A Study of male germ cells of Raphidopalpa-foveicollis and effects of Radiations on them

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Abstract— **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 red pumpkin beetle (Raphidopalpa-foveicollis) severely damages vegetables belonging to cucurbitaceae family., 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 cannibilism. More recently integrated pest management (IPM) methods are being employed in which a combination of natural and applied control have been used. The effects of both ionizing and non-ionizing radiation on the male germ cells of coleopteran beetle Raphidopalpa-foveicollis has 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**

I. INTRODUCTION

The Coleoptera is the largest order in not only the arthropoda but in the entire animal kingdom consisting about 330000 species. They are adapted to various modes of life, viz. terrestrial, aquatic, arial, fossorial, subterranean cavernicolous etc. and are mostly concealed in habit. The order represents a heterogeneous assemblance of very minute size.

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 control of insects can be accomplished in many ways, and may broadly be subdivided into:

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1. **Applied control measures:** Which depend upon the man for their application or success and can be influenced by him to a considerable degree.
2. **Natural control measures:**- It does not depend upon man for their continuance or success and cannot be influenced by him.

The present work is of great agricultural importance. The beetle under study, ***Raphidopalpafoveicollis*** is destructive pest.. Red pumpkin beetle, destroy leaves and flowers of cucurbit plants.

The effects of ionizing radiations especially of X-rays and CO^{60}rays have extensively been studied by numerous workers at the cytogenetic level. Muller (1927) made pioneering discovery of artificial induction of mutation in drossophila by X-rays radiation since then the progress in radiation cytogenetics has been reviewed from time to time by various workers (Sax 1941; Lea 1946; Cateheside 1948; Hallaender 1954; Bacq and Alexander 1961; Ray Chaudhary 1961; Evans 1962; Manna 1969; Savage 1970). So far as the effects of radiation on chromosomes during cell division are concerned studies of Mohr (1919); Dictiecus and Carlson (1938a); Carlson (1938b; 1940 1941,1953), Carlson and Harington (1955) are important.

The X-rays are generally used for experimental work while the X-rays CO^{60} rays are used on large scale in the sterilization programme. Uses of neutrons, however, have been rarely made. The α, β, γ, x-rays, protons and neutron radiations are ionizing radiations i.e. high energy radiations because of the high photon energy of radiation. The primary effect of its interaction with living matter consists of ionization. Only genetically effective non-ionizing radiation is Ultra-violet radiation, which gives biological effects similar to those induced by high-energy radiations. Unit of radiation is rad. One rad is equal to 100 ergs of the energy/gm absorbed by the material, roughly equivalent to the roentgen.

1000 rad = 1 kilo rad or k. rad.

X-rays consist of little more than the densely ionized “tail”. X-rays are natural. X-rays of very short wavelength having quanta of very high energy and producing only Compton recoil dectrons. Ionizing radiation also dissipates energy in tissue by excitation i.e. by raising on electron in an atom or molecule to a state of high energy. Ultra-Violet light can cause excitation but not ionization. Ionizing radiations affect any cell by direct damage to the chromatin, inhibiting mitosis, meiosis, or differentiation, causing necrosis and pyknosis. The germinal cells are no exception and the major effect of ionizing radiations on them has to do with nuclear chromatin sensitivity so that higher levels of exposure will demonstrate graphically the irrevocable changes brought about in these nuclear components. Such inhibition of maturation as occurs may not be evident at the time of irradiation but will become evident as any particular cell reaches the stage where mitosis or differentiation is supposed to occur.

The main objective in the present study is to try and control these insect pests by hampering their reproduction by radiation. The proposed research work, it may be mentioned here that the studies on the male germ cell cycle of beetles undertaken have not been done so far. It will not be out of place to mention here that for the first time an attempt is being made to study the effects of different doses of X-ray irradiation on various developing cells during the process of spermatogenesis.

II. MATERIAL AND METHODS

Live specimen of male coleopteran beetle was collected during rainy season and from various localities. The red pumpkin beetles were collected from agricultural fields of cucurbit nurseries in the premises of I.G.A.U., Raipur during the month of August and December (2010).

The collected insects were then maintained in the laboratory for further use. They were starved for 1-2days 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.

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 acetocarmine 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 post-mortem 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 thickness, were stained with haemotoxylin□ and 8□benzene. Sections cut at 6 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

$\text{Low Dose} = \frac{G-15T8}{15\ W}$ For 20 minutes

$\text{High Dose} = \frac{G-15T8}{15\ W}$ For 30 minutes

For X-ray radiation, exposure to

Low Dose = $60K_V$ – 80 MAS (At 160 station) 100 Cmsdistance between tube and object.

High Dose = $65K_V$ – 80 MAS (At 160 station) 100 Cmsdistance between tube and object.

For CO^{60} radiation, exposure to

Low Dose – 3 Rads

High Dose – 5 Rads

OBSERVATION

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. Histogically 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.

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 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.

With high dose of X-ray radiation exposure in red pumpkin beetles the spermatogonia are affected, their chromatin material become condensed, and cells become shrunken, cell boundaries are obliterated and distorted. Secondary spermatocytes become necrotic. Spermatids become reduced in size and the chromatin of nucleus becomes condensed at the inner margin of the nuclear membrane. Vacuoles fuse to become reduced sperms and acrosome appears as a granule near the membrane.

Low dose of CO^{60} radiation exposure to red pumpkin beetles affects the germinal epithelium of the testis. In spermatogonia, primary spermatocytes, secondary sperma-tocytes, become deeply stained and vacuole reduced. In lesser mealworm the follicles become smaller. The chromosomes of spermatogonia become deeply stained due to the 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 spermatogonia 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.

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.

III. RESULT AND DISCUSSION

The present research work includes the study of the structure of male germ cells and the effects of irradiation on the species of coleopteran beetles ***Raphidopalpafoveicollis.*** In the species the testis has been found surrounded by a fibrous sheath. In the case ***Raphidopalpafoveicollis*** the sheath the comparatively thin,

The spermatogonial cells, primary and secondary spermatocyte cells, spermatids and sperms have been found in groups within the testicular lumen. Mostly the central part of the cavity has been found to be filled with spermatids, developing and nature sperm bundles are compared to the other groups of cells.

IV. SPERMATOGONIAL CELLS

In the species under study, only one distinct nucleolus has been observed in the nucleus of the cell. In ***Raphidopalpafoveicollis*** it is largest in size and measures 2.0 mm., It is remarkable o note have that in ***Raphidopalpafoveicollis*** one or two clear vacuoles are found within he nucleolus of the cell, in ***Raphidopalpafoveicollis***, a clear unstained perinucleolar ring has been found surrounding the nucleolus. The nucleolarvacuole as well as the perinucleolar ring remain unstained throughout the process.

V. PRIMARY SPERMATOCYTE CELLS

In the species, under investigation the primary spermatocyte cells are abundant in the testis and remain packed together, therefore their cell boundaries fail to keep their regular rounded shape. Their cell membrane and nuclear membrane are distinct. The nucleoplasm is granular while cytoplasm is almost clear in appearance. A well stained is clearly distinct in the nucleus of the cells. ***Raphidopalpafoveicollis*** contains one or two nucleolar vacuoles in the nucleolus. In the present study there could not observe these “bouquet” or rosette like structures. During diplotene, the chromosome loops become shorter at certain points the crossing over and chiasma have also been observed in some of these cells. During diakinesis, some of the chromosomes form ‘rings’ whereas in others these look like “rod” and dot shaped.

VI. SECONDARY SPERMATOCYTE CELLS

The primary spermatocyte cells, after the first mitotic division form the cells known as secondary spermatocyte. These cells are smaller in size as compared to primary spermatocytes. It is remarkable here to note that the primary spermatocyte cells (are found in the groups and packed together), the cells in a group are of the same age and all start dividing at one time, with the result, the newly formed secondary spermatocyte cells are also of the same age and are found in the groups. A clear nucleolar vacuole is visible, inside the nucleolus. An unstained perinucleolar ring around the nucleolus is seen up to the prophase stage in ***Raphidopalpafoveicollis***. All the secondary spermatocyte cells of one group have been found exhibiting prophase, metaphase, anaphase and telophase stages in all the three species. The size of the secondary spermatocyte cells in ***Raphidopalpafoveicollis*** is larger measuring 7.0 mm. to 8.0 mm.

Spermatids-

During the present investigation in ***Raphidopalpa-foveicollis*** it has been found that the newly formed spermatids are round having deeply stained and condensed chromatin material within the nucleus. However, in its latter stages the chromatin material aggregates at the inner margin of the nuclear membrane. Due to this condensation of the chromatin material at one side, a clear nuclear vesicle is seen within the nucleus in all the species.

It may be mentioned here that the acrosome appears as a deeply stained granule situated near the nuclear membrane. However, the acroblast could be seen in ***Raphidopalpaf-oveicollis***. The acrosomal granule gradually grows at the anterior tip of elongating sperm nucleus.

During the present investigation in ***Raphidopalpafoveicollis*** only one control has been found near the nuclear membrane, The acrosomal granule appears as a deeply stained body at the inner margin of the nuclear membrane of the spermatid.

Sperm Formation.

During the present study, the nucleus of spermatid gradually elongates and becomes oval, fusiform and lastly filamentous. During the elongation it has been found that vacuoles are lost. The elongated sperm nucleus contains within itself a single tube-like vacuole, which is gradually lost due to further condensation of the nucleus. It may be mentioned here that during condensation the nucleus assumes the form of a plate like structure. The flattened nucleus now contains an elongated narrow cavity within itself. Due to further condensation and elongation of the nuclear material the hollow space within it is totally lost and it becomes filamentous and stains deeply.

During the present study of ***Raphidopalpafoveicollis***, the nucleus of spermatid gradually elongates and becomes oval, fusiform and lastly filamentous. In all the three species undertaken the acrosome and centriole have been found to be situated at the anterior ends. The chromatin material appears as deeply stained granules within which some vacuoles are seen. it contains 1-4 vacuoles. During further elongation it has been found that such vacuoles are lost. The elongated sperm nucleus contains within itself a tube like vacuole which is gradually lost due to further condensation of the nucleus.

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, X-ray and CO^{60} radiation, beetles do not show any remarkable change. High dose of Ultra-violet radiation has no effect but high dose of X-ray and CO^{60} radiation affects the germinal epithelium of the testis as it becomes shrunken.

Kaufman and Hollaender (1946), Swanson's (1944) Russell (1961), Allenberg (1933,1936), Eberhardt (1941), Muller (1940), Mcclintock (1939, 1941a), Catcheside (1939), Dempster (1940), Eberehratt (1939), Sax (1942), Giles (1940, 1943), Thoday (1942), Kotval and Gray (1947) have studied the mutational changes (chromosomal aberrations) in male germ cells of various insects. The principle aberrations studied are as described by Lea and the pertinent data have reviewed by Kaufmann and giles for ionizing radiation and Swanson and Stadler for Ultra violet radiation.

It is thus, inferred that the affected spermatogonia by radiation exposure treatment cause interruption of spermatogenesis at the spermatogonial level. 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.

The overall effect of irradiation exposure treatment on the germ cells within the testis of ***Raphidopalpa-foveicollis***, gradually decreased respectively which showed pronounced distortion in general epithelium damage of younger germ cells, loosening of germ cells, epithelium of testis follicle broken at few places. Some follicles shrunken, the spermatids become pyknotic but the nuclei of spermatogonia and the spermatids become slightly distended. Most of the spermatozoa become small in size than control insects. Some spermatozoa shrunken in shape, it seems their tails did not develop properly. Such spermatozoa tended to clump together, from head to tail and therefore are assumed to be functionless.

With the high dose of X-ray radiation exposure in red pumpkin beetles the spermatogonia affected their chromatin material become condensed, they become shrunken, cell boundaries obliterated and distorted. Secondary spermatocytes become necrotic. Spermatids become shorter and their chromatin of nuclear material has condensed at the inner margin of the nuclear membrane. Vacuoles fused to become reduced.

Low dose of CO^{60} radiation exposure to red pumpkin beetles affects the germinal epithelium of the testis. Spermatogonia, primary spermatocytes, secondary spermatocytes changed their nuclei, as they become deeply stained and vacuole become reduced. In lesser meal worm the follicles become4 smaller. The chromosomes of spermatogonia become deeply stained due to the condensation of nuclear material. Primary spermatogonia become deeply stained due to the condensation of nuclear material. Primary spermatocytes showed shrunken effect. Secondary spermatocytes and spermatids showed distortion effect.

On exposure to high dose of CO^{60} radiation to red pumpkin beetles the germinal epithelium of testis shrunken. Follicles are damaged and shrink. The cell boundaries of spermatogonia become obliterated and distorted. Primary and secondary spermatocytes become necrotic. Therefore, their cytoplasm and nuclear material is difficult to distinguish from one another. Acrosome of spermatids becomes indistinct and flagellum seen very short. The nucleus is not seen distinctly and does not take stain properly. The sperms become distorted, indistinct, vacuoles disappears, acrosome not clearly seen showing the stickiness and clumping of chromosomes. In lesser meal worm the germinal epithelium of the testis become shrunken. The chromosomes of spermatogonia become abnormal in shape and the chromatin material not seen clearly. They become necrotic, elongation of spermatids and sperms is affected. Sperms becomes distorted and acrosome not clearly seen. In sal heart woodborer beetles do not show any remarkable change in testis. Follicles showed damages as they shrink and become smaller. The cell boundaries of spermatogonia 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 reduced.

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. It is very remarkable here that the effects of radiation on sperms showing the partial aspermia state and clumping of chromosomes undergo abnormal mitosis. The sperms fail to form sperm bundles and showing the disturbed physiological state of gonad and adds to infertility.

Taking into account of existing knowledge of cytology of the male germ cell cycles in beetles, it has been decided to study and discussed this phenomenon in the three species of coleopteran beetles i.e. ***Raphidopalpafoveicollis,*** with special reference to the effect caused by various doses of irradiation on their germ cells, which has not been studied so far.

Sterility is nothing but the inability to produce offspring's. The sterile insects cannot contribute a genome to the next generation. The sterility may develop due to infecundity of females, aspermia or sperm inactivation in males, inability to mate and dominant lethal mutations in gametes. The last one is most promising in the pest control.

The types of cells present in the testis determine the type of sterility developed by the irradiation in male insects. Secondary spermatogonia are extremely sensitive to the radiation producing aspermia in the males. The next sensitive stage is the young primary spermatocytes which in screwworm, were killed by a dose of only 500 rads.

The ultra-violet radiation is the only non-ionizing radiation capable of fragmenting chromosomes. However, being a photochemical radiation it is selectively absorbed and its effects on the hereditary material of the cell reveal aspects of radiation biology let untouched by studies in which the ionizing radiation is employed.

The effects of ionizing radiations especially of X-ray and CO^{60} ray have extensively been studied by numerous workers at the cytogenetic level.

Radiation may produce different types of sterility in insects particularly in males. The sterility is more effective then only other method and without creating any adverse effect.

With high dose of U.V. radiation exposure the secondary spermatocytes and sperms show changes in red pumpkin beetle and lesser mealworm. In red pumpkin beetle, and the 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.

Therefore various criteria of effects of irradiation of the testis; induced mutation chromosomal aberrations, reduced fertility. Semisterility and permanent sterility – all involving the germinal epithelium and bypassing the sertoli and interstitial cells.

The affected spermatogonia by radiation exposure treatment cause interruption of spermatogenesis at the spermatogonial level. This seems significant because discontinuity of spermatogenesis will be the result when all or most of the spermatogonia are damaged and this will lead of permanent sterility. Hypertrophied as well as atrophied sperms (in some cases) fail to form sperm bundles in males showing disturbed physiological state of the Gonad and adds to infertility.

Hence, the application of radiation technology can be helpful to the population of such destructive insect's pests. Effects of radiation on male germ cells of these coleopteran beetles will help in controlling these insect pests. If other methods of control such as pesticide spray etc. are employed there is a danger of environmental poisoning affecting non-target useful organisms. Therefore, we would also suggest radiation as an alternative strategy, because applying radiation technology can be a specific one and no other organism will be affected during the process. Pesticide use can be avoided which has been proven to be hazards to non-target organisms in nature. Hence, the present work is of great agricultural importance.

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 Ultra-violet 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.

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