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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 ultrastructural 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}