CHAPTER - VII

SUMMARY
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The coleopteran beetles have a world-wide distribution and are found on land, water and also in the air due to their great amount of adaptability in the environment. They are known to cause diseases and also damage food, fruits and vegetables. The Sal borer, which infests Sal trees, is a deadly pest destroying the Sal forest wealth of both M.P. and Chhattisgarh. The red pumpkin beetle severely damages vegetables belonging to cucurbitaceae family. The poultry beetle serves as a reservoir for Salmonella, Pasteurella and other diseases causing agent.

Since, most insects are harmful, researches on measures to control their growth and reproduction have been undertaken over the years. Until recently there have been two main aspects of controlling insects. Firstly, applied control such as by use of chemicals (pesticides and chemosterilants). Secondly, natural control, such as biological control, by increasing the presence of natural predators of insects like birds, fishes, reptiles, mammals and by cannabilism. More recently integrated pest management (IPM) methods are being employed in which a combination of natural and applied control have been used.

The objective of the present study is two fold. One is to see the effects of radiation on the male germ cells i.e. what type of effect is produced on exposure to radiation in these cells and the other is an attempt to apply radiation technology to control these destructive insect pests, with a view to make them sterile, and disrupt their reproductive capabilities.

The effects of both ionizing and non-ionizing radiation on the male germ cells of three coleopteran beetles viz. *Raphidopalpa foveicollis,*
*Alphitobius diaperinus* and *Hoplocerambyx spinicornis* have been investigated in the present study. The study also aims to find out as to what extent radiation alters the process of spermatogenesis and also what changes, it brings about in the quality and quantity of off-springs produced. As observed by some scientists [Muller (1927) Carlson (1941), Sax (1941), Reynolds (1941), Mitchell (1942), Bishop (1942), Knipling (1955), Rogers (1955), Manna (1969), Savage (1970)] radiation may produce different types of sterility in insects particularly in males.

*Raphidopalpa foveicollis*, is a red coloured pumpkin beetle found feeding on gourd, cucumber and various cucurbits. It feeds on flower buds, leaves and fruits of cucurbits causing severe damage. The adult beetle measures about 7 m.m. in length and 4 m.m. in width. The colour is orange-red. Males are small in size than females.

*Alphitobius diaperinus*, commonly known as the lesser mealworm is abundantly found in dirt floors of poultry houses. These beetles serve as reservoirs of Mark’s disease, virus Salmonella species, Pasteurella species and other infective agents which are causative or carriers of the disease. This beetle is small black in colour and rounded on anterior margin. Prothorax wider then long, legs rather short, length 4-5mm, possesses chewing mouth parts and two pairs of wings. Males are smaller in size than females.

*Hoplocerembyx spinicornis*, is a well known borer, infesting the Sal trees of forest areas of Madhya Pradesh and Chhattisgarh State. They cause great damage to the forest by boring the stem bark of Sal trees. To get-rid of this borer, both the State’s have taken extensive drastic measures
like deforestation of the infected Sal trees and also employing labourers to catch and destroy (manual eradication) the population of these insects. Pesticide sprays have proved hazardous to non-target organisms and they are not effective in killing this beetle since it penetrates the bark and reproduces inside the stem of the tree. It feeds on xylem sap. It is a large dark brown coleopteran beetle, which varies greatly in size; length 18.5 - 65.0 mm, width in anterior part of elytra 5-20 mm. The antennae measure 20-33 mm. Males have longer antennae compared to females.

Live specimens of male coleoperan beetles were collected in different seasons and from various localities. The red pumpkin beetles were collected from agricultural fields of cucurbit nurseries in the premises of Indira Gandhi Agricultural University, Raipur (Chhattisgarh) during the month of August and December (2000 and 2001).

Specimens of lesser mealworm were collected from the local poultry farms (Sarin poultry farm, Jai Shree poultry farm) in Raipur throughout the year in 2001.

Specimens of Sal heartwood borer were obtained from the heavily infested Sal trees of forests in Shahdol (Amarkantak) and Mandla (Dindori) district of Madhya Pradesh and Bastar, Kavardha and Raipur district of Chhattisgarh State. These were collected during the rainy season in the month of June and July (2000 and 2001) at which time they are abundant. Collection of these beetles was done with the help of local adivasis and the forest officers of the area.

The collected insects were then maintained in the laboratory for further use. They were starved for 1-2 days to reduce their body fat.
All these beetles show sexual dimorphism hence it was easy to
differentiate the males. Male beetles of red pumpkin beetles and lesser
mealworm are comparatively smaller in size than females and can be easily
identified. The males of Sal borer beetle can be distinguished by their long
antennae. Females have comparatively short antennae.

For the study of normal male germ cells the male beetles were
dissected under the Binocular microscope. Testes were removed and put in
normal saline solution (0.6%) which is a hypotonic saline solution, for 15 to
20 minutes. The testes were further fixed in acetic alcohol (2:5) and
Carnoy’s fluid for histological studies.

Squash preparations were made using aceto-carmine stain. Smear
preparation of testis was made by dissecting out the testis from the insect
with the help of fine forceps and needles in living condition. Material was
transferred to a very clean slide and smear prepared by teasing the
testicular fibrous sheath under the Binocular microscope. The contents of
testicular lumen were allowed to flow and a uniform film was made. Then the
material was fixed in acetic alcohol (2:5) immediately to avoid postmortem
changes. The material was dehydrated, stained with haemotoxylin stain
(alcoholic), cleared in xylol and mounted.

For histological studies paraffin blocks were prepared after fixation in
carnoy’s fluid and by dehydrating the material in ascending grades of alcohol
and clearing in methyl benzoate and benzene. Sections cut at 6μ and 8μ
thickness, were stained with haemotoxylin stain.
For exposure to radiation, two different doses i.e. Low and High dose were selected. Beetles were exposed to these doses of ultraviolet, X-rays and CO.$^{60}$ radiation for different durations. Two types of radiation doses were selected, as follows:

*For Ultra-violet radiation, exposure to*

(a) Low dose = \[
\frac{G-15 \text{ T 8}}{15 \text{ W}}
\] for 20 minutes

(b) High dose = \[
\frac{G-15 \text{ T 8}}{15 \text{ W}}
\] for 30 minutes

*For X-ray radiation, exposure to*

(a) Low Dose = \(60K_v - 80\) MAS (At 160 station) 100 Cms distance between tube and object.

(b) High Dose = \(65K_v - 80\) MAS (At 160 station) 100 Cms distance between tube and object.

*For CO.$^{60}$ radiation, exposure to*

(a) Low Dose – 3 Rads

(b) High Dose – 5 Rads

The Ultra-violet radiation facility was availed from Department of Plant Pathology Indira Gandhi Agricultural University, Raipur (Chhattisgarh). X-ray radiation facility was availed at a private Nursing Home at Raipur. The CO.$^{60}$ radiation facility was availed from the Deptt. of Radiation and Oncology, Cancer Hospital, Sector-1, Bhilai, District Durg (Chhattisgarh). Photomicrography was done using Leico equipment at the Indira Gandhi Agricultural University, Raipur (Chhattisgarh).
The effects on radiation exposed male germ cells were observed and compared with normal controls.

Red pumpkin beetle possesses a single testis. It is dorsally placed at the right side of the fifth abdominal segment. The testis is round and orange coloured. Posteriorly it opens into the vasa differentia. It is externally covered with fat body. Histologically the testis is covered by a fibrous sheath, inside which are, a large number of follicles separated by partitions. Within these follicles stages of spermatogenesis are seen viz. spermatogonia, primary and secondary spermatocytes, spermatids and sperm bundles. The follicle contains many cysts each of which consists of a clone of germinal cells. These cysts are arranged in order of increasing maturity i.e. from the periphery to the centre of the testis. The primordial germ cells, spermaogonia and spermatocytes are observed at the periphery. The central lumen is filled with the mature sperms. Spermatogonia are large and seen rarely. Several of these actively dividing cells are seen in testicular lumen. Primary and secondary spermatocytes are abundant. Developing spermatids generally occupy central part of the cavity. They are round in shape, having deeply stained acrosomal granule. The sperms are seen in bundles with deeply stained nucleus.

Lesser mealworm beetle has a pair of testes, cotton like in appearance, dirty white in colour, located at the latero-dorsal side of the abdomen. They are fixed in a groove like structure within the abdomen. Histology of testis shows four distinct parts within the follicle. A germarium containing primary spermatogonia, a zone of growth where transformation of
spermatogonia into spermatocyte occurs, a zone of maturation where the spermatocytes are converted into spermatids and the zone of transformation where spermatids change into spermatozoa. A number of follicles are found enclosed within the testicular epithelium. The spermatogonia are large in size, primary and secondary spermatocytes are comparatively small in size having 2-3 vacuoles and clear cytoplasm. The sperms are found both isolated and in bundles. The mature sperm consists of deeply stained filamentous nucleus and lightly stained cytoplasmic tail.

In Sal heartwood borer the paired testes are located at latero-ventral side of the alimentary canal. The testes are oval in shape and muddy white in colour. Posteriorly, they open in the vasa differentia. They are anteriorly broad and posteriorly pointed structures. Histologically, the testis consists of a thick covering of a fibrous sheath in which a number of follicles separated by many partitions are present. Follicle consists of 4 distinct zones viz. a thick germarium, a zone of growth, a zone of maturation and a zone of transformation. All germ cells are enclosed within a layer of non-germinal cells forming cysts. Divisions within cyst appear synchronously. Follicle sheath has two cellular layers outer which is continuous and inner present in germarium. The outer layer of the sheath is a thin lamina and the inner a thicker basal lamina. In the cytoplasm of the outer cells flattened nuclei are present. The cells of inner layer, besides being discontinuous, differs from the outer ones in that their cytoplasm is some what denser. Spermatogonia are large in size and remain separated from each other and exhibits no cytoplasmic connections between them. Spermatocytes are abundant in the
zone of maturation. Primary Spermatocytes are large in size and have a well distinct nucleus. Secondary Spermatocytes are small in size and contain a prominent nucleus and vacuoles. The sperms are large in size and more in number. It is remarkable to note that in Sal heart woodborer the elongated sperm nucleus contains a tube like vacuole inside, which is lost with the condensation of chromatin material. The elongating spermatids assume the shape like "Trypanosomes".

On exposure to radiation, beetles become hyperactive, their mobility increases. Even high dose of radiation is not lethal for these beetles.

On exposure to low dose of Ultra-violet radiation exposure all these three beetles do not show any remarkable effects on the testes and on germ cells.

With high dose of Ultra-violet radiation exposure the secondary spermatocytes and sperms show changes in red pumpkin beetle and lesser mealworm. In red pumpkin beetle, the and secondary Spermatocytes become shrunken. Spermatids change their shape and size, become shorter. In lesser mealworm the secondary Spermatocytes, cell wall distorted. Spermatids condensed their nuclear material. In Sal heart woodborer no remarkable effect is found in any of the germ cells of the testes.

With low dose of X-ray radiation, minor changes are observed in the secondary spermatocytes, spermatids and sperms of red pumpkin beetles. The secondary spermatocytes and spermatids shrink, change their shape and sperms reduces their vacuoles. In lesser mealworm the primary spermatocytes, secondary spermatocytes and sperms showed effects. They shrink and become indistinct. In Sal heartwood borer no remarkable change is seen.
condensation of nuclear material. Primary spermatocytes showed shrunken effect. Secondary spermatocytes and spermatids exhibit distortion. In Sal heart woodborer, the testis does not show any remarkable change. Secondary spermatocytes and spermatids showed distortion. Flagellum of spermatids is very short and acrosome becomes indistinct.

On exposure to high dose of CO.$^{60}$ radiation in the red pumpkin beetles the germinal epithelium of testis shrinks. Follicles are shrunken and damaged. The cell boundaries of spermatagonia become obliterated and distorted. Primary and secondary spermatocytes become necrotic. Acrosome of spermatids becomes indistinct and flagellum becomes very short. The nucleus is not seen distinctly and does not take stain properly. The sperm becomes distorted, indistinct, vacuoles disappear, acrosome not clearly seen, showing the stickiness and clumping of chromosomes.

In lesser mealworm the germinal epithelium of the testis becomes shrunken. The chromosomes of spermatagonia become abnormal in shape and the chromatin material is condensed, due to the shrunken effect. Primary and secondary spermatocytes are not clearly seen. In Sal heart woodborer, Follicles showed damage as they shrink and become smaller. The cell boundaries of spermatagonia become distorted. Secondary spermatocytes showed necrotic effects. In spermatid the nucleus become indistinct and cell become distorted. Most of the spermatids disappear. Number of sperms are reduced. The sperms fail to form sperm bundles and show disturbed physiological state of spermatogenesis and indicates infertility.
It is thus inferred that the affected germ cells by high dose of CO$^{60}$ radiation exposure treatment cause interruption of spermatogenesis. This seems significant because discontinuity of spermatogenesis will be the result when all or most of the spermatogonia are damaged and this will lead to permanent sterility.

To conclude, the study reveals that CO$^{60}$ radiation has a severe effect on the male germ cells in all these three beetles. However, X-ray and Ultraviolet radiation exposure results in interference of spermatogenesis and damage of spermatogonia and sperms. This technology can be applied as part of Integrated Pest Management (IPM) for the control of these insects pests.