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Inherited influence of low dose gamma radiation on the reproductive potential and spermiogenesis of the cowpea weevil, *Callosobruchus maculatus* (F) (Coleoptera: Chrysomelidae)

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ABSTRACT

The southern cowpea weevil, *Callosobruchus maculatus* (F) is a severe agriculture pest worldwide. In the current work, newly emerged adult males of *C. maculatus* have been irradiated with a low dose of 20Gy gamma radiation. The inherited deleterious effects on the fecundity, hatchability, adult emergence, and the sterility percent were recorded for the progenies F1 and F2 of the irradiated parental males. The fecundity, hatched larvae, the number of males and females were reduced in both F1 and F2. The sterility percent was high in F1 (70.8%) and increased in the F2 (88.3%) generation. Histopathological effects were also documented in the testes of F1 and F2 progenies. The spermatids and sperms have exhibited a variety of abnormalities. In the early spermatids, the nebenkern outer cell membrane was ruptured. The spermatid nucleus loses its homogeneous texture and has multiple foci of dense chromatin, as well as, profiles range has little dense material. In some groups, the nucleus had a peculiar ring of chromatin. The sperms had shown a variety of aberrations. The sperms irregularity distributed in lysed cysts by unusual manner. Also, some sperms had remarkably enlarged axoneme and small rounded nucleus. Many of the sperm cells were observed with two axonemes, abnormal mitochondria derivatives, and more than two accessory bodies. These results indicate that the low dose of 20 Gy induces semi-sterility in *C. maculatus* through generations. The same technique would help to improve using of sterile insect technique for other agriculture pests.

1. Introduction

The southern cowpea weevil, *Callosobruchus maculatus* (F) is considered as a cosmopolitan serious stored pulses pest. It attacks varieties of cowpea beans and causes a great damage to many other stored legumes. The fertilized females oviposit their eggs on the outer surface of the seeds. The produced larvae feed on the seeds content and cause great damage by turning those to useless either for cultivation or for human consumptions (Aly, El-Sayed, & El-Bishlawy, 2005; Lale, 1998). Several methods have been applied to control *C. maculatus* such as the use of insecticides, biological control system, plant extracts, and mating interruption methods (Ajayi & Lale, 2000; Khani & Asghari, 2012; Lale & Abdulrahman, 1999; Lale & Mustapha, 2000; Ngamo et al., 2007).

Irradiation as a phytosanitary treatment has raised great acceptance and has used to control insects in fresh commodities, stored products and ornamentals have grown (IAEA, 2002). Sterile insect technique (SIT) and autocidal control methods, can be used as an alternative way to control stored grain pests (Bakri, Mehta, & Lance, 2005) and to maintain low pest infestation levels in infected regions or to eradicate pests (Ahmed, 2001; Fetoh, 2011).

Several researchers had reported the effect of various doses of gamma radiations on different developmental stages in many coleopteran pests. The effect of gamma radiation on *Callosobruchus* sp. had demonstrated by many authors (Ahmed, 2001; Ahmed, Younes, & Al-Arab, 1977; Pajni, Cheema, & Kaur, 1997).

The influence of irradiation on male germ cells in Coleopteran

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insects had documented. Cork (1957) worked on flour beetle, *Tribolium confusum*. Mercier (1979) studied the colorado potato beetle, *Leptinotarsa decemlineata*, Rahim and Norimah (1990) and Boshra (1994) worked on stored pulse beetle, *Callosobruchus chinensis*. Saha and Shahjahan (1998) studied the effects of neem and radiation on hide beetle, *Dermestes maculatus*. The effect of gamma radiation on larval stages of the Khapra beetle, *Trogoderma granarium* were reported by Abdel, 1999; Abdel & El-Naggar, 2000. Khare and Khare (2015) recorded the effect of various doses of radiations (UV rays, X-ray and ^{60}Co gamma rays) on the germ cells of three Coleopteran populations, Sal heart-wood borer, *Hoplocerambyx spinicornis*, lesser meal-worm, *Alphitobius diaperinus* and Red pumpkin beetles, *Raphidopalpa foveicollis*.

The first documented study of inherited sterility was documented on the codling moth, *Cydia pomonella*. Irradiated male with sub-sterilizing doses of radiation mated with virgin fertile females will produce fewer F1 offspring and most of them will be completely sterile. The prospect for expanded application of inherited sterility as part of an area-wide integrated approach is considerable (IAEA, 2016).

The aim of the present work is to evaluate the deleterious effect of a low dose of 20 Gy gamma radiation on the reproductive potential and the ultrastructure spermatogenesis aberrations induced in F1 and F2 progenies of the irradiated parental males of *C. maculatus*.

2. Materials and methods

2.1. Insect colony

Strain of cowpea weevil, *C. maculatus* (F) was obtained from the colony reared in Entomology Department, Faculty of Science, Ain Shams University and maintained on cowpea seeds, *Vignaun guiculata* (L).

2.2. Irradiation technique

Newly emerged adult males (1 day old) were irradiated to several doses using ^{60}Co Indian gamma cell (Gy 4000 A), located at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The dose rate was 1.589 KGy/hr. A preliminary experiment was conducted using several doses of 90, 70, 50, 40 and 20 Gy to evaluate the effect of gamma radiation on the reproductive potential of males *C. maculatus*. The dose 20 Gy was selected because this dose was the lowest dose which able to induce high sterility in the F1 generation and in the same time gave enough progeny to produce the F2 generation in five replicates.

2.3. Biological assay

After exposure to radiation, the newly emerged adult males were transformed into the lab where two irradiated males were coupled with two virgin non-irradiated females in 9 mm Petri dishes with some cowpea seeds. Five replicates were made for the control and the irradiated parents, where they incubated at 27 ± 2 °C and relative humidity of 60 ± 5%. Fecundity, hatchability, the number of males, the number of females, the number of adults, the male ratio and the percentage sterility were estimated for two generations of the irradiated parental males.

2.4. Percentage sterility

The percentage sterility was calculated according to Chamberlain's formula (1962):

$$\%\ sterility = 100 - \left(\frac{a \times b}{A \times B} \times 100 \right)$$

Where:

a = number of eggs per female in the treatment
b = Percentage of hatched eggs in the treatment
A = number of eggs per female in control
B = Percentage of hatched eggs in control.

2.5. Transmission electron microscope

The control and irradiated adult males were dissected in 2.5% glutaraldehyde fixation. The internal reproductive organs were removed immediately and placed in ice-cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate and 0.17 M sucrose at PH 7.4 for 2 h at 4 °C. The testes were post-fixed in 1% osmium tetra oxide in 0.1 sodium cacodylate and 0.17 M sucrose at 4 °C for 90 min. Then embedded in pure Epon 812 resin. Sections 70–90 nm thick were cut on an ultramicrotome (LEICA EM UC6, Germany) fitted with a Diatome 45° diamond knife onto 100 mesh formvar coated copper grids and stained with 30% uranyl acetate in methanol for 30 min. Then followed by aqueous lead citrate for 7 min and viewed in a Transmission Electron Microscope (JEM 1400, Japan) at Faculty of Agriculture, Cairo University, Cairo, Egypt. Electron micrographs were taken at several magnifications.

2.6. Statistical analysis

The results were analyzed with ANOVA and followed by *post-hoc* analysis using LSD-test with the help of SPSS version 20 for Windows. The level of significance used was $p < 0.05$.

3. Results

3.1. Biological study

A preliminary experiment was conducted using several doses of 90, 70, 50, 40 and 20 Gy to evaluate the effect of gamma radiation on the reproductive potential of males *C. maculatus*. The dose 20 Gy was selected because this dose was the lowest dose which able to induce high sterility in the F1 generation and in the same time gave enough progeny to produce the F2 generation in five replicates. Some biological aspects of the F1 and F2 progenies were observed after irradiating *C. maculatus* parental males with 20 Gy dose of gamma radiation and crossed to normal females. The recorded biological aspects were: eggs/female, hatched larvae, adult emergence (M & F), male sex ratio, and percentage sterility.

Comparing between the fecundity (number of eggs) in the three tested groups the control (68.25 ± 1.89), the F1 (35.80 ± 11.29) and F2 (5.60 ± 2.16), ($P = 0.000$), it was cleared that number of eggs produced by the control was significantly higher than that produced by the F1 ($P = 0.005$) and F2 ($P = 0.000$). Moreover, the F1 fecundity was significantly high in comparing to F2 generations ($P = 0.004$). The overall hatchability (number of larvae produced) was significant between the three tested groups ($P = 0.000$). Number of hatched larvae produced by the control (64.00 ± 1.87), with hatchability percentage 93.77 ± 0.87%, was significant comparing to those produced either by F1 generation (13.80 ± 4.75, $P = 0.000$) or F2 generation (5.002.35, $P = 0.000$). However, comparing hatchability percentage between the 20Gy-irradiated F1 and F2 generations (40.93 ± 9.21% and 68.91 ± 16.17%, respectively), there was no significant difference ($P = 0.056$) (Fig. 1).

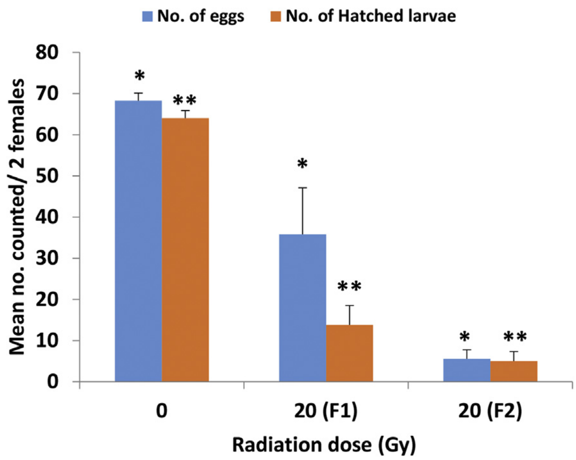

Fig. 1. Mean number of eggs laid and number of hatched larvae after crossing irradiated males of cowpea weevil, *C. maculatus* (20 Gy dose of gamma irradiation) with normal virgin females to produce F1 and also number of eggs laid and number of hatched larvae from crossing males and females from F1 to produce F2. The asterisks indicate significant differences at (P < 0.05). Confidence bars are shown for ± SE.

The overall number of adults was significant between the three tested groups ($P = 0.000$). Comparing number of adults produced by the control (34.75 ± 2.10), there was a significant difference either with F1 (13.25 ± 4.96, $P = 0.000$) or the F2 (2.50 ± 0.05, $P = 0.000$) generations. There was a significant difference also comparing the 20 Gy irradiated F1 and F2 ($P = 0.045$). The number of males was significant within the three tested groups ($P = 0.000$). It was 13.25 ± 0.48, 5.50 ± 1.89, and 0.50 ± 0.5 for the control, F1, and F2 generations, respectively. Furthermore, the number of males produced in the F1 generation was significantly higher than that produced by the F2 generation ($P = 0.024$). Also, the number of females was significant within the three tested groups ($P = 0.000$). The females produced by the control (21.50 ± 1.66) was significantly higher either comparing those produced by the F1 (7.75 ± 3.09) or F2 (2.00 ± 0.00) generation ($P = 0.000$). However, comparing the number of females in F1 and F2 generations, there was no significant difference ($P = 0.071$). The male sex ratios were 38 ± 1.00%, 42 ± 3.00%, and 17 ± 17% in the control, F1, and F2 progenies, respectively with no significant difference ($P = 0.162$) (Fig. 2).

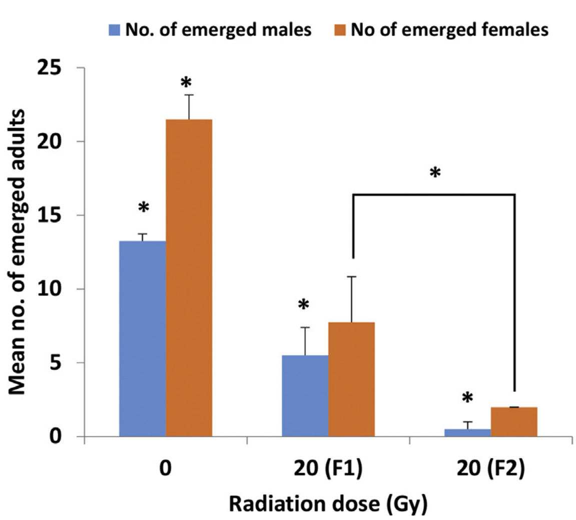

Fig. 2. Mean number of emerged adults in the F1 and F2 generations after crossing irradiated males of cowpea weevil, *C. maculatus* (20 Gy dose of gamma irradiation) with normal virgin females. The asterisks indicate significant differences at (P < 0.05). Confidence bars are shown for ± SE.

The adult percentage sterility was significant between the control (0.00 ± 0.00) and the individuals of F1 (70.87 ± 8.70) and F2 (88.31 ± 7.01), ($P = 0.000$). There was a significant difference in percentage sterility between the F1 and F2 generations ($P = 0.049$) (Fig. 3).

3.2. Electron microscopic studies

Transmission electron microscopy of testes of control male *C. maculatus* showed that, the testes are consists of testicular follicles which are composed of aggregations of round or oval cysts. Cysts are formed by the grouping of germ cells of the same stage in clusters (Fig. 4a, d, e, f, g). Cysts involved spermatogonia, spermatocytes, spermatid and sperm. The spermiogenesis processes undergo a complex series of rearrangements. In early stage of spermatid, mitochondria become spherical and are clustered together (Fig. 4a). They undergo a complex series of reorganization and fusions that lead to their integration into a large spherical mass, the nebenkern (nbk) (Fig. 4b). It is composed of outer membranous array of vesicles enclosing coiled spireme (Fig. 4c). In later stages of spermatid morphological transformation process, the nebenkern divided into two tortuously interwoven mitochondria which are closely associated with the developing axial filament (Fig. 4d). Spermatids at different stages of spermiogenesis can be distinguished. In electron micrograph the nucleus migrates to the anterior tip of the cell and the cytoplasm is occupied by two unequal mitochondria derivatives and the axial filament (axoneme) appears behind them (Fig. 4d and e). Another morphologic nucleus transformation changes process was observed in electron micrograph where, chromatin condenses forming an elongated dark mass in the nucleus and presence of two mitochondria derivatives (the presperm) (Fig. 4f and g). Presperm has large nucleus which has dense chromatin (Fig. 4f) and aligned by several membranous layers, the acrosomes (Fig. 4g). Sperms are differentiated in shape from the presperm by the great extension of the tail.

Sperm within one cyst bundle are arranged in the same direction and appear to be held closely together by an electron dense material and enclosed by a membranous sheath (Fig. 4h and i). Each individual sperm is surrounded by membranous sheath (Fig. 4j). A very thin layer of cytoplasm is surrounding the nucleus of the sperm. The head points anteriorly where acrosome is existing. Behind the nucleus, there are two unequal mitochondrial derivatives. Sperms don't have distinct cristae and filled with dark

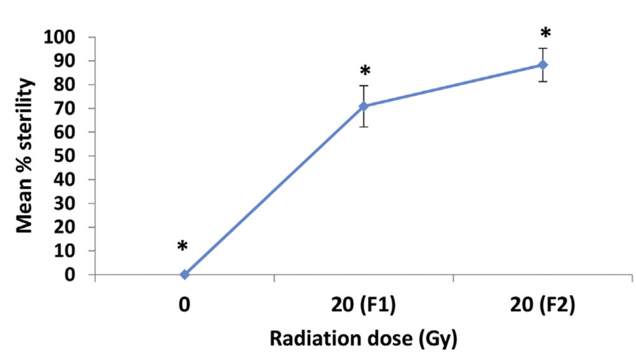

Fig. 3. Mean percentage sterility in the F1 and F2 generations after crossing irradiated males of cowpea weevil, *C. maculatus* (20 Gy dose of gamma irradiation) with normal virgin females. The asterisks indicate significant differences at (P < 0.05). Confidence bars are shown for ± SE.

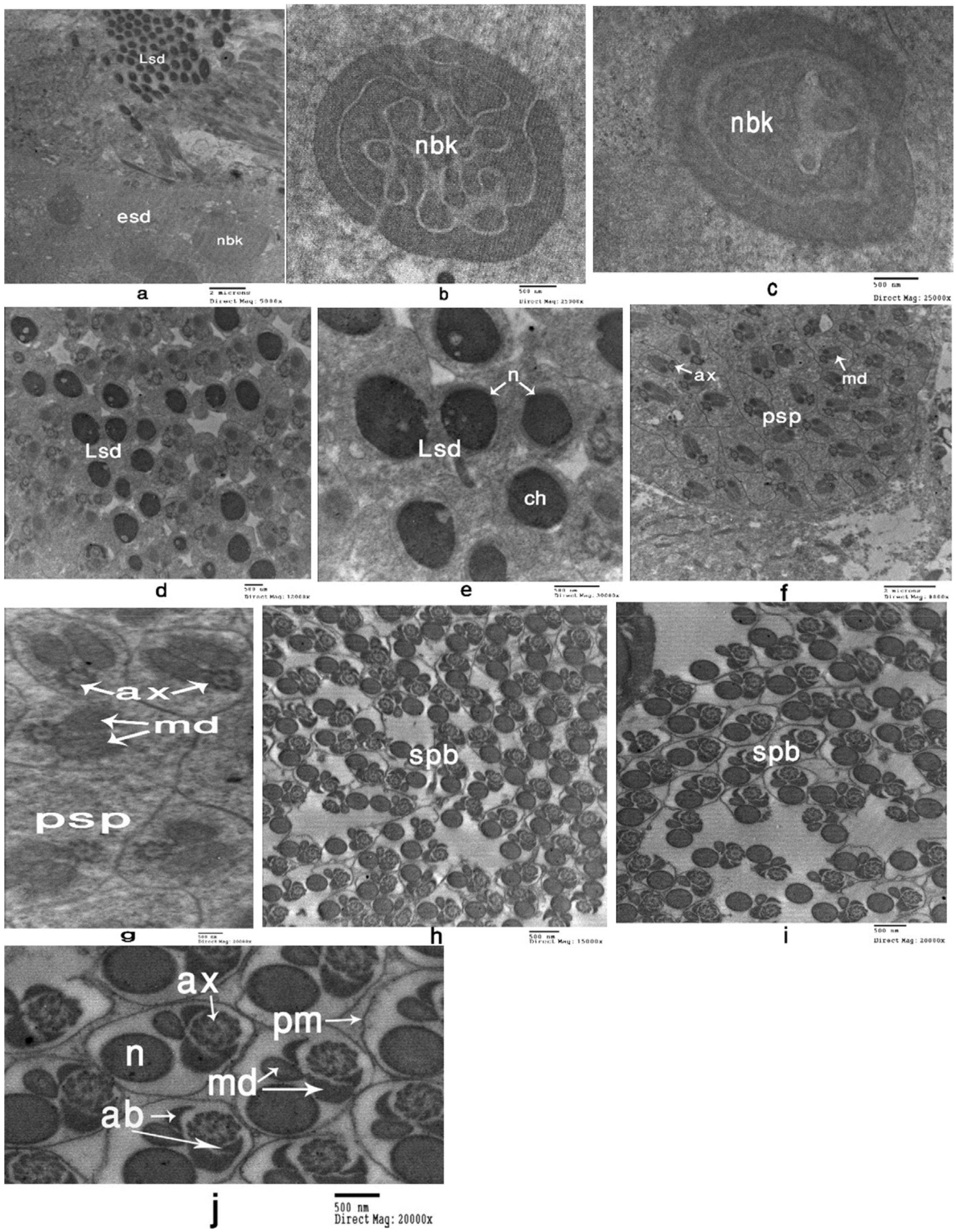

Fig. 4. (a–j): Electron micrograph of non-irradiated germ cells of *C. maculatus* showing a:early spermatid (esd) and nebenkern (nbk), b&c: the nebenkern, d: the late spermatids (Lsd),e:higher magnification of late spermatid (Lsd) showing, nucleus with condense chromatin (ch), f:presperm (psp), g:higher magnification of presperm (psp) showing two mitochondria derivatives (md) and axoneme (ax), h&i: the sperm bundle (spb), j: higher magnification of sperm bundle showing, sperm cyst involve nucleus (n), two mitochondria derivatives (md),axoneme (ax) and two accessory bodies (ab) included within plasma membrane (pm).

granular substance (Fig. 4j). Two dusky curved-shape accessory bodies are flanking the axoneme and are running along the middle line behind the two mitochondrial derivatives (Fig. 4j). An electron lucid material spaces out all components from each other which are enclosed in the plasma membrane of the cell. The sperm tail has three distinct elements; the axoneme, two unequal mitochondrial derivatives and two accessory bodies (Fig. 4j). The axoneme is typically formed of 20 fine microtubules, which are organized as a wheel like shape of (9 + 9+ 2). In this pattern, nine singlets are surrounding the two central microtubules which are encircled by nine doublets (Fig. 4j).

3.3. Histopathological studies

Histopathological investigations of the testes for F1 progeny showed that, at early spermatid, the nebenkern has deformity of their outline (Fig. 5a) and disintegration of their crista are detected (Fig. 5b). Nuclear membrane has protruding outline (Fig. 5b). The late spermatids exhibit a variety of abnormalities (Fig. 5c and d). The spermatid nucleus loses its homogeneous texture and has multiple foci of dense chromatin, as well as, profiles range has little dense material (Fig. 5d). Some groups also has abnormal figures, the nucleus had a peculiar ring of chromatin. The profiles range from little dense material to relatively large amount of dense material (Fig. 5d). The structures of sperm has abnormal appearance (Fig. 5e, f, g, h). The sperm consists of plasma membrane which

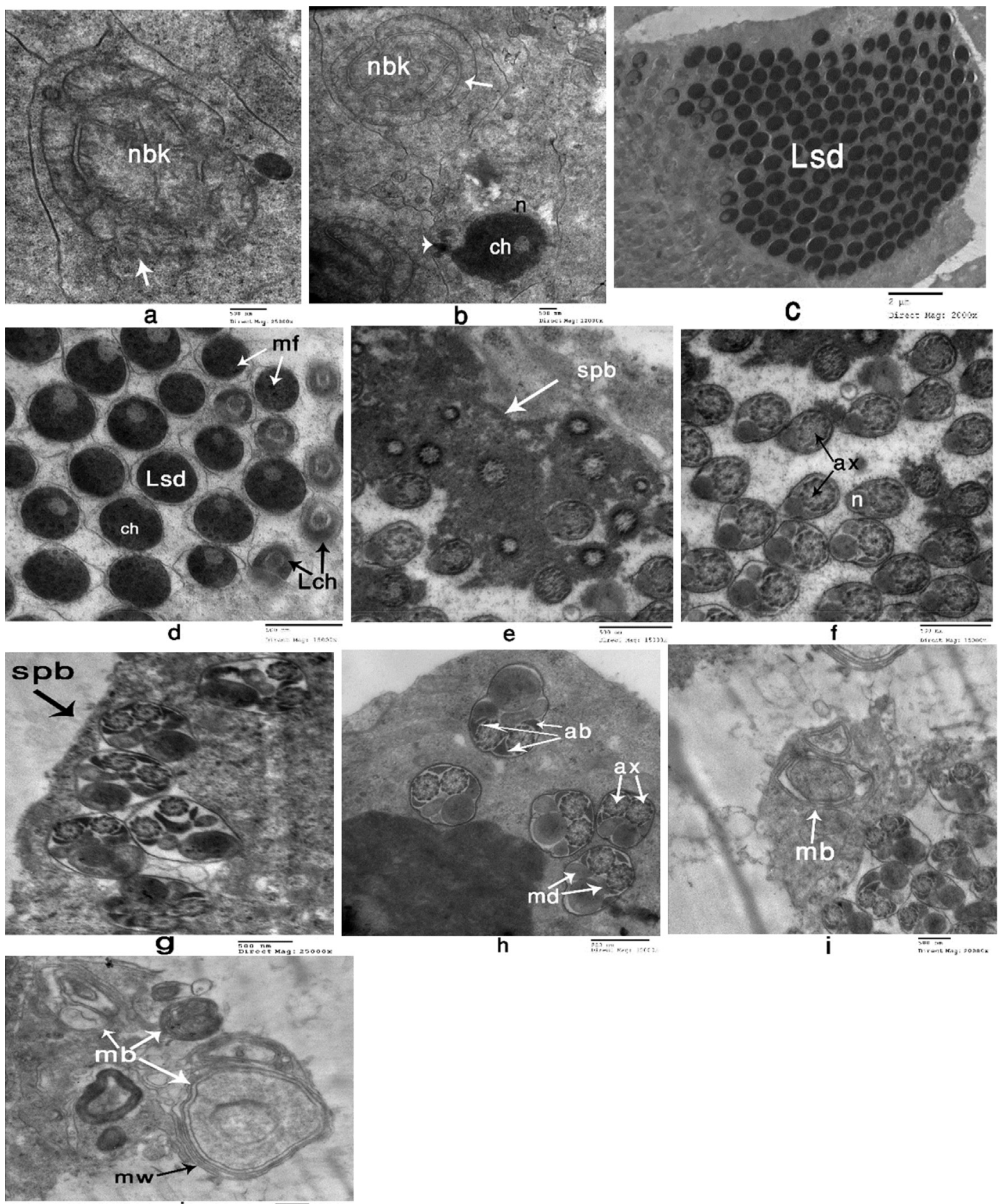

Fig. 5. (a–j): Electron micrograph of F1 progeny resulted from irradiated parental male *C. maculatus* with 20 Gy dose of gamma radiation showing, a: nebenkern (nbk)with deformity membrane (arrow), b:deteriorated crista of nebenkern (nbk) (arrow) and protruding of the nucleus(n) (arrow head), c: late spermatid (Lsd), d: higher magnification of late spermatid (Lsd) showing nucleus(n) with multiplefoci (mf) (arrow), less dense chromatin (Lch) (arrow)e:abnormal sperm bundles (spb), f: higher magnification of sperm bundle showing, enlarged axoneme (ax)and disappearance of two mitochondria derivatives (md),g: abnormal sperm bundles (spb), h: higher magnification of sperm bundles (spb) showing, sperm cysts had two axonemes (ax), abnormal mitochondria derivatives (md) and more than two accessory bodied (*ab*)i:appearance of myeloid bodies (mb) (arrow), j: higher magnification showing myeloid whorls (mw) (arrow).

encloses relatively small round nucleus and remarkable enlarged axoneme, they are surrounded by a matrix of similar electron density. They are generally spherical or pear shaped (Fig. 5e and f). Cross section through a cyst of mature spermatozoa showed a high frequency of structural abnormalities and malformation of the sperm flagellum, it includes supernumerary axonemes and multiple flagellar bodies enclosed within a single plasma membrane (Fig. 5g and h). Large spherical or ellipsoidal bodies were also observed. These bodies were lined by intact membranes filled with myeloid whorls (Fig. 5i and j). The myeloid whorls are formed by phagocytic activity and phagolysosomes. These are known as myelin body (Fig. 5i and j).

In the F2 offspring, the nucleus of the late spermatid has abnormal appearance, suffers chromatolysis or chromatekinetic (Fig. 6a). The sperms exhibit a variety of abnormalities. Deteriorated and ruptured sperm bundles. Wide spread cytolysis is observed in the follicles which filled with cell debris to such extend the sperm bundle cannot be identify (Fig. 6b) Sperms could be observed not enclosed within a relatively thin plasma membrane (Fig. 6b). Many of the sperm cells had two axonemes, abnormal mitochondria derivatives and more than two accessory bodied (Fig. 6c). Nucleus and the other organelles were disappeared except the axonemes and only one mitochondria remained with enlarged vacuolation in the cells (Fig. 6d). Few sperms had recorded the cytoplasm were completely disappeared while nucleus and axoneme were atrophy or small in size (Fig. 6d and e). In oblique sections two axonemes were also detected in individual sperm (Fig. 6f and g).

4. Discussion

4.1. Biological studies

The effect of ionizing radiation doses on the stored product insects had been early reviewed by Watters (1968); Tilton (1974); Hallman (2013). It was proposed that a dose of 500 Gy would be necessary to stop reproduction of all stored product pests, although coleopteran insects could be controlled with lower doses. The organisms could be reproductively sterilized with relatively low doses that displayed no other gross effects to the organism (Hunter,

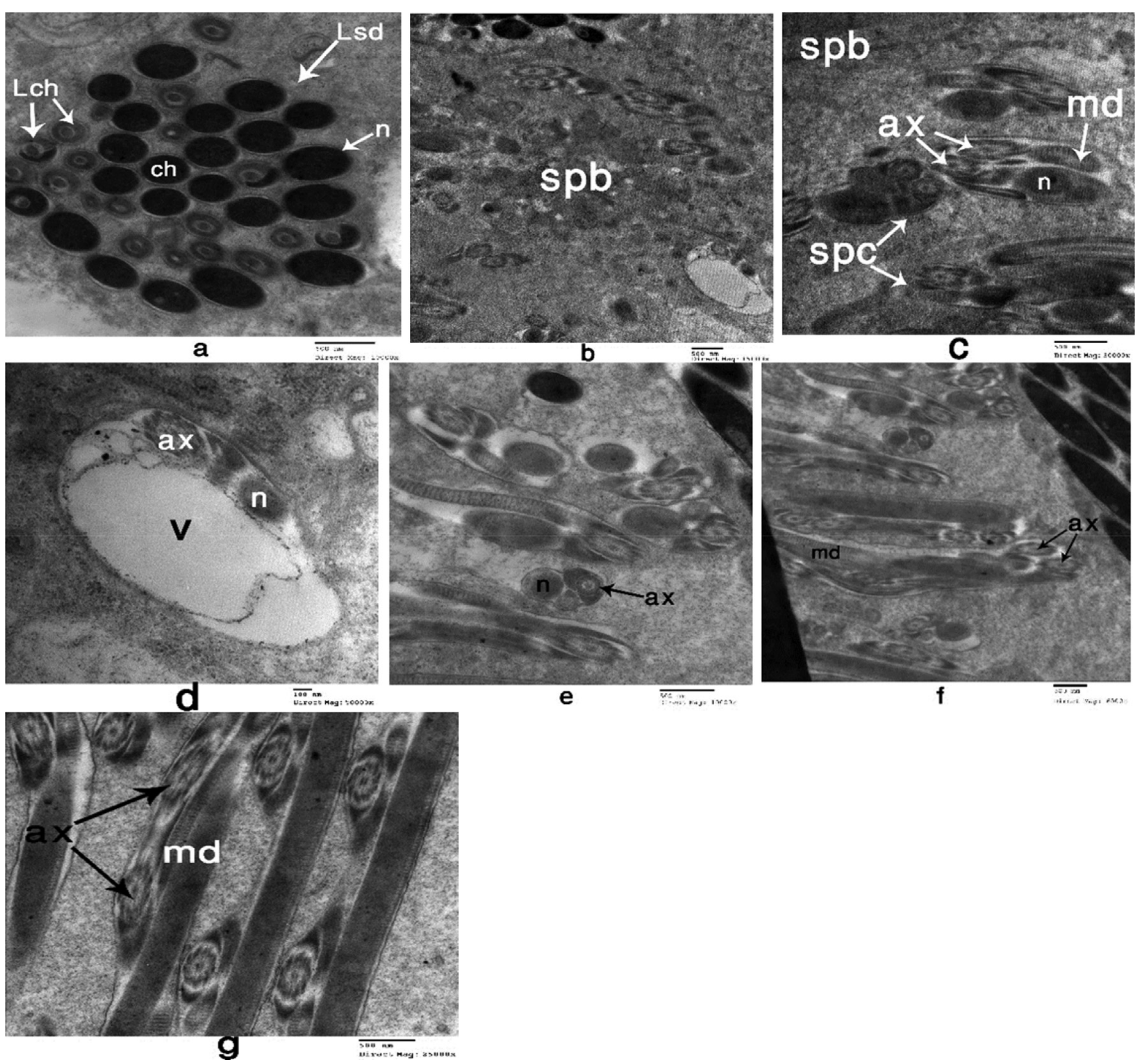

Fig. 6. (a–g):Electron micrograph of F2 progeny resulted from irradiated parental male *C. maculatus* with 20 Gy dose of gamma radiation showing, a: the late spermatid (Lsd) showing nucleus(n) with malformed chromatin (ch) which less dense (Lch), b: ruptured sperm bundles and their contents dispersed throughout the lumen of the testes, c: higher magnification of sperm bundles (spb) showing, sperm cysts (spc) had two axonemes (ax) and abnormal mitochondria derivatives (md) d: Sperm (sp) with enlarged vacuolation (v) in the cells, e: the cytoplasm were completely disappeared while the nucleus (n) and the axoneme (ax) small in size, f&g: oblique sections showed sperm cells with two axonemes (ax)and abnormal mitochondria derivatives (md).

1912). Farghaly, El Sharkawy, El Alfawy, Risk, and Bader (2014) reported that fecundity of irradiated insects was dose dependent.

The results of the present study had proved that irradiation of parental male *C. maculates* with the low dose as 20 Gy induce semi-sterile males, affected not only F1 progeny, but the deleterious effect was inherited to F2 generation. Fecundity, hatchability, the number of male and female adults emerged were significantly decreased. The sterility percent was (70.8%) in F1 and increased in the F2 generation (88.3%). Thus, instead of preventing males from achieving complete reproduction for one generation, the harmful effect was inherited to the next generations. There was no skewed sex ratio in our study. It is in agreement with the study of Carpenter, Bloem, and Bloem (2001) who didn't detect a skewed sex ratio while studying the results of male offspring for *Cactoblastis cactorum* (Lepidoptera). On the other hand, this result is in contrary to other reported studies for other insect pests (Saour, 2014; Seth & Sharma, 2001). Khan and Islam (2006) had recorded significantly less number of females produced after *Musca domestica* irradiation, but the overall female ratio between generations was not affected by radiation doses.

High dosages of gamma radiation were utilized to prompt sterility and reduce the biological activities in *Callosobruchus* sp. Ahmed, Abou-Donia, Ahmed, and Salem (1981) had irradiated adult males *C. maculatus* with 150 Gy which brought about 96.5% infertility in the subsequent eggs. Fertilization by normal males could roughly invalidate insemination by irradiated males as the egg hatchability expanded from Zero to 47.8%. The adult males of *C. maculatus* irradiated as 1-day-old with gamma-ray doses of 75, 100 or 150 Gy, a sterilizing dose, were fully competitive with normal males at most ratios. As the sterile to fertile flooding proportion was expanded to 5: 1 or to 10:1, the percentage of egg infertility was expanded to 86.36 and 95.25%, respectively (Ahmed & Younes, 1981). The impact of gamma ray with dose of 0, 1, 1.5, 2 and 2.5 krad on biology of two generations of *C. maculatus* had greatly affected the hatching proportion, and it achieved the most minimal proportion 30.62% at the dose 2.5 krad for the first generation, while the most astounding ratio for hatching was 94.15% at the dosage 0 krad. The larval and pupal stage period and death proportion expanded at these doses, while the number of insects produced was extremely diminished likewise, the productive rate of the female was declined and which was 32.9% at 2.5 krad dosages (Mohamed & Rashed, 2008). Hallman (2013) had reported that the 100 Gy dose of gamma radiation to adults will prevent the reproduction of *C. maculatus*.

The pulse beetle, *Callosobruchus chinensis* males irradiated as completely developed pupae with the sterile dose 70 Gy. The dose was more effective in reducing percent hatchability from 88.23 to 32.44 when normal males were supplanted by sterile males (Haiba, 1981). Hussain and Lmura (1989) mentioned that the sterilized males of *C. chinensis* irradiated as 1-day-old grown adults at 80 Gy were fit for contending sexually with the normal ones and the percentage of egg hatchability decreased as the proportions of 9: 1 and 15:1. A dosage of 1 kGy of gamma radiation totally killed adult *C. chinensis* in a week, yet the dose of 0.5 kGy required two weeks to accomplish a similar level of mortality (Roy & Prasad, 1993). Boshra (1994) reported that the dose required to disinfest and/or sterilize both males and females was 120 Gy. Ionizing treatment dose less than 100 Gy had utilized to sterilize the adults and elimination of the eggs, larvae, and pupae without bringing about troublesome healthful outcomes (Diop, Marchioni, Ba, & Hasselmann, 1997). IAEA (2002) had prescribed that the 100 Gy is the minimum dose required to sterilize the adult of *C. chinensis*.

The effect of various doses of gamma radiation on the progenies of irradiated parents was also recorded in other insects. The deleterious effects of 4 different doses of gamma radiation on the pupae of male Hessian flies, *Mayetiola destructor* were studied by Jeffrey, Stuart, Wells, and Roger (1997). The inherited sterility in the spiny bollworm, *Earias insulana* (Boisd) had recorded by Sallam, El-Shall, and Mohamed (2000). F1 sterility of irradiated *Spodoptera litura* was higher than P sterility and that F1 males inherited more sterility than did F1 females (Seth & Sharma, 2001). The acquired deleterious impacts of gamma radiation were seen in the F1, F2 and F3 generations of *Culex pipiens* (Hassan, Mounier, Kotb, Gabarty, & Tharwat, 2016).

The inheritance of sterility in Lepidoptera and some other insects, when compared with full sterility give points of interest for pest control. When partially sterile males inseminate the wild females, the radiation-induced harmful effects are inherited by the F1 generation. The lower dose of radiation used to induce F1 sterility increases the quality and competitiveness of the released insects (IAEA, 2016).

4.2. Ultrastructure studies

The germ cells of insects, in particular, the secondary spermatogonia, are generally agreed to be sensitive to radiation. The radiosensitivity of the germ cells depends on the developmental stage at the time of irradiation and the principle by which it is appraised. The final effect exerted by ionizing radiations relies on the genetic makeup and age of the organism (Coggins, 1973; Mathur, 1960).

Radiation-induced aberrations in the spermatids and the sperms of the progenies F1 and F2 generations of the irradiated parental males of *C. maculatus* were observed. These aberrations had been also recorded in other irradiated beetles. *Dermaste sfrischii* exposed to X-ray dose of 3 krad and abnormalities were observed in spermatids and sperm only. Malformed nuclei, nebenkern and acrosomes were detected. Spermiogenesis was not inhibited and resulted in the formation of pseudo spermatids (Hodges, 1983). The impact of the 80 Gy irradiation doses on the spermatogenesis of red palm weevil, *Rhynchophorus ferrugineus*, had evaluated some abnormalities in maturing spermatids that showed supernumerary axonemes and mitochondria (Paoli et al., 2014). The influence of the low dose of ^{60}Co-radiation exposure to red pumpkin beetles, *Raphidopalpa foveicollis* affects the germinal epithelium of the testes. In lesser mealworm, *Alphitobius diaperinus* the follicles become smaller. The testes of Sal heart woodborer, *Hoplocerambyxs pinicornis* does not have any remarkable change. At high dose of radiation, the follicles of the germinal epithelium are diminished and damaged in testes of the red pumpkin beetles, *R. foveicollis*. In lesser mealworm, *A. diaperinus* the germinal epithelium of the testes becomes shrunken. The chromatin material is condensed and the chromosomes of spermatogonia become abnormal in shape due to the shrunken effect. Primary and secondary spermatocytes are not clearly observed. In Sal heart woodborer, *H. spinicornis* the follicles had damaged as they shrunk and become smaller. The cell boundaries of spermatogonia were distorted. Secondary spermatocytes demonstrated necrotic effects. The nucleus of the spermatids becomes indistinct and the cell becomes distorted. Most of the spermatids were disappeared and the number of the sperms was reduced. The sperms fail to form sperm bundles and indicated a disturbed physiological state of spermatogenesis and infertility. The high dose of ^{60}Co radiation had caused interruption of spermatogenesis. Gamma radiation had a severe effect on the male germ cells in all these three beetles (Khare & Khare, 2015).

All the mitochondrial developmental stages are very sensitive to radiation (Coggins, 1973; Mahomud & Shoman, 2009). The nebenkern of the F1 progeny of *C. maculatus* had shown abnormal structure. The nebenkern in the spermatids is the initial stage of the mitochondria organelles (Dallai, 2014; Pratt, 1962, 1970). In late spermatid, the nebenkern divides forming the mitochondrial derivatives which extend caudally along the developing tail, in front of the axoneme (Mohammad, Shaarawi, & Kamel, 2003). The nebenkern may develop under the influence of the centriole and centriole adjunct (Gatenby, 1941). Coggins (1973) had proposed that the mitochondria fail to fuse into a nebenkern may be due to temporary interference with the genes controlling this formation process and/or with the breakdown of the old or the formation of the new bounding membrane. Abnormalities in the mitochondria derivatives for the offspring of irradiated *C. maculatus* parental males were also observed. In normal males, mitochondria derivatives appeared unequal in shape (Khaled et al., 2015). In F1 and F2 progenies, the malformations recorded were included the disappearing of one or the two mitochondria derivatives from the germ cells, and in other cells they were reduced in size. This could lower the overall energy available to the sperm and decrease its ability to fertilize any ova.

The axoneme of the sperm cells originates from a single centriole present in the sperm, which is believed to be the mobile element of the sperm, arises behind the nucleus in the sperm head and extends longitudinally flanked by the accessory bodies along the sperm tail (Dallai, 2014; Mohammad et al., 2003). Production of enlarged axonemes and presence of two axonemes in the spermatids and sperms were also observed in F1 and F2 progenies of irradiated parental males of *C. maculatus*. Hodges (1983) had reviewed the supernumerary abnormal structure of the axonemes in many insects. He suggested that the appearance of many axonemes in the irradiated *Dermamestes frischii* beetle with X-ray radiation resulted from suppression of either the first or second meiotic division. Explanations for supernumerary axonemes were suggested earlier by many researchers. Creighton and Evans (1941) had predicted that these abnormalities occurred from the suppression of either the first or second meiotic divisions. Also, the number of chromosomes lost corresponded to the number of centrioles gained. They postulated that each kinetochore of the lost chromosome had taken on the role of a centriole and might responsible for the formation of the axoneme. Tahmisian and Devine (1961) proposed that radiation caused an intracellular induction of these organelles of an irradiated grasshopper, *Melanoplus differentialis*. Du Plessis and Soley (2011), had documented that the axoneme gives structure to the flagellum, yet is flexible. An excess of axonemes may increase the rigidity of the flagellum and decrease the amount of thrust available to the sperm or produce multiple-tailed sperm. In irradiated red palm weevils, *R. ferrugineus,* two to four axonemes were observed (Paoli et al., 2014). Their observation of the doublet orientation allowed them to exclude axonemal folding within the same cytoplasm.

Large spherical or ellipsoidal bodies which are lined by intact membranes and filled with myeloid whorls, were observed in testes of F1 progeny of irradiated parental males of *C. maculatus*. These are named myelin bodies, myelin is a fatty insulating substance. Salem and Degheele (1989) reported the presence of a myelin bodies in the testes of a sterile stock of the southern cowpea weevil, *C. maculatus.* Moreover, the abnormal sperm cells degenerated into myeloid whorls forming myelin bodies. Hruban, Slesers, and Hopkins (1972) documented that the lamellar bodies in *Musca domestica* differ from the myeloid bodies described in other tissues, in having straight rather than concentric laminae. The lamellar appearance was ascribed to the linear arrangement of the lipoprotein macromolecules.

Irradiated germ cells of other insects have been studied after exposure to radiation. Production of supernumerary centrioles, axonemes, mitochondrial material, flagella and nuclear outgrowths in the spermatids of X-irradiated insects had been recorded by Gatenby (1941); Tahmisian and Devine (1961). Spermatids of the Dipteran fruit fly, *Drosophila melanogaster* had suffered fissures, fragmentation, partial lysis or pycnosis with impaired nuclei after irradiation (Abro, 1964). The hemipteran leaf hopper, *Circulifer tenellus* irradiated with ^{60}Cobalt radiation had observed sperm depletion. Vacuolation of the testicular sacs and reduction in various stages of spermatogenesis (Ameresekere, Georghiou, & Sevacherian, 1971). In *Schistocerca gregaria* supernumerary centrioles and mitochondria, up to six of the former, are frequently observed in spermatids which have developed from irradiated spermatocytes and early spermatids (Coggins, 1973). Supernumerary organelles may be formed, two or more acroblasts, centrioles, and axonemes were recorded in *Dermestes frischii* and in other insects (Hodges, 1983). The effect of 40 Gy gamma radiation on heteropteran pentatomid bug, *Nezara viridula* male gonad cells, showed structural abnormalities in the mitochondria derivatives and supernumerary axonemes in the flagella of spermatids and sperms of the irradiated males (Ibrahim, 2016). Histopathological effects of gamma radiation on spermatogenesis in the Dipteran insect, *Culex pipiens* were recorded. Abnormal distributions of the developmental stages of spermatogonia and spermatocytes led to a general decrease in the rate of spermatogenesis, deformity of sperm, inhibiting the sperm movements and reduce the fertility (Hassan et al., 2016).

Cells which irradiated as spermatocytes may fail, or slow to have normal maturation division (s), or may have morphologically abnormal spermatids (Mandl, 1964). Any of these radiation-induced abnormalities might be expected to have an effect on the insects' fecundity. Coggins (1973) reported that the reduction in hatchability may well be explained by dominant lethal mutations, carried in the sperm nuclei and/or by a decrease in sperm motility due to the supernumerary axonemes and hence a reduction in the number of eggs fertilized.

At low radiation doses, the relationship of dose to the dominant lethal mutation in the mature insect's sperm is approximated to linearity, but producing an asymptotic approach towards full sterility at high doses (Robinson, 2005).

5. Conclusion

Our results have indicated that, the 20 Gy dose of irradiation is able to induce semi-sterility and have deleterious effects on the reproductive potential and spermatogenesis of *C. maculatus*. We recommend that the low dose of gamma radiation could be utilized in SIT and autocidal programs to control the cowpea beetle.

Author contribution

HI, SF, MA, MA, DM and EE designed research and wrote the manuscript. HI, SF, MA, MA, DM and EE conducted the biological experiments and the ultrastructure work. All authors read and approved the manuscript.

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