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Latent effect of gamma irradiation on reproductive potential and ultrastructure of males' testes of *Culex pipiens* (Diptera; Culicidae)

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ABSTRACT

Laboratory male pupae of *Culex pipiens* were exposed to 23, 41, 74 and 128 Gy doses of gamma radiation according to the LD_{25}, LD_{50}, LD_{75} and LD_{90} calculation, respectively. The inherited deleterious effects of gamma radiation were observed in the F_1, F_2 and F_3 generations. Levels of sterility index in the F_1 and F_2 were higher than those of untreated control but in the F_3 generation there was a semi-sterility compared with the control. Ultrastructure of normal males' testes of *C. pipiens* was studied using transmission electron microscopy. Histopathological responses were observed in the irradiated testes of *C. pipiens*. Gamma radiation had greatly affected the testes, such as (i) rupture, necrosis, degeneration and small vacuoles were reported in the testicular wall (ii) an abnormal distribution of the developmental stages of spermatogonia and spermatocytes leading to a general decrease in the rate of spermatogenesis; and (iii) deformity of sperm inhibitting the movements and the fertility of the sperm led to the decrease in the reproductive potential of *C. pipiens*. Consequently, these radiation doses are consistent with those used in the already established Sterile Insect Technique (SIT) programmes against *Culex pipiens*.

1. Introduction

Mosquitoes are carriers of various vertebrate blood parasites. In Egypt *Culex pipiens* (Diptera; Culicidae) is widely distributed and is the main carrier of Rift Valley Fever virus Darwish and Hoogstraal (1981), *Wuchereria bancrofti* (falariasis) Gad, Hammad, and Farid (1996) and Western Nile virus (Pelah, Abramovich, Markus, & Wiesman, 2002). Hassan et al. (2003) studied the possibe experimental transmission of Hepatitis C virus (HCV) by *C. pipiens*.

The high hopes for mosquitoes control placed on residual insecticides were soon belied by the discovery of resistance in mosquito vector. This discovery has once again stressed the value of using other methods for mosquito's control. On the other hand, releases of sterile individuals have permitted the successful regional extermination of primary pests, such as the New World screw worm, *Cochliomyia hominivorax* Coquerel, the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, and the Mexican fruit fly, *Anastrepha ludens* Loew (Klassen & Curtis, 2005; Krafsur, 1998). Sterile Insect Technique (SIT) was tested for mosquito control in the 1970s by using several sterilizing approaches, such as chemosterilization, ionizing radiation, cytoplasmic incompatibility, or chromosometranslocations (Benedict & Robinson, 2003; Dame, Curtis, Benedict, Robinson, & Knols, 2009). Research is currently being performed to improve the different technical steps (Robinson, Grantham, & Clark, 2009). Some field trials have shown encouraging potential of the releases of sterile mosquitoes in reducing wild population of *Anopheles albimanus* Weidhaas, Breeland, Lofgren, Dame, and Kaiser (1974), *Aedes aegypti* Harris et al. (2012), *Aedes polynesiensis* O'Connor et al., (2012) and *A. albopictus* Bellini, Medici, Puggioli, Balestrino, and Carrieri (2013).

Many tissues show negligible damage in mature insects, and the reproductive organs are sensitive to gamma radiation because the germinal cells usually seem 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 appeared that

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innate genetic factors determine the time and mode of post-radiation mortality (Hasan, Khalequzzaman, & Khan, 1989). The histopathological effects of gamma irradiation are morphological modifications occurring in mosquito spermatozoa during their transit through the male and female reproductive tract (Ndiaye, Mattei, & Thiaw, 1997). At full sterilizing radiation doses, non-dividing somatic cells are also damaged and the radiation decreases the overall quality of the insect; e.g. vigor, longevity and mating competitiveness (Franz, 1999).

The structure of testes and the development of the germinal cells have been described in *Drosophila melanogaster* by Bairati (1967) and (Coulthart & Singh, 1988; Wang et al.,1992; Joly & Bressac, 1994). Several authors have also studied the male germinal cells of Culicidae. The spermatogenesis in 18 species of mosquitoes was studied by Wandall (1986), whereas the ultra-structural diversity in the spermatids was revealed by Ndiaye et al. (1997). Moreover, the ultrastructure of spermatozoa of *Chrysomya megacephala* has been recently described by Name, Pujol-Luzc, and Báob (2010).

The aim of the present work is to evaluate the latent effect of gamma irradiation on the reproductive potential and ultrastructure of *Culex pipiens* males' testes.

2. Materials and methods

2.1. Insect rearing technique

In this study, *Culex pipiens* L mosquito was obtained from the Medical Entomology Research Center. The mosquito was reared for several generations, in the Insectary of Medical Entomology at the Department of Zoology Faculty of Science Al-Azhar University, under controlled laboratory conditions (27 ± 2 °C, 70 ± 10% rh and 12-12 light–dark regime).

2.2. Irradiation process

Gamma cell-40 (cesium-137) irradiattor unit was used in performing this study located at National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The dose rate of the radiation unit was 2.3 Gy/min.

2.3. The latent effect of gamma irradiation on the reproductive potential of irradiated males Culex pipiens

The susceptibility of males pupae of *Culex pipiens* to different doses of gamma irradiation and the determination of (LD_{25} - 23 Gy), (LD_{50} - 41Gy), (LD_{75} - 75 Gy) and (LD_{90} - 128Gy) are given in detail in (Selim, 2015).

To evaluate the effect of gamma radiation (LD_{25}, LD_{50}, LD_{75}, and LD_{90}) on the reproductive potential of males *C. pipiens* at 1st (F_1), 2nd (F_2) and 3rd (F_2) generation. Male pupae were exposed to gamma radiation doses (LD_{25}, LD_{50}, LD_{75} and LD_{90} Gy) to give irradiated parents. The irradiated male's parents collected by an aspirator allowed mating with normal females to give the irradiated F_1 generation. Males which emerged from the first irradiated generation were collected and allowed to mate with normal females to give the irradiated second generation. Males which emerged from the F_2 irradiated generation were collected and allowed to mate with normal females to give the irradiated F_3 generation. The control's data (non-irradiated ♂♂ X non-irradiated ♀♀) of F_1, F_2 and F_3 generation were recorded.

Three replicates were performed for each dose level through the three generation (each one had 10 irradiated males vs 10 non-irradiated females). Adult males that emerged from the pupal stage which were treated with each dose level were collected and transferred with normal females obtained from the colony to the wooden cages (20 × 20 × 20 cm) using an electric aspirator recommended by (WHO), and fed on a 10% sugar solution for three days. Then, the adult males and females were left for one day without the sugar solution. On the 5th day, the starved females were allowed to take a blood meal from a pigeon and allowed to oviposite on dechlorinated water (oviposition traps) in the cages. The number of eggs/raft was counted using binoculars and then the mean value was calculated.

The eggs were sorted into two categories (hatched and non-hatched eggs) according to the method used by Hassan, Zayed, and Ahmed (1996). The non-hatched eggs were further classified into embryonated and non-embryonated eggs by the apparent confirmation of the presence of an embryo under a dissecting microscope. Hatched and non-hatched embryonated eggs were considered fertilized, while non-hatched and non-embryonated eggs were regarded as unfertilized ones (Rak & Ishii, 1989). The egg-hatchability was calculated and the sterility percentage was estimated according to the formula of Toppozada, Abdallah, and El-Defrawi (1966).

2.4. Histopathological and ultrastructure on male's testes of Culex pipiens

Males that resulted from irradiated pupae by the LD_{25}, LD_{50}, and LD_{75} were killed with chloroform after 3 days of feeding on the 10% sugar solution. The head, thorax, legs and wings were removed under a stereo microscope, with fine dissecting needle to clarify the anatomy of mosquitoes. Testes were obtained anatomically with fine two dissecting needles according to El-Shaikh (2002) and put in 5% glutaraldehyde. Specimen tissues were made ready for Transmission Electron Microscopy TEM (JEOL 1010 Transmission Electron Microscope) was used in examining stained sections at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

2.5. Statistical analysis

One way analysis of variance (ANOVA) using SPSS (statistical package for social sciences, ver.15.0) was involved in analyzing experimental data and the significance among the samples was compared at $P \leq 0.05$. Results were represented as mean ± SE ($n = 3$).

3. Results

3.1. The latent effect of gamma irradiation on the reproductive potential of irradiated males Culex pipiens

The results obtained represented the reproductive potential of adult males *Culex pipiens* at F_1, F_2 and F_3 generation resulted from irradiated male pupae (parent). In the F_1 generation, the fecundity of non - irradiated females crossed with irradiated males resulted from irradiated male pupae was decreased by increasing the irradiation dose level. Also, the statistical analysis revealed a significant decrease ($p < 0.01$) in the mean number of eggs, where it was 591.7, 446.0 and 347.7 eggs/ten$_{♀♀}$ at LD_{25}, LD_{50} and LD_{75},respectively, compared with 838 ± 7.2 eggs/ten$_{♀♀}$ for the control. Also, there was a reduction in the hatchability percent, where it was 72.6, 51.5 and 23.0% at the doses LD_{25}, LD_{50} and LD_{75},respectively, compared with 94.0% for the control group (Table 1).

The non-hatched eggs were sorted into two categories; embryonated (with embryonic development) and non-embryonated (without embryonic development). The percent of embryonated eggs was 47.8, 54.1 and 69.1% at the doses LD_{25}, LD_{50}

Table 1
The reproductive potential of males *Culex pipiens* resulted from irradiated pupae in the F_1 generation.

Dose (Gy)	No.of eggs laid/ten♀♀ (mean ± SD)	No. of hatched eggs (mean ± SD)		No. of non - hatching eggs (mean ± SD)					Sterility Index (S.I.) %
		Total	%	Total	Embryonated		Non-Embryonated		
					No.	%	No.	%	
LD_{25} (23 Gy)	591.7 ± 16.9**	429.33 ± 13.01**	72.6	162.33 ± 4.72**	77.66 ± 2.08**	47.8	84.66 ± 5.03**	52.2	45.5
LD50 (41 Gy)	446.0 ± 32.**	229.66 ± 16.4**	51.5	216.33 ± 16.07**	117 ± 7.93**	54.1	99.33 ± 9.01**	45.9	70.8
LD_{75} (75 Gy)	347.7 ± 22.4**	80 ± 14.7**	23	267.66 ± 7.63**	185 ± 5.0**	69.1	82.66 ± 11.54**	30.9	89.8
LD_{90} (90 Gy)	–	–	–	–	–	–	–	–	–
Control	838.0 ± 7.2	788 ± 2.0	94	50 ± 8.71	8.33 ± 2.08	16.6	41.66 ± 6.65	83.4	–

(*) = Significant < 0.05, (**) = highly Significant, P < 0.01, ns = non-significant > 0.05, SD = standard deviation and (LD_{90}) = (–) at this dose the irradiated pupae did not emerge from pupal stage.

and LD_{75}, respectively, compared to 16.6% for the control. On the other hand, the percent of non-embryonated eggs was 52.2, 45.9 and 30.9% at the doses LD_{25}, LD_{50} and LD_{75}, respectively, vs. 83.4% for the control (Table 1).

A positive correlation between the sterility index and the dose level was observed, where it was 45.5, 70.8 and 89.8% at the doses LD_{25}, LD_{50} and LD_{75},respectively, compared to 0.0% for the control group (Table 1).

In the F_2 generation, the fecundity of non-irradiated females crossed with irradiated males resulted from the 1st generation was decreased by increasing the irradiation dose level. Also, the statistical analysis revealed a significant decrease ($p < 0.001$) in the mean number of eggs, where it was 680, 576.7 and 527.3 eggs/ten♀♀ at the doses LD_{25}, LD_{50} and LD_{75},respectively, compared with 797 eggs/ten♀♀ for the control. Also, there was a reduction in the hatchability percent, where it was 86.5, 77.3 and 61.1% at the doses LD_{25}, LD_{50} and LD_{75}, respectively, compared with 95.2% for the control group (Table 2).

The percent of embryonated eggs was 34.6, 47.7 and 48.8% at doses LD_{25}, LD_{50} and LD_{75}, respectively, vs. 20.9% for the control. On the other hand, the percent of non-embryonated eggs was 65.4, 52.3 and 51.2% at the doses LD_{25}, LD_{50} and LD_{75}, respectively, compared with 79.1% for the control. The sterility index was 22.5, 41.2 and 57.5% at LD_{25}, LD_{50} and LD_{75}; respectively, compared to 0.0% for the control group (Table 2).

On the other hand, in the F_3 generation, the fecundity of non-irradiated females crossed with irradiated males resulted from F_2 generation was not significantly different ($p < 0.01$) in the mean number of eggs, where it was 770, 806 and 785 eggs/ten♀♀ at LD_{25}, LD_{50} and LD_{75}, respectively, compared with 790 eggs/ten♀♀ for the control (Table 3). Also, the percent of non-hatching eggs (embryonated and non-embryonated) were more decreased than those found in the F_1 and F_2 generation; in addition semi sterility was recorded in F_3 generation (Table 3).

According to previously obtained results, the inherited harmful impacts of gamma radiation were observed in the F_1, F_2 and F_3 generations. Fecundity and hatchability percentage of non-irradiated female mated with irradiated males was critically different from untreated controls at F_1 and F_2 generations but not seriously different from untreated controls at F_3. Levels of sterility index in the F_1 and F_2 generations were higher than those of untreated control but semi sterility in the F_3 generation was recorded.

3.2. Histopathological and ultrastructure on males' testes of Culex pipiens

3.2.1. Ultrastructure of normal males' testes of Culex pipiens

Ultrastructural examination of normal males' testes of *C. pipiens* consisted of simple, undivided sacs (single follicle). The testicle is composed of an external wall surrounding the germinative cells. A peritoneal sheath, a muscular layer, a basement membrane, a follicular epithelium, tracheoles, and an epithelium at the base of the follicle are what form the testicular wall. The cytoplasm of peritoneal sheath containing rounded reddish-brown grains pigments, which gives the organ its characteristic color (Fig. 1 A).

Ultrastructure of testes follicle of males' *C. pipiens* demonstrated three zones of development (Fig. 1 B) are commonly recognized below the germarium, in which the germ cells divided to produce spermatogonia (Sg) which located in the apex of the follicle, the free spermatogonia were large rounded cells with nucleus occupying most of the cell (Fig. 1B). Spermatogonia divided mitotically to produce spermatocytes (Sc); which divided meiotically to produce spermatids (Sm) (Fig. 1B) which are transformed into sperm inside the cysts (Fig. 1D).

The differentiation of the spermatids in *C. pipiens* happens within cysts. The spermatic cells are perfectly aligned inside each cyst also; there are a definite number of spermatozoa in each cyst in the same stage of maturation (Fig. 1C). The spermatozoon of the examined species is very long and filiform, including the head and tail regions (Fig. 2A). The head is made up of an acrosome and nucleus. The structure of the acrosome is composed of two regions: distal and proximal. The distal region (dr) which comprises around

Table 2
The reproductive potential of males *Culex pipiens* resulted from irradiated pupae in the F_2 generation.

Dose	No.of eggs laid\ten ♀ (mean ± SD)	No. of hatched eggs (mean ± SD)		No. of non-hatching eggs (mean ± SD)					Sterility Index (S.I.) %
		Total	%	Total	Embryonated		Non-Embryonated		
					No.	%	No.	%	
LD_{25} (23 Gy)	680.0 ± 26.6**	588.3 ± 19.5**	86.5	91.7 ± 9.8**	31.7 ± 3.1**	34.6	60.0 ± 7.0**	65.4	22.5
LD_{50} (41 Gy)	576.7 ± 23.**	446.0 ± 33.1**	77.3	130.7 ± 21.**	62.3 ± 11.0**	47.7	68.3 ± 10.**	52.3	41.2
LD_{75} (75 Gy)	527.3 ± 18.**	322.3 ± 18.**	–	205.0 ± 10.0**	100.0 ± 5.**	48.8	105.0 ± 5.0**	51.2	57.5
LD_{90} (90 Gy)	–	–	–	–	–	–	–	–	–
Control	797.0 ± 51.2	758.7 ± 45.0	95.2	38.3 ± 6.5	8.0 ± 1.0	20.9	30.3 ± 5.5	79.1	–

(*) = Significant < 0.05, (**) = highly Significant, P < 0.01, ns = non-significant > 0.05, SD = standard deviation and (LD_{90}) = (–) at this dose the irradiated pupae did not emerge from pupal stage.

Table 3
The reproductive potential of males *Culex pipiens* resulted from irradiated pupae in the F_3 generation.

Dose	No.of eggs laid\ten ♀ (mean ± SD)	No. of hatched eggs (mean ± SD)		No. of non - hatching eggs (mean ± SD)					Sterility Index (S.I.) %
		Total	%	Total	Embryonated		Non-Embryonated		
					No.	%	No.	%	
LD_{25} (23 Gy)	770 ± 44.44 ^{ns}	728.33 ± 49.07 ^{ns}	94.6	41.66 ± 11.54 ^{ns}	8.33 ± 2.88 ^{ns}	20	33.33 ± 10.40 ^{ns}	80	2.6
LD_{50} (41 Gy)	806.66 ± 45.09 ^{ns}	768.33 ± 37.52 ^{ns}	95.2	38.33 ± 7.63 ^{ns}	7.33 ± 2.51 ^{ns}	19.1	31 ± 5.29 ^{ns}	80.9	2.6
LD_{75} (75 Gy)	785 ± 37.74 ^{ns}	753.33 ± 33.29 ^{ns}	95.9	41.66 ± 7.63 ^{ns}	8 ± 1 ^{ns}	19.2	33.66 ± 7.02 ^{ns}	80.8	0.6
LD_{90} (90 Gy)	–	–	–	–	–	–	–	–	–
Control	790 ± 36.05	748.33 ± 32.53	94.7	41.66 ± 7.63	6 ± 1	14.4	35.66 ± 6.65	85.6	–

(*) = Significant < 0.05, (**) = highly Significant, P < 0.01, ns = non-significant > 0.05, SD = standard deviation and (LD_{90}) = (–) at this dose the irradiated pupae did not emerge from pupal stage.

45% of the total length of the acrosome is anterior to the nucleus, and the proximal region (pr) is closely attached to the nucleus (Fig. 2a).

The tail region can be divided into five zones: the 1st zone, where the cytoplasm can be observed, showed a small identification of the nucleus. Its region is also characterized by the presence of microtubules, but they are not organized neither in a centriole nor in an axoneme (Fig. 2 b).

The 2nd zone (overlap zone) is characterized by the emergence of the complete axoneme (with the 9 + 9 + 2 configuration of microtubules) and the two mitochondrial derivatives, centriolar adjunct associated with the nucleus (Fig. 2c).

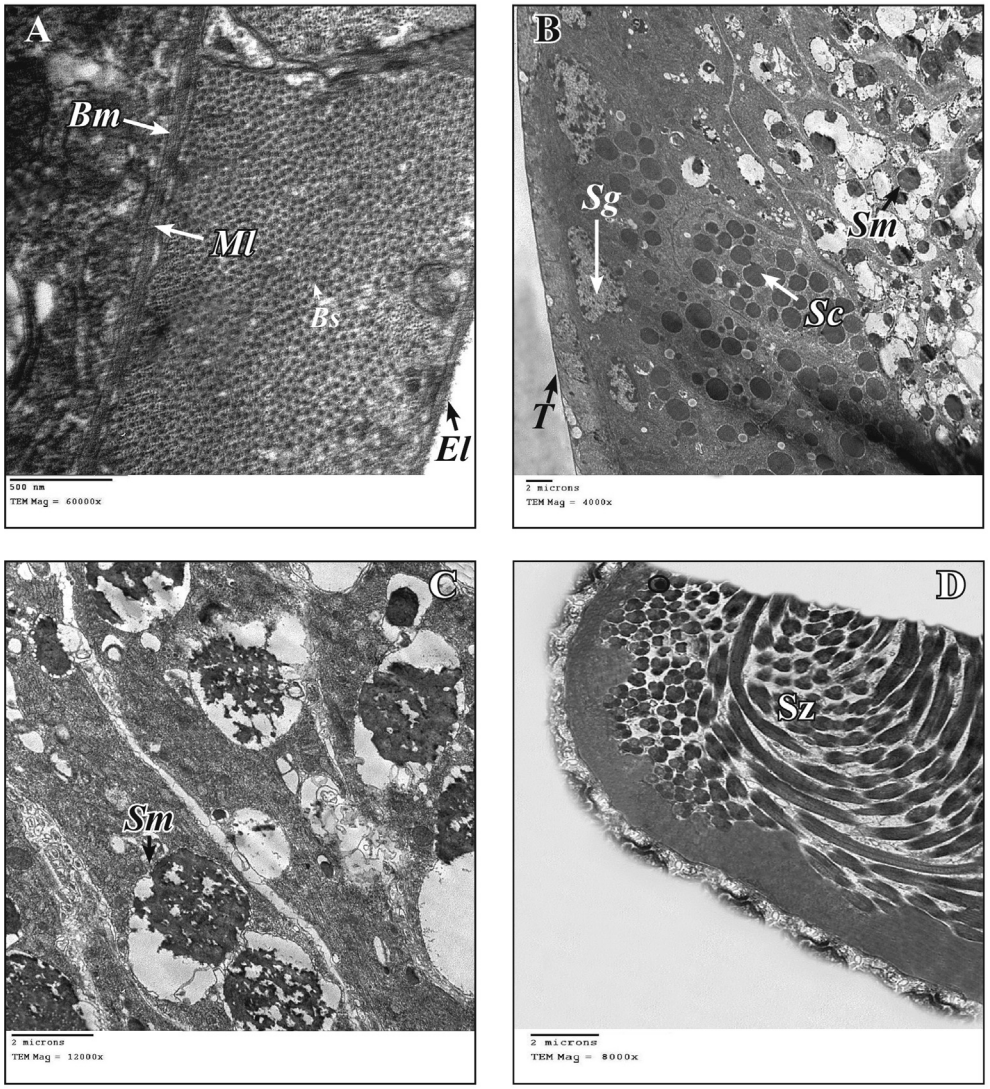

Fig. 1. (A–D) Photomicrograph of normal testis follicle (A): showing testis wall and it is possible to see; external layer (El), peritoneal sheath (Ps), muscle layer (Ml), basement membrane (Bm). (B); showing three developmental zone, spermatogonia (Sg), spermatocytes (Sc), spermatids (Sm). (C): showing maturation of spermatids. (D): showing development of the spermatozoa.

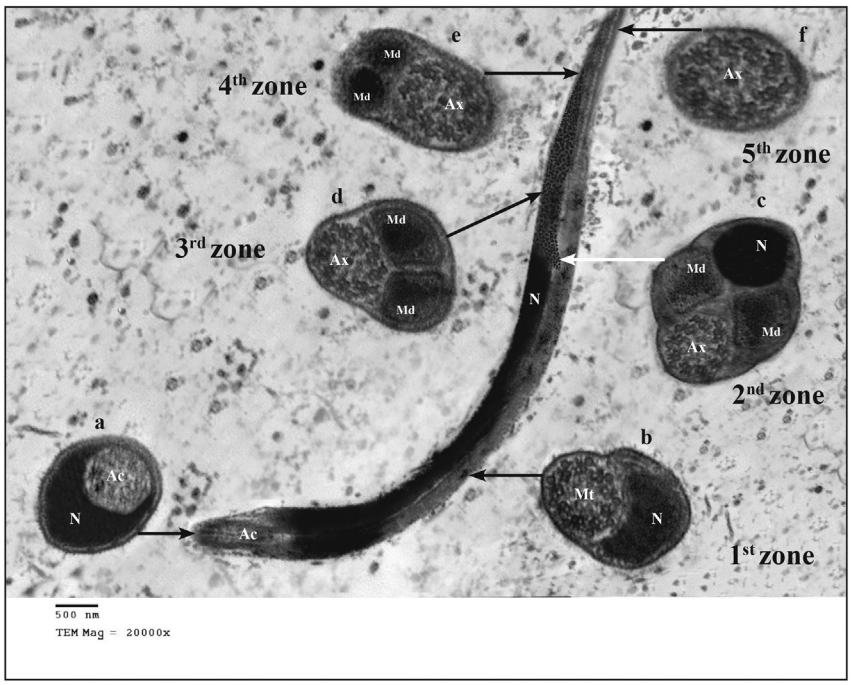

Fig. 2. Photomicrograph of normal spermatozoa **(A)**. **(a)**: showing cross-sections in head region and it is possible to see; acrosome (Ac), nucleus (N). **(b–f)** cross-sections in tail region **(b)**:1st zone showing, microtubules (Mt), nucleus (N). **(c)**: 2nd zone showing, axoneme (Ax), two mitochondrial derivatives (Md), centriolar adjunct (Ca) surrounded by nucleus (n). **(d)**: 3rd zone showing, axoneme (Ax), two mitochondrial derivatives (Md). **(e)**: 4th zone showing small two mitochondrial derivatives (Md), axoneme (Ax).**(f)**: 5th zone showing axoneme (Ax).

The two mitochondrial derivatives are positioned side-by-side near the midline and ventral to the axoneme in the 3rd zone (Fig. 2d).

In the 4th zone, the two mitochondrial derivatives decrease in size and are positioned ventral to the axoneme (Fig. 2 e).

The 5th zone (end piece) is characterized by disappearance of the two mitochondrial derivatives and the reduced number of microtubular elements (Fig. 2 f).

3.2.2. Ultrastructure of irradiated males' testes of Culex pipiens

Histopathological effects of gamma radiation (LD_{25}, LD_{50} and LD_{75} Gy) on irradiated males' testes of *C. pipiens* were studied by using TEM. There was a clear deformity and cell degeneration effect such as;

Ultrastructural examination of the irradiated testicular wall region showed that at the LD_{25} dose the external wall rupture at the different zone, also necrosis, degeneration and small vacuoles were observed (Fig. 3A). At the dose level LD_{50} Gy and LD_{75} Gy, the external wall seemed to have more irregulars; the definite layer of the testicular wall degenerated and had become un clear (Fig. 3 B&C).

Irradiation induced obvious effects on the developmental zone (spermatogonia, spermatocytes and spermatids) that lost the synchrony. At the dose LD_{25} Gy, the developmental zone appeared as one zone and it is difficult to identify (Fig. 4 A). At the dose level LD_{50} Gy, the spermatogonia and spermatocytes appeared vacuolated in their cytoplasm and around them (Fig. 4B). The highest histopathological response was observed by photomicrograph examination at the dose LD_{75} Gy, the developmental zone appeared as one zone, vacuolated and the cell structure degenerated (Fig. 4C).

The reduction in the number of sperm was observed in the irradiated *C. pipiens* compared with the control at the dose level LD_{25} Gy (Fig. 5A). Many of the sperm bundles were ruptured and their contents dispersed throughout the lumen of the testes at the dose level LD_{50} Gy (Fig. 5B). The abnormalities observed in the developing sperms at the dose level LD_{75} Gy (Fig. 5C). TEM in the irradiated sperm exposed to LD_{75} Gy showed rupture in the axoneme and mitochondrial derivatives membrane, abnormalities of the axoneme and mitochondria derivatives was observed (Fig. 5D).

4. Discussion

The present study showed that the applied dose of radiation was inversely proportional to the fecundity and fertility when the *C. pipiens* males were exposed as pupae to all tested doses of gamma radiation. Similar results were obtained by Al-Taweel, Ahmed, Kadhum, and Hameed (1990) on *Ephestia cautella*: Seth and Reynolds (1993) on the tobacco hornworm, *Manduca sexta* (Linnaeus), with 100 Gy; El-Dessouki, El-Awady, Sallam, El-Naggar, and Shible (1996) reported the reduction of the fecundity on the cotton leafworm, *Spodoptera littoralis* (Boisd.) exposed to 25, 50, 75 and 100 Gy doses of gamma radiation. Salem, Fouda, Abas, Ali, and Gabarty (2014) demonstrated the decrease in the reproductive potential of *Agrotis ipsilon* when exposed to 50, 100, 150 Gy of gamma radiation.

The inherited harmful impacts of gamma radiation in *C. pipiens* were observed in the F_1, F_2 and F_3 generations. Fecundity and hatchability percentage of non-irradiated females mated with irradiated males was significantly different from untreated controls at F_1 and F_2 generations but not significantly different from untreated controls at F_3. Levels of sterility index in the F_1, F_2 and F_3 generations were higher than those of untreated control but the sterility in the F_3 generation was less than $F_1$and F_2 generation. These results are in agreement with Jeffrey, Stuart, and WellsoRoger (1997) who exposed pupae of male Hessian flies, *Mayetiola destructor*, to 4 different doses of gamma radiation (0.0, 15, 30, 40, and 60 Gy) and the deleterious effects through three generation

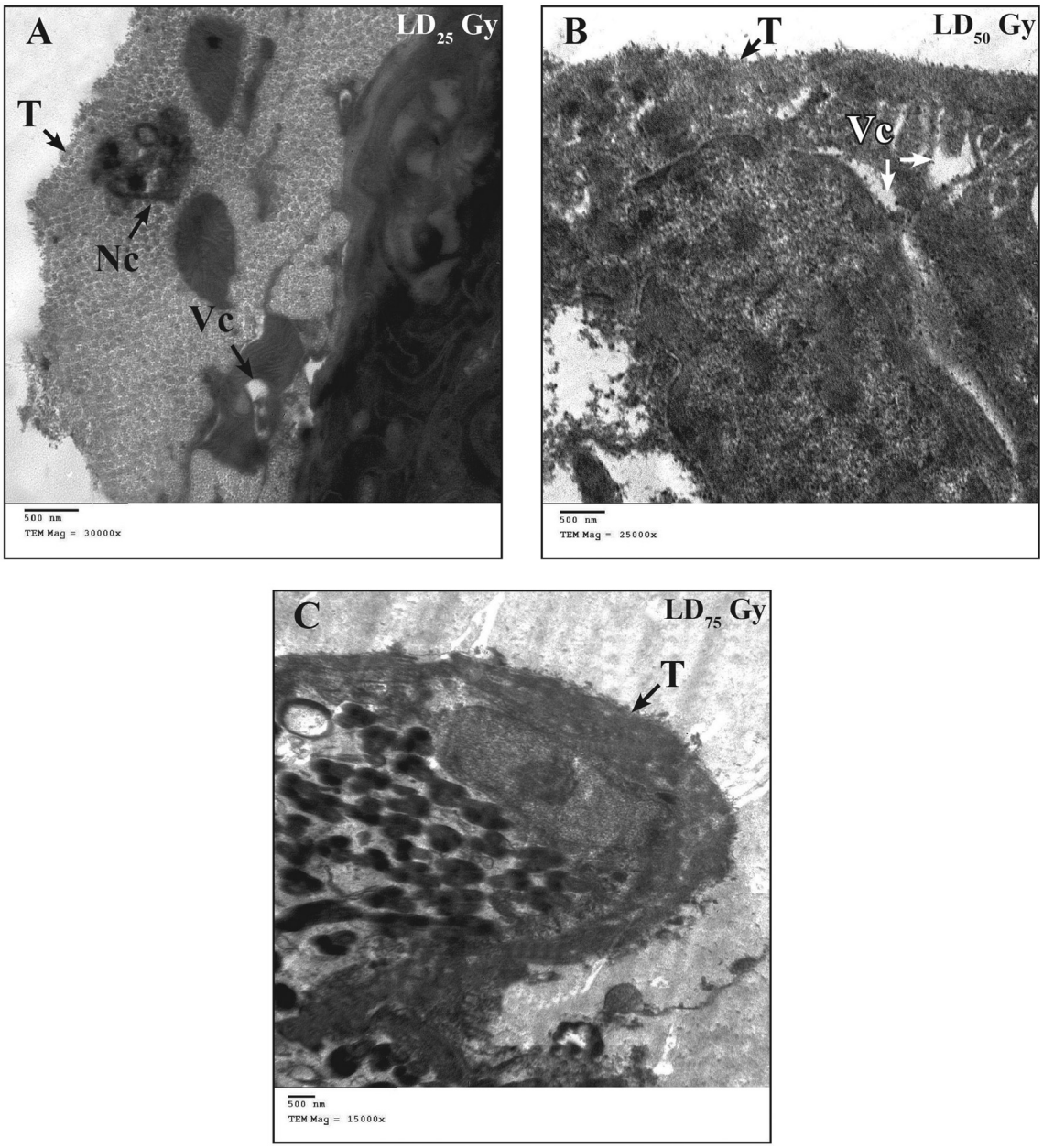

Fig. 3. (A–C) Photomicrograph of testicular wall region in males' *C. pipiens* irradiated with LD_{25}, LD_{50} and LD_{75} Gy of gamma radiation. Testicular wall (T), vacuoles (Vc), necrosis (Nc).

was investigated. Sterility index was completed in the F_1 and F_2, but semi-sterility was found in the F_3. The inherited sterility in the spiny bollworm, *Earias insulana* (Boisd), was studied by Sallam, El-Shall, and Mohamed (2000) they reported that female reproductive potential decreased at the three doses of irradiation (100, 150 and 200 Gy) throughout P_1, F_1, F_2 and F_3 generations as compared to the control. The progeny of F_1 males was obviously more sterile than their irradiated male parents. The impact continued in the F_2 population, however, F_3 males almost regained their fertility.

The male internal reproductive organs in *C. pipiens* comprised testes, vas deferens, accessory glands, seminal vesicle, and ejaculatory duct resemble the pattern structure observed in other Diptera (Joly, Bressac, Devaux, Lachaise, & Lemullois, 2003; Sinclair, Borkent, & Wood, 2007).

Transmission Electron Microscopy studies in the testicular wall of *C. pipiens* as well as in *Ceratitis capitated*, *Anastrepha ludens* (Tephritidae) and *Chrysomya megacephala* (Calliphoridae), the testes were made up of an external and pigmented wall which surrounds the germ cells. The testicular wall in both species consists of a peritoneal sheath and muscular layer. In the peritoneal sheath it is possible to see the presence of a great number of vesicles containing pigment, which gives the organ its characteristic color. Báo and Dolder (1991), stated that pigmented epithelium is responsible for forming an outer physical barrier which protects the testis. According to Valdez (2001), these vesicles could store nutrients temporally, en route from the blood to the transforming germ cells. The muscular layer is presumably associated with the peristaltic contractions, and possibly contributes to the displacement of the spermatid bundle and frees spermatozoa into the testes and toward the posterior duct, leading out of the testis (Chapman, 1998).

The spermatids found in the testes, at the distal and medial regions of this organ, are encapsulated by a somatic cell, forming

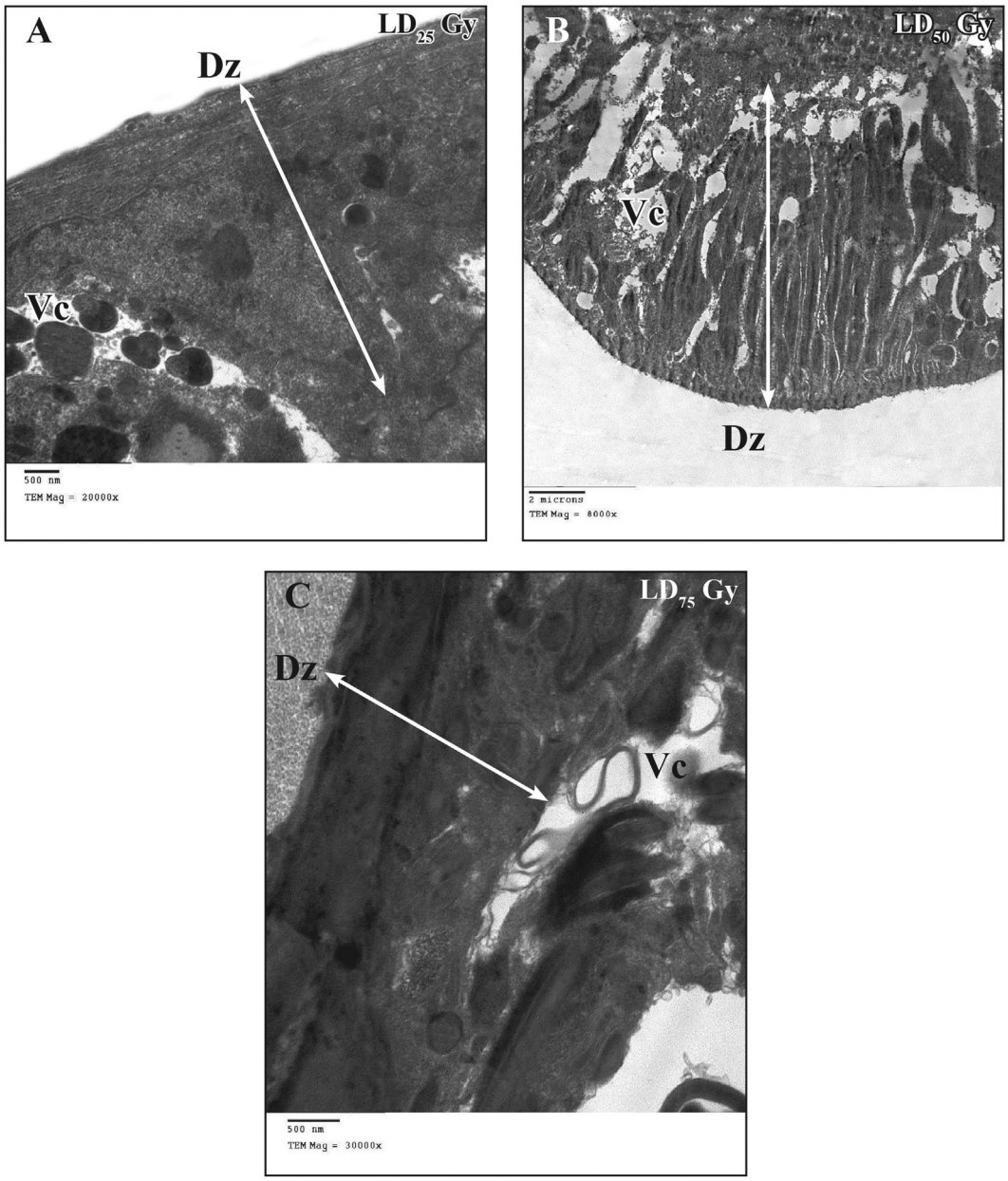

Fig. 4. (A–C) TEM in the developmental zone of males' *C. pipiens* irradiated with LD_{25}, LD_{50} and LD_{75} Gy of gamma radiation. Developmental zone (Dz), vacuoles)(Vc).

the cyst, where they complete the differentiation process. These aspects of testicular organization were also described in some Tephritidae (Valdez, 2001), in *Chrysomya megacephala* (Name et al., 2010). In *C. pipiens*, as in most insects, the development of the germinative cells takes place within such cysts.

The structure of spermatozoa in *C. pipiens* is similar to the general structure of insect sperm (Jamieson, 1987). They are filiform with head and tail regions as in the majority of the Diptera and do not show great variations in length, as found in the spermatozoa of *Chrysomya megacephala* (Name et al., 2010).

Histopathological effects of gamma radiation on spermatogenesis of *C. pipiens* were studied by using TEM. There is gradation and pronounced deformities with increasing the radiation dose (LD_{25}, LD_{50} and LD_{75} Gy) such as;

Rupture, degeneration, vacuoles and necrosis in the irradiated testicular wall region was reported in the present study. The deformity effects induced by gamma radiation has been recorded by Mahmoud and Shoman (2009) they worked on *Ceratitis capitata* and found that the peritoneal sheath appeared thicker due to vacuolation, the muscle layer contain large amount of degenerated mitochondria, coated vesicles and vacuoles.

The synchrony was lost between the developmental zones spermatogonia and spermatocytes, irregular view of spermatogonia and spermatids are observed. Moreover, it is difficult to differentiate stages of spermatogenesis, because of an increase in vacuolation, tubules and space around them. and their Spermatogenesis deformity induced by gamma radiation have been observed previously by Shen and Berryman (1967) who found that increased vacuolation in the testis and dispersion of spermatocytes of *Rhyacionia buoliana* irradiated by gamma rays. Cells in the process of spermatogenesis are very radiosensitive and apparently are easily killed (Hasan et al., 1989). Spermatogenesis, as reported by Tilton and Brower (1983), occurs normally in the pupal stage in Coleoptera and often for most of the adult life. Hence, pupal or adult

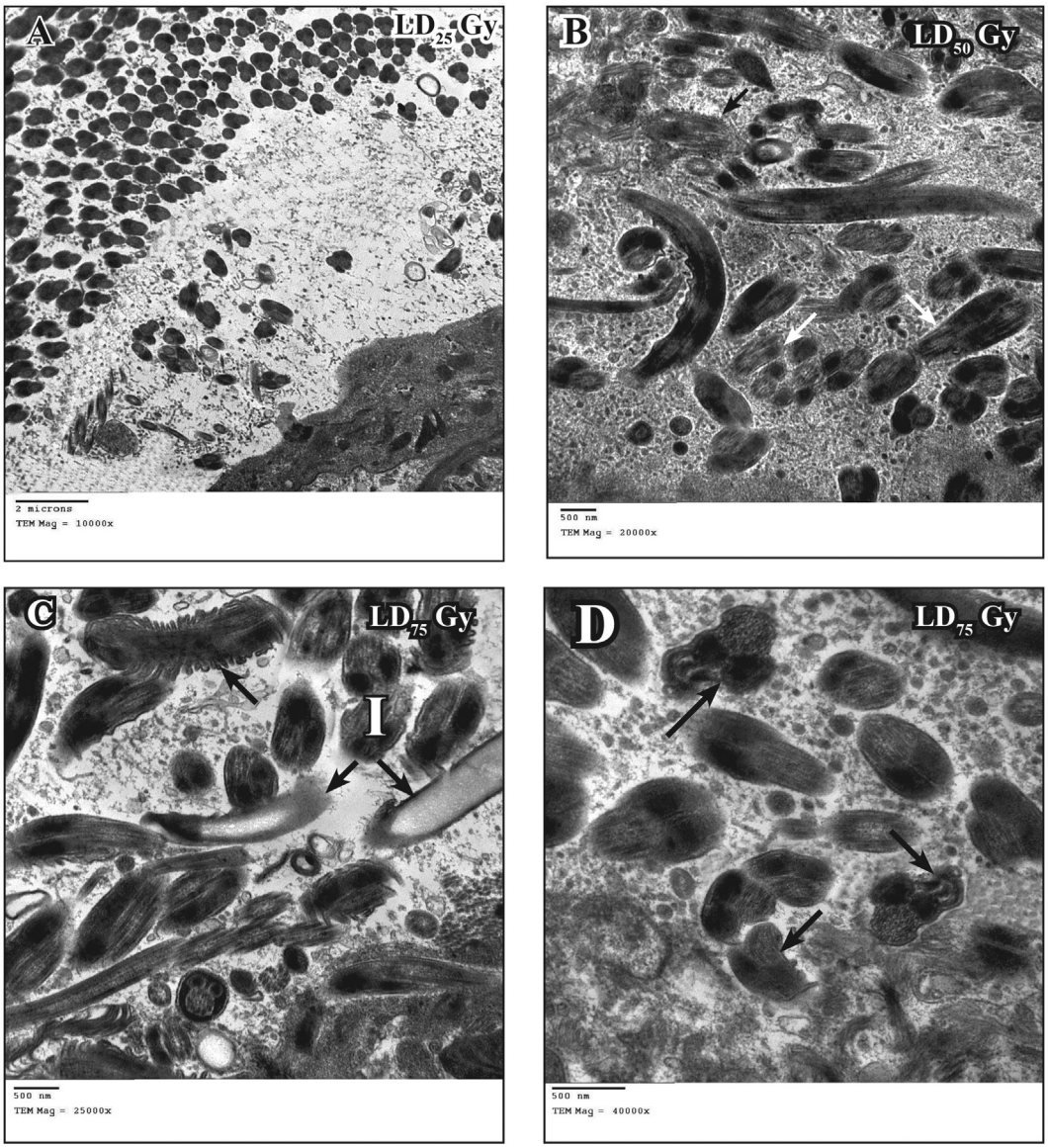

Fig. 5. (A–E) TEM in the spermatozoa of males' *C. pipiens* irradiated with LD_{25}, LD_{50} and LD_{75} Gy of gamma radiation. (A): showing reduction in the number of sperm. (B): Arrows showing rupture in the sperm content. (C) Arrows showing deformity spermatozoa and deformity in cross section of spermatozoa (I). (D): Arrows showing rupture in the axoneme and mitochondrial derivatives membranes.

irradiation leaves rather similar impacts, and, accordingly, histological damage is expected to be similar. Abnormalities in the spermatids of *Dermestes frischii*, were observed only in cells treated as primary or secondary spermatocytes (Hodges, 1983). Irradiation, as stated by Mahmoud and Shoman (2009), has basic impacts on the reproductive function of *Ceratitis capitata* and can affect normal cells, especially the rapidly growing ones, such as the spermatogenic cells with a distinct sensitivity to irradiation during their development from spermatogonia to sperm.

Ultrastructural abnormalities observed in the developing sperms of adult *C. pipiens*, irradiated as pupae with sterilizing dose level (LD_{25}, LD_{50} and LD_{75} Gy), appear to result in the production of non-functional sperms or decrease in sperm production. The reduction in sperm number was observed previously by Mahmoud and Shoman (2009) in *C. capitata*. Sperm bundles were ruptured and their contents dispersed throughout the lumen of the testes reported before by Abd-Alla (1995) in the testes of a1 day old adult *Sitotroga cerealella* exposed to 700 Gy and examined 24 h post-irradiation. Abd-El Meguid and Haiba (1995) when exposed male pupae of *Phthorimeae operculella* to sterile dose (30 K rad) of gamma irradiation, they reported that testes of irradiated males showed degeneration of eupyrene sperms and defects were very apparent in the mitochondrial derivatives and axoneme. What was noticed by Hasan (1995) is that high doses of gamma radiation can inactivate sperm or at least produce dominant lethals in cells, and, on the other hand, lower doses can have serious effects on sperm production, especially if the timing of the treatment has impacted the developing sperm cells.

Finally, it can be concluded that the inherited deleterious effects of gamma radiation were observed in the F_1, F_2 and F_3 generations. Fecundity and hatchability percentage of non-irradiated female mated to irradiated males was significantly different from untreated control at F_1 and F_2 generations but non-significantly different from untreated controls at F_3. Levels of sterility index in the F_1 and F_2 generations were higher than those that of untreated control but the semi sterility were recorded in the F_3 generation.

Ultrastructure of normal males' testes of *C. pipiens* was studied in detail using transmission electron microscopy (TEM). Histopathological and cytotoxic responses were observed in the irradiated testes of *C. pipiens*. Results revealed that gamma radiation greatly affects the testes such as; (i) rupture, necrosis, degeneration and small vacuoles were reported in the testicular wall. (ii) an abnormal distribution of the developmental stages of spermatogonia and spermatocytes leading to a general decreases in the rate of spermatogenesis; and (iii) sperm deformity, which inhibits the movements and the fertility of the sperm which leads to the decrease in the reproductive potential of *C. pipiens*.

References

Abd-Alla, M. S. (1995). *Histological and biological studies on the effects of gamma radiation on the angoumois grain moth, Sitotroga cerealella (Oliv.)*. Ph.D. Thesis. Cairo, Egypt: Faculty of Agriculture, Ain Shams University.

Abd-El Meguid, A., & Haiba, I. (1995). The effect of gamma irradiation on the testes of male *Phthorimaea operculella* Zeller. An ultrastructural study. *Arab Journal of Nuclear Sciences and Applications, 28*(1), 137–148.

Al-Taweel, A. A., Ahmed, M. S. H., Kadhum, S. S., & Hameed, A. A. (1990). Effect of gamma radiation on the progeny of irradiated *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) males. *Journal of Stored-Products Research, 26*(4), 233–236.

Bairati, A. (1967). The structure and ultrastructure of the male genital apparatus of the *Drosophila melanogaster* Meig. I. The testis. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie (Vienna, Austria: 1948), 76*, 56–99.

Báo, S. N., & Dolder, H. (1991). Testicular organization in adult *Ceratitis capitata* (Diptera: Tephritidae): RA mutant and wild-type lineages. *Revista Brasileira De Biologia, 51*(2), 313–319.

Bellini, R., Medici, A., Puggioli, A., Balestrino, F., & Carrieri, M. (2013). Pilot field trials with *Aedes albopictus* irradiated sterile males in Italian urban areas. *Journal of Medical Entomology, 50*(2), 317–325.

Benedict, M., & Robinson, A. S. (2003). The first releases of transgenic mosquitoes: Anargument for the sterile insect technique. *Trends in Parasitology, 19*, 349–355.

Chapman, R. F. (1998). *The insects: Structure and function* (Edn 4). Cambridge, United Kingdom: Cambridge University Press.

Coulthart, M. B., & Singh, R. S. (1988). Differing amounts of genetic polymorphism in testes and male accessory glands of *Drosophila melanogaster* and *Drosophila simulans*. *Biochemical Genetics, 26*, 153–164.

Dame, D. A., Curtis, C. F., Benedict, M. Q., Robinson, A. S., & Knols, B. G. (2009). Historical applications of induced sterilisation in field populations of mosquitoes. *Malaria Journal, 8*, S2.

Darwish, M., & Hoogstraal, H. (1981). Arboviruses infesting human and lower animals in Egypt., A review of thirty years of research. *The Journal of the Egyptian Public Health Association, 56*, 1–112.

El-Dessouki, S. A., El-Awady, S. M., Sallam, H. A., El-Naggar, S. M., & Shible, M. M. (1996). Cotton leafworm: Inherited effects of low doses of gamma radiation on the progeny of irradiated males. In *6th conf. Nucl. Sci. Appl. Cairo* (pp. 15–20) (March).

El-shaikh. (2002). *A study on the effect of blood on oogenesis and protein metabolism in Culex pipiens L. (Diptera: Culicidae)*. M. Sc. Thesis. Cairo, Egypt: Faculty of Science, Al-Azhar University.

Franz, G. (1999). In *Proceedings of the seminar "sterile insect Technique as an environmentally friendly and effective insect control system"*, 12–13 November, Funchal, Madeira, Portugal.

Gad, A. M., Hammad, R. E., & Farid, H. A. (1996). Uptake and development of *Wucheria bancrofti* in *C. pipiens* L. and *Ae. Caspius* Pallas. *Journal of the Egyptian Society of Parasitology, 26*(2), 305–314.

Harris, A. F., McKemey, A. R., Nimmo, D., Curtis, Z., Black, I., Morgan, S. A., et al. (2012). Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature, 30*, 828–830.

Hasan, M. (1995). *Comparative sensitivity of Tribolium spp. to gamma irradiation throughout ontogeny*. Ph.D. Thesis. UK: Newcastle University.

Hasan, M., Khalequzzaman, M., & Khan, A. R. (1989). Development of *Tribolium anaphe* irradiated as larvae of various ages with gamma rays. *Entomologia Experimentalis et Applicata, 53*, 92–94.

Hassan, M. I., Mangoud, A. M., Etewa, S., Amin, I., Morsy, T. A., El hady, G., et al. (2003). Experimental demonstration of Hepatitis C virus (HCV) in an Egyptian strain of *C. pipiens* complex. *Journal of the Egyptian Society of Parasitology, 33*(2), 373–384.

Hassan, M. I., Zayed, A. B., & Ahmed, M. S. (1996). The influence of symbiotic bacteria on digestion and yolk protein synthesis in *C. pipiens* L. (Diptera: Culicidae), 2001 *Journal of the Egyptian German Socirty of Zoology B, 21*(E), 269–284. Entomol.

Hodges, R. J. (1983). Morphology of the X-irradiated testes of *Dermestes frischii* kugelann (Coleoptera: Dermestidae). II. Spermatids and sperm. *International Journal of the Invertebrate Reproduction and Development, 6*, 51–63.

Jamieson, B. G. M. (1987). *The ultrastructure and phylogeny of insect spermatozoa*. Cambridge, U.K: Cambridge University Press.

Jeffrey, J., Stuart, G., Wellso, & Roger, H. (1997). Induction and inheritance of chromosome rearrangements in the hessian fly *Mayetiola destructor* (Diptera: Cecidomyiidae). *Annals of the Entomological Society of America, 90*(4), 480–486.

Joly, D., & Bressac, C. (1994). Sperm length in Drosophilidae (Diptera): Estimation by testis and receptacle lengths. *International Journal of the Insect Morphology and Embryology, 23*(2), 85–92.

Joly, D., Bressac, C., Devaux, J., Lachaise, D., & Lemullois, M. (2003). The sperm roller: A modified testicular duct linked to giant sperm transport within the male reproductive tract. *Journal of Structural Biology, 142*, 348–355.

Klassen, W., & Curtis, C. F. (2005). History of the sterile insect technique. In V. A. Dyck, J. Hendrichs, & A. S. Robinson (Eds.), *Sterile insect Technique. Principles and practice in area-wide integrated pest management* (pp. 3–36). The Netherlands: Springer. Dordrecht.

Krafsur, E. S. (1998). Sterile insect technique for suppressing and eradicating insect populations: 55 years and counting. *Journal of Agricultural Entomology, 15*, 303–317.

Mahmoud, E. A., & Shoman, A. A. (2009). Studies on the radio- protective role of melatonin on the testes fine structure of medflies *Ceratitis capitata* (Wied.) sterile males. *Isotope and Radiation Research, 41*(2), 1421–1441, 4.

Name, K. P. O., Pujol-Luzc, J. R., & Báob, S. N. (2010). Structure and ultrastructure of spermatozoa of *Chrysomya megacephala* (Diptera: Calliphoridae). *Micron, 41*, 853–860.

Ndiaye, M., Mattei, X., & Thiaw, O. T. (1997). Ultrastructural diversity of the manchette in spermatids of mosquitoes (Diptera: Culicidae). *Acta. Zoologica (Stockholm), 78*, 47–49.

O'Connor, L., Plichart, C., Sang, A. C., Brelsfoard, C. L., Bossin, H. C., & Dobson, S. L. (2012). Open release of male mosquitoes infected with a *Wolbachia* biopesticide: Field performance and infection containment. *PLoS Neglected Tropical Diseases, 6*(11), e1797. http://dx.doi.org/10.1371/journal.pntd.0001797.

Pelah, D., Abramovich, Z., Markus, A., & Wiesman, Z. (2002). The use of commercial saponin from *Quillaje saponaria* bark as a natural larvicidal agent against *Ae. aegypti* and *C. pipiens*. *Journal of Ethnopharmacology, 81*(3), 407–410.

Rak, S. S., & Ishii, T. (1989). Characters eggs and egg masses obtained by induced oviposition in *C. pipiens* L. *Journal of Science College of General Education University of Tokushima, 22*, 25–31.

Robinson, R. A., Grantham, M. J., & Clark, J. A. (2009). Declining rates of ring recovery in British birds. *Ringing & Migration, 24*, 266–272.

Salem, H. M., Fouda, M. A., Abas, A. A., Ali, W. M., & Gabarty, A. (2014). Effects of gamma irradiation on the development and reproduction of the greasy cutworm, *Agrotis ipsilon* (Hufn.). *Journal of Radiation Research and Applied Sciences, 7*(1), 110–115.

Sallam, H. A., El- Shall, S. S. A., & Mohamed, H. F. (2000). Inherited sterility in progeny of gamma irradiated spiny bollworm, *Earias insulana* (Boisd). *Arab Journal of Nuclear Sciences and Applications, 33*(1), 263–270.

Selim, T. A. (2015). *Influence of gamma radiation on some biochemical parameters and inherited sterility in Culex pipiens L. (Diptera: Culicidae)*. M.Sc Thesis.in Entomol. Faculty of Science, Al-Azhar University.

Seth, R. K., & Reynolds, S. E. (1993). Induction of inherited sterility in the tobacco horn worm *Manduca sexta* (Lepidoptera: Sphingidae) by sub sterilizing doses of ionizing radiation. *Bulletin of Entomological Research, 83*, 227–235.

Shen, S. K., & Berryman, A. A. (1967). The male reproductive system and spennatogenesis of the European pine shoot moth, *Rhyacionia buoliana* (Lepidoptera: Olethreutidae), with observations on the effects of gamma irradiation. *Annals of the Entomological Society of America, 60*, 767–774.

Sinclair, B. J., Borkent, A., & Wood, D. M. (2007). The male genital tract and aedeagal components of the Diptera with a discussion of their phylogenetic significance. *Zoological Journal of the Linnean Society, 150*, 711–742.

Tilton, E. W., & Brower, J. H. (1983). In E. S. Josephson, & M. S. Peterson (Eds.), *Preservation of food by ionizing radiation* (pp. 269–316). Florida: CRC Press.

Toppozada, A., Abdallah, S., & El-Defrawi, M. E. (1966). Chemosterlization of larvae and adults of the egyptian cotton leafworm, *Prodenia littura* by apholate, metepa and hempa. *Journal of Economic Entomology, 59*, 1125–1128.

Valdez, J. M. (2001). Ultrastructure of the testis of the Mexican fruit fly (Diptera: Tephritidae). *The Morphology, Histology, and Fine Structure, 94*(2), 251–256.

Wandall, A. (1986). Ultrastructural organization of spermatocysts in the testes of Aedes aegypti (Diptera: Culicidae). *Journal of Medical Entomology, 28*, 374–379.

Wang, F., Hanske, M., Miedema, K., Klein, G., Ekblom, P., & Hennig, W. (1992). Laminin in the male germ cells of Drosophila. *The Journal of Cell Biology, 119*, 977–988.

Weidhaas, D. E., Breeland, S. G., Lofgren, C. S., Dame, D. A., & Kaiser, R. (1974). Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. IV. Dynamics of the test population. *The American Journal of Tropical Medicine and Hygiene, 23*, 298–308.