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Effects of gamma radiation on the reproductive organs in the red flour beetle *Tribolium castaneum* (Herbst)

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Abstract: Gamma radiation effects on the gonads of *Tribolium castaneum* were assessed by cytological techniques. Pupal treatments of 15 Gy resulted in the significant reduction of testes and ovaries leading to sterility in males and infecundity in females, respectively. The present findings thus provide an important information for designing a sterile male technique for this storage pest species.

Key words: Gamma radiation, *Tribolium castaneum,* reproductive organs

Introduction

Although many tissues show negligible damage in mature insects, the reproductive organs are sensitive to gamma radiation because the germinal cells usually show moderate to severe damage (Tilton & Brower, 1983). The rapidly dividing germinal cells that are still in the process of differentiation are particularly radiosensitive, and because of their active division they express radiation damage quickly. In some cases, as with larvae or adults, it appears that innate genetic factors determine the time and mode of post-radiation mortality (Hasan *et al.*, 1989). Hasan (1995) noticed that high doses of gamma radiation can inactivate sperm or at least produce dominant lethals in cells and lower doses can have significant effects on sperm production, especially if the timing of the treatment has affected the developing sperm cells. Cells in the process of spermatogenesis are very radiosensitive and apparently are easily killed (Hasan *et al.*, 1989). Tilton & Brower (1983) reported that in Coleoptera, spermatogenesis normally occurs in the pupal stage and often for most of the adult life. Pupal or adult irradiation has therefore rather similar effects and accordingly, histological damage is expected to be similar.

The irradiated females usually lay reduced number of eggs, and the rate of this decrease depends on the species, the stage or age at which radiation takes place, the dose and other factors (Tilton & Brower, 1983). Females lacking functional ovaries exhibit the ultimate state of infecundity. Where the females with rudimentary ovaries were irradiated in a pre-adult stage, the ovaries have never been developed, but high doses delivered to the adult can cause severe tissue damage and regression of the ovaries, although radiation does not always cause decreases in oviposition or fecundity (Hasan, 1995). Here we report the gamma irradiation effects on gonads of the red flour beetles *Tribolium castaneum* and the cytological changes that lead to sterility characterized by infecundity.

Materials and Methods

The test insects: A local strain of *T. castaneum* was used for the histological studies. Pupae were collected from culture maintained in an incubator at temperatures ranging from 29° to 31°C with an uncontrolled RH. They were sexed by the microscopic examination of the exogenital process of the female (Halstead, 1963).

Radiation treatments: Ten 2-day old pupae of both sexes were irradiated with a dose of 15 Gy. The dose rate was 10 Gy/min from a deep-therapy radiation unit of Co^{60} installed at the Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Saver, Dhaka. After irradiations, pupae were kept in a jar containing standard food media for eclosion. Fresh adults were dissected for gonads. Adults from unirradiated pupae were used as control.

Dissection and staining procedures: Beetles were dissected under dissecting microscope in a drop of Ringer's physiological solution on a wax-fixed petridish. A pair of dissecting forceps was used to open the abdominal cavity and then the reproductive organs were taken out. The surrounding tracheoles and fat body were removed. For dissection and fixations techniques described by Anwar *et al.* (1971) were followed. Dissected organs were fixed in Bouin's solution for one day that stained the specimen yellow but provided inadequate contrasts for photography (Eltringham, 1930). The specimens were washed in saturated aqueous solution of lithium carbonate for 30 minutes to remove the yellow colour. Then they were washed again in distilled water for 30 min, stained in 0.5% methylene blue chloride in distilled water for 45 min and passed gradually through 30%, 50%, 70%, 90% and 100% ethanol for 15 min each to make them histoclear. Temporary squash preparations were made of the specimens, stained with acetic orcein, mounted in DPx, and then covered with slide covers. The slides were examined with a Pantex trinocular contrast microscope for histological studies. Photographs were taken using a microscope with 25 mm film (Kodacolor 200).

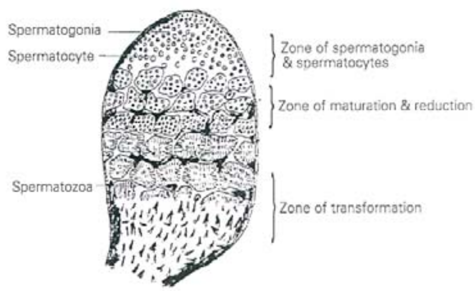

Plate : 1. Longitudinal section of testicular follicle of *Tribolium*

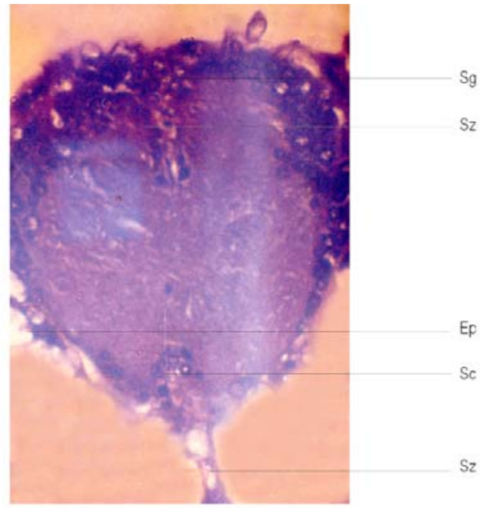

Plate : 2. Unirradiated tesicular follicle of *T. castaneum* (RAJstrain). Vs-Vesicula seminalis, Ep-Epithelium, Sc-Spermatocyte, Sg-Spermatogonia Sz-Spermatozoa.

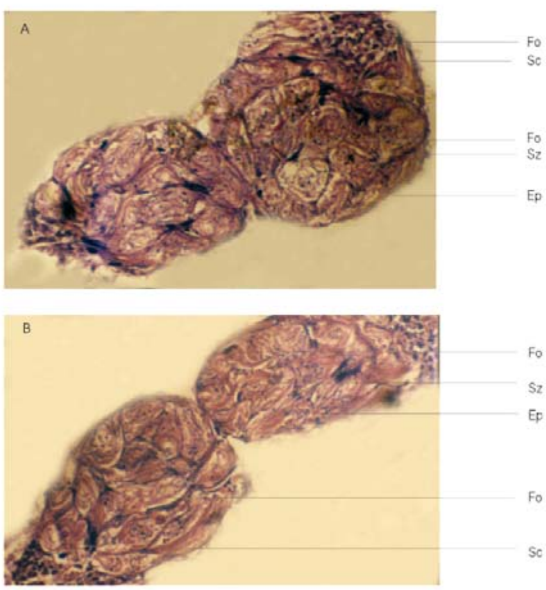

Plate : 3. Unirradiated testicular follicles of *T.castaneum* (RAJ Strain) (A). Irradiated testicular follicles of *T. castaneum* (RAJ Stain) 10 days after irradiation on pupae with 15 Gy (B). Fo-Testicular follicle, Ep-Epithelium,Sc-Spermatacyte, Sz-Spermatozoa.

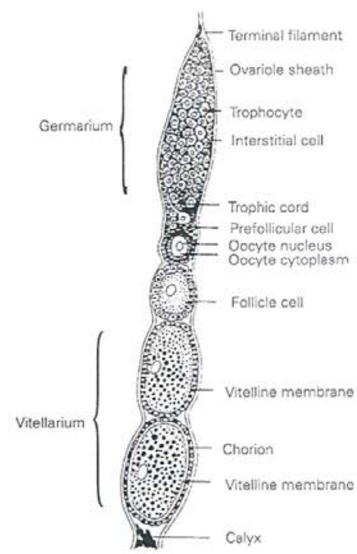

Plate : 4. Diagram of a single ovariole of *Tribolium* showing the different stages of oocyte development

Results and Discussion

Radiation effects on testes: A schematic drawing of the longitudinal section of testis (Plate 1) shows the location of different germ cells, and the progressive development of the sperm from the spermatogia, zones of spermatogonial cells, spermatocytes, spermatids and mature spermatozoa, starting from the apex to the base of the testis. Examination of the irradiated testis revealed that the organ was very sensitive to gamma radiation of 15 Gy. Normal testicular follicles had vericula seminalis, epithelium, spermatogonia spermatocytes and spermatozoa (Plate 2), whereas reduction in the size of the irradiated testes (Plate 3A) compared with those of unirradiated testis (Plate 3B) was apparent. Reduction in growth of the testis after irradiation may account for the decreases in or absence of spermatogenic activity as a possible consequence of radiation damage to the germ cells. However, the measurement of the size alone was not a prime indicator of the exact degree of damage.

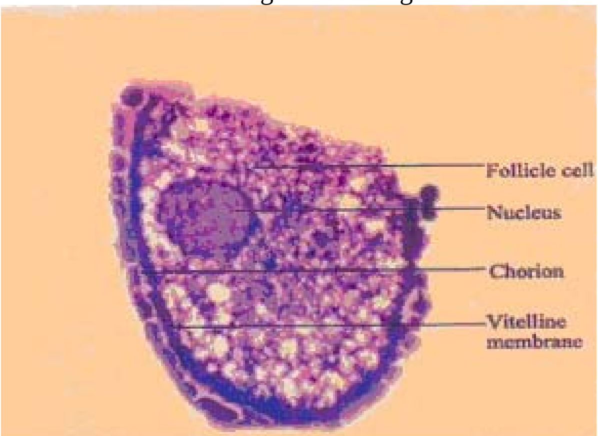

Plate : 5. Showing a unirradiadiated chorion of *T. castameun* (RAJ strain).

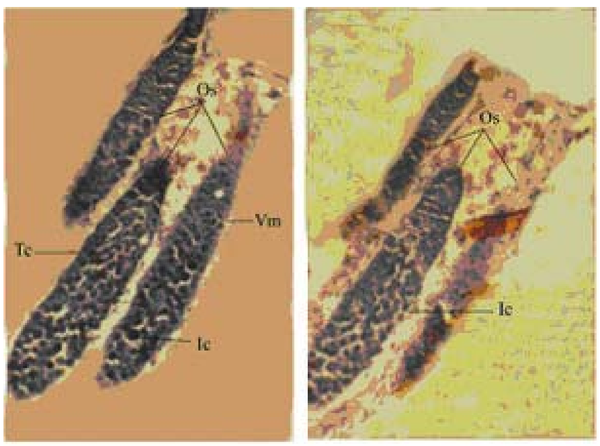

Plate : 6. Ovarioles of adult unirradiated *T. castaneum* (RAJ strain) 10 days after emergence (A) and ovaries of irradiated *T. castancum* (RAJ strain) 10 days after irradiation on pupae with 15 Gy(B). Os-ovariole Sheath, Tc-Trophocyte, Vm-Vitelline membrane, Ic-Interstitial cell.

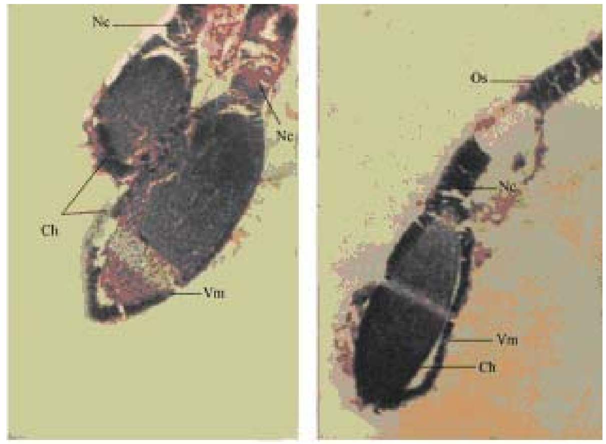

Plate : 7.Ovaries of unirradiated *T. castaneum* (RAJ strain) 10 days after emergence (A) and ovaries of irradiated *T. castaneum* (RAJ strain) 10 days after irradiation on pupae with 15 Gy (B). OS-Ovariole sheath Ch-Chorion, Vm-Vitelline membrane, Nc-Nutritive cell

Radiation effects on ovaries: A schematic drawing of a single ovariole is shown in Plate 4 for interpreting the different stages of the oocyte development. Plate 5 shows In an unirradiated chorinon of the female *T. castaneum* (Plate 5), vitelline membrane, nulceus and follicle cells are clearly seen. A comparison has been made between unirradiated ovariole sheath and irradiated one (Plate 6). Reduction in the size of ovariole sheath and ruptured vitelline membrane are obvious in Plate 6B. Irradiation dose of 15 Gy did not arrest the development of egg follicles. The irradiated ovaries did not show much difference from unirradiated ones (Plates 7A and 7B) except in size, but no measurements were made.

Gamma irradiation with a dose of 15 Gy on the late pupae of *T. castaneum* resulted in atrophied reproductive organs, resulting in a reduction in size of the gonads. Previous study by Hasan (1995) revealed that the degree of atrophy increased with the increase of irradiation dose where very high doses resulted in lacking follicles in testes and ovarioles. Cell death may occur as a result of the failure of the structurally changed chromatin material to undergo mechanical separation at the first post-irradiation cell division (Muller, 1940). Any attempt to examine the relation between genetic damage and radiation-induced lethality must take into account the response of a wide variety of cell types ranging from the simplest virus to the most complex of animals.

Annan (1955) concluded that the irradiated females were more susceptible to a reduction of fertility than the irradiated males. King (1957) observed two overall effects of radiation: (i) the most common was an abnormal distribution of the developmental stages of oogenesis leading to a general decreases in the rate of oogenesis; and (ii) the inhibition of cell division particularly in oogonial cells. LaChance & Bruns (1963) also observed periods of development when the volumes of the ovarioles were reduced by one-half or more by irradiation. Studies reported by LaChance & Bruns (1963) and LaChance & Leverich (1968) on female *C. hominivorax* indicated that gamma radiation not only showed down the rate of ovarian growth but also caused cytopathological changes in developing egg follicles. Rami Reddy *et al.* (2006) noticed that the radiation sensitivity of the insect decreased with increasing age in *Callosobruchus chinensis* (L.). They also showed that the longevity, fecundity and fertility of the surviving adult insects were adversely affected by the electron treatment (200 kV equal to 10 kGy) and the insects failed to complete their life cycle. The apparent reduction in the volume of ovarioles was probably not due to a reduction in their number but to morphological change in the ovarioles. It is suggestive from the earlier investigation that irradiation with a dose of 15 Gy could reduce the size of gonads while 40 or 50 Gy may produce deformities in the shape of the developing follicles and nurse cell nuclei (Akhter, 2005). Similar evidence comes from Hasan (1995) who noticed that irradiation with doses from 5 to 20-krad not only delayed ovarian growth, but also produced deformities in the shape of the developing follicles and nurse cell nuclei in *T. brevicornis.* It indicated that treatment with these doses was sufficient to stop the process of oogenesis in *T. brevicornis* adults completely. The present cytological studies on the internal reproductive organs of *T. castaneum* could provide important information for designing a sterile male technique for this important pest of storage products.

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