Effect of gamma radiation on some biological aspects and ultrastructure of female ovaries of the house fly, Musca domestica L. (Diptera: Muscidae)

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Abstract

The present study aimed at evaluating the effect of lethal doses of gamma radiation (LD_{25}, LD_{50} and LD_{75}) on reproductive potential of females resulted from irradiated pupae, and the latent effect on first generation (F1). In addition, ultrastructure alterations in resulted female ovaries were studied using transmission electron microscope. The numbers of eggs laid were 158.3 and 70.0 eggs/5♀♀ at LD_{25} and LD_{50}, respectively, compared with 536.7 eggs/5♀♀ for control, and while no eggs were deposited by females resulted from pupae exposed to LD_{75} of gamma radiation. Also, the sterility index recorded 94.0 and 77.7 at LD_{25} and LD_{50}, respectively in F1 females. Otherwise, the growth index of F1 larvae and pupae recorded 7.2 and 6.4 at LD_{25} and LD_{50}, respectively, compared with 8.7 for control. Gamma radiation affected the ultrastructure of resulted female ovaries such as degeneration of ovarian sheath, distortion, and irregularities in the outlines of follicular epithelial cells and lysis of oocyte. Also, shrinkage nuclei with the unclear components were observed, as well as dispersion of vacuoles around cytoplasm with irregularity and shrinkage of nurse cells. So, these radiation doses can be used in the Sterile Insect Technique (SIT) programs against Musca domestica.

1. Introduction

The housefly, Musca domestica L., is a mechanical vector for the transmission of many pathogens to human and animals including bacterial pathogens of salmonellosis, anthrax, ophthalmia, shigellosis, typhoid fever, tuberculosis, cholera and infantile diarrhea; protozoan pathogens such as amebic dysentery; helminthic pathogens of pinworms, roundworms, hookworms and tapeworms and viral pathogens (Lal, 2014; Scott et al., 2014). Also, it transmits pathogens responsible for eye diseases such as trachoma and epidemic conjunctivitis, and infect wounds or skin with diseases such as cutaneous diphtheria, mycoses, yaws, and leprosy (Keiding & World Health Organization, 1986). The connection of M. domestica with excreta, carcasses, garbage and other septic matter, and intimate association with animal pathogens and humans contribute its role in transmission of these diseases (Scott et al., 2014).

The Sterile Insect Technique (SIT) is a non-disruptive and species-specific pest control strategy that is effectively used against several insect species (Hendrichs, Robinson, Cayol, & Enkerlin, 2002) depending on releasing large numbers of sterile males into the field where they mate with wild females of the same species and cause decline in wild population as reproduction is blocked (Ogaaqwu, Wilson, Cobblah, & Annoh, 2012). Reproductive sterility is induced by exposing the insects to X-rays, electron beams, or most commonly gamma rays from a Cobalt-60 or Caesium-137 source (Bakri, Mehta, & Lance, 2005; LaChance, 1975; Robinson, 2005). Insect fitness components will decrease as flight ability, longevity, startle activity, and mating competitiveness affected by radiation (Calkins & Parker, 2005). In the present study, lethal doses (LD_{25}, LD_{50} and LD_{75}) of gamma radiation were used to evaluate their effect on some biological aspects of the house fly, M. domestica and the changes induced in resulted female ovaries to avoid M. domestica hazards.

2. Materials and methods

2.1. M. domestica culture

The housefly, M. domestica were reared and maintained continuously for several generations in Medical Entomology Insectary, Animal House, Zoology Department, Faculty of Science, Al-Azhar university, Cairo according to the method described by El-Bermawy, Elkattan, Ahmed, and Abdel-Gawad (2011).

2.2. Irradiation process

The irradiation process was performed using Gamma cell-40 (cesium-137 irradiation unit), at National Center...
2.3. Effect of gamma irradiation on reproductive potential of irradiated *M. domestica* females

Five days old pupae were irradiated with 3, 5, 7, 9 and 11Gy doses in order to determine the lethal dose levels (LD_{25}, LD_{50} and LD_{75}). The fecundity (No. of eggs laid/ per female) and Fertility (No. of eggs hatched) were detected in the resulted females. Response percentages of non-hatched eggs were corrected using Abbott’s Formula (Abott, 1925) and LD_{25}, LD_{50} and LD_{75} were calculated according to the method of Finney (1971).

2.4. The latent effect of gamma irradiation on *M. domestica* through first generations

*M. domestica* pupae were exposed to gamma irradiation doses (LD_{25}, LD_{50} and LD_{75}) to give irradiated parents. The females resulted from irradiated pupae were allowed to mate with normal males to give the irradiated first generation and the control group (non-irradiated females and non-irradiated males mated together) of first generation were recorded.

2.5. Reproductive potential of irradiated *M. domestica* females

Four replicates were used for each dose level. Each replicate has five irradiated females and five non-irradiated males from the colony. The adults were fed with dry diet (milk powder) and sucrose solution (cotton pads soaked in 10% sucrose solution) for three days and the females were allowed to lay eggs on an artificial diet rafts. The number of eggs was counted by using binocular and then the mean value was calculated. The egg-hatchability was calculated by using the equation described by El- Sheikh, Bosly, and Shalaby (2012) as Egg-hatchability % = A/B × 100 where, A = Total No. of hatched eggs and B = Total No. of eggs laid. According to the formula of Toppozada, Abdallah, and El-Defrawi (1966), Sterility percentage = 100 − [a × b/A × B] 100 where, a = Number of eggs laid/female in treatment, b = Percentage of hatched eggs in treatment, A = Number of eggs laid/female in control and B = Percentage of hatched eggs in control.

2.6. Effect of gamma irradiation on the mature and the immature stages of *M. domestica*

The larvae, which succeeded to emerge from eggs laid by irradiated females, were observed daily until pupation and adult emergence. The larval mortality percent was estimated by using Briggs (1960) equation: Larval mortality % = (A − B/A) × 100 where, A = number of larvae and B = number of pupae. Larval period was calculated as the intervals between the commencement of first instar larvae and the commencement of pupation, it was calculated for each larva and then the mean value was taken. According to El- Sheikh et al. (2012), Pupation % = A/B × 100 where, A = number of pupae and B = number of larvae. Pupal mortality % = A − B/ A × 100 (El- Sheikh et al., 2012) where, A = number of produced pupae and B = number of observed adults. Pupal period was calculated as the interval between the commencement of pupation and the commencement of adult emergence, it was calculated for each one and then the mean value was taken. The emerged adults were counted and the adult emergence percent was calculated as Adult emergence % = A/B × 100 where, A = number of emerged adults and B = number of tested pupae. The Growth index = a/b where, a = percent of adult emergence and b = mean development (days).

2.7. 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 ≤ 0.05. Results were represented as Mean±SD.

2.8. Histopathological and ultrastructure alterations in female ovaries of irradiated *M. domestica*

Females resulted from pupae irradiated with the LD_{25}, LD_{50} and LD_{75} of gamma rays were used in making Semi thin sections (1µm thick) to study the ultrastructure alterations in ovary. Ovaries were obtained anatomically with fine two dissecting needles according to El-Sheikh (2002) under a stereo microscope 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.

3. Results

3.1. Susceptibility of irradiated females crossed with non-irradiated males

The effects of gamma radiation on fecundity of *Musca domestica* females resulted from 5-days old pupae irradiated with 3, 5, 7, 9, and 11Gy and crossed with non-irradiated males are given in (Table 1). The number of eggs/♀ decreased from 486.3 ± 159.4 at 0Gy to 94.0 ± 11.9 at 11Gy. Meanwhile, at 3, 5, 7, and 9Gy the
Gamma radiation affects the egg-hatchability percentage as compared with the control group. At the highest doses (11 and 9 Gy) the egg-hatchability percent recorded 48.9 and 58.3%, respectively, while at the lowest doses (3 and 5 Gy) the egg-hatchability percent recorded 89.3 and 76.2%, respectively, compared with 91.7% for the control group (Table 1). From the aforementioned results, The LD25, LD50, LD75, LD90, LD95, and LD99 recorded 6.8, 11.2, 18.5, 29.0, 37.9, and 62.8 Gy, respectively, based on the susceptibility of non-hatching eggs percentage resulted from irradiated female crossed with normal male (Table 2).

3.2. The latent effect of gamma irradiation lethal doses on some biological aspects of M. domestica resulted from irradiated pupae

Results in (Table 3) indicated the effect of lethal doses (LD25, LD50 and LD75) on the reproductive potential of irradiated females crossed with non-irradiated males. The fecundity was significantly decreased (p < 0.001) at LD25 and LD50 doses. Where, it recorded 158.3 ± 40.3 and 70.0 ± 10.0 eggs/5 ♀, respectively, vs. 536.7 ± 98.7 eggs/5 ♀ for the control group. On the other hand, LD75 caused complete inhibition (0.0 eggs/5 ♀) in egg deposition by irradiated females. A positive correlation between hatchability and doses was observed, where the hatchability percent decreased as the gamma radiation doses increased. The hatchability percent recorded 67.2 and 43.8% at LD25 and LD50 doses, respectively compared to 88.8 number of eggs/5 ♀ recorded 317.8 ± 51.1, 250.0 ± 39.2, 156.3 ± 33.3, and 120.0 ± 18.7, respectively.

### Table 1. Susceptibility of irradiated females crossed with non-irradiated males to gamma radiation.

| Dose (Gy) | Eggs laid/5 ♀ females (Mean±SD) | Hatched eggs (%) | Non-hatched eggs (%) | Corrected response (%) |
|-----------|---------------------------------|------------------|---------------------|------------------------|
| Zero      | 486.3 ± 159.4                   | 91.7             | 8.3                 | 0                      |
| 3         | 317.8 ± 51.1                    | 89.3             | 10.7                | 2.6                    |
| 5         | 250.0 ± 39.2                    | 76.2             | 23.8                | 16.8                   |
| 7         | 156.3 ± 33.3                    | 66.7             | 33.3                | 27.2                   |
| 9         | 120.0 ± 18.4                    | 58.3             | 41.7                | 36.4                   |
| 11        | 94.0 ± 11.9                     | 48.9             | 51.1                | 46.6                   |

Gy: gray; SD: standard deviation; a = non-significant (P > 0.05); b = significant (P < 0.05); c = highly significant (P < 0.01); d = very highly significant (P < 0.001).

### Table 2. Lethal doses of gamma radiation affecting the non-hatched eggs of irradiated females of Musca domestica crossed with non-irradiated males.

| LD (Gy) | Dose (Gy) | Lower limit (Gy) | Upper limit (Gy) | Slopes±SE |
|---------|-----------|------------------|------------------|-----------|
| 25      | 6.7921    | 5.8579           | 7.8553           | 3.1065 ± 0.1407 |
| 50      | 11.1977   | 10.0963          | 14.0679          |           |
| 75      | 18.4609   | 16.6472          | 25.8594          |           |
| 90      | 28.9523   | 21.5333          | 45.2361          |           |
| 95      | 37.8993   | 33.5213          | 63.3094          |           |
| 99      | 62.8028   | 54.4917          | 119.3386         |           |

SE: standard error.

### Table 3. Effect of gamma irradiation (lethal doses) on the reproductive potential of irradiated females mated with non-irradiated males of M. domestica.

| No. of eggs laid/5 ♀ | Hatchability | Sterility Index (SI) |
|---------------------|--------------|----------------------|
| Control             | 536.7 ± 98.7 | 88.8                 |
| LD25                | 158.3 ± 40.3 | 67.2                 |
| LD50                | 70.0 ± 10.0  | 43.8                 |
| LD75                | 0.0          | 0.0                  |

Gy: gray; SD: standard deviation; a = non-significant (P > 0.05); b = significant (P < 0.05); c = highly significant (P < 0.01); d = very highly significant (P < 0.001).
Table 4. The effect of gamma irradiation (lethal doses) on some biological aspects of irradiated females mated with non-irradiated males of *M. domestica*.

| Dose (Gy) | Larval Mort. | Larval Period | Pupation | Pupal Mort. | Pupal Period | Emergence | Development | Growth Index |
|-----------|--------------|---------------|----------|-------------|--------------|-----------|-------------|-------------|
| Control   | 1.8 ± 0.6    | 4.9 ± 0.4     | 98.3     | 2.1 ± 1.4   | 6.3 ± 0.1    | 97.8 ± 1.6| 11.2 ± 0.5  | 8.7         |
| LD25 (7.0) | 13.8 ± 2.2^d | 5.3 ± 0.7^f  | 86.5     | 13.9 ± 4.7^d| 6.6 ± 0.1^*  | 86.1 ± 4.7^d| 11.9 ± 0.8^d| 7.2         |
| LD50 (11.0)| 28.4 ± 3.2^d | 4.9 ± 0.3^d  | 71.7     | 24.1 ± 3.1^d| 6.8 ± 0.3^d  | 75.8 ± 3.1^d| 11.8 ± 0.6^d| 6.4         |

Gy: gray; SD: standard deviation; a = non-significant (P > 0.05); b = significant (P < 0.05); c = highly significant (P < 0.01); d = very highly significant (P < 0.001).

for the control group. Data in Table 3 revealed that, the egg duration was insignificantly (p > 0.05) affected by LD25 and LD50 doses as compared with the control group. A very retarded effect on the sterility index was noticed, where it recorded 77.7 and 94.0 at the doses of LD25 and LD50 respectively (Table 3).

The effect of gamma irradiation lethal doses (LD25 and LD50) on some biological aspects of larvae succeeded to emerge from eggs laid by irradiated females represented in Table 4. The larval mortality percent recorded 13.8 ± 2.2 and 28.4 ± 3.2% at LD25 and LD50, respectively, compared to 1.8 ± 0.6% for control group. Meanwhile, the pupal mortality was 13.9 ± 4.7 and 24.1 ± 3.1% at LD25 and LD50 respectively, compared to 2.1 ± 1.4% for the control. The mean duration of the resulted larvae was insignificantly (p > 0.05) affected by LD25 and LD50 as compared with the non-irradiated group. In addition, the mean duration of the resulted pupae was significantly (p < 0.01) affected by LD50 as compared with the control group. The adult emergence percent decreased to record 86.1 ± 4.7 and 75.8 ± 3.1% at LD25 and LD50 compared to 97.8 ± 1.6% for the control group. A very slight decrease in growth index by gamma radiation lethal doses (LD25 and LD50) was recorded. Where, it was 7.2 and 6.4 as compared with 8.7 for the control group (Table 4).

3.3. Histopathological and ultrastructure alterations induced by lethal doses of gamma irradiation on female ovary of *M. domestica*

Comparing, the ultrastructure of ovaries in non-irradiated females (Figure 1) and in irradiated females (Figure 2) appeared that the LD25 of gamma radiation caused deterioration in the vitellogenic region including degeneration of ovarian sheath, distortion, and irregularities in the outlines of follicular epithelial cells. In (Figure 2(a)), the ovarian sheath began to separate from the follicular epithelial cells, cytoplasm of the oocyte region began to lysis and disappearance of Golgi complex. The internal structure of the nucleus seemed unclear with irregular nuclear membrane; in addition, the components of the nucleus seemed to be unclear with irregularity in the nuclear membrane. Also, unclear Golgi complex and vacuoles were dispersed around cytoplasm, whereas the nurse cells are small in size with reduction in its number (Figure 2(b)). On the other hand, the lethal dose LD50 of gamma radiation caused the lysis of oocyte, and the disappearance of its components also, the shrinkage of the nucleus (Figure 3(a,b)). In addition, the nurse cells in the vitellarium region degenerated, showed irregularity, as well as shrinkage in the shape of ring canals (Figure 3(c)). Meanwhile, LD25 of gamma radiation caused disappearance of oocytes and nurse cells, destruction in the follicular epithelial cells and the nucleus discharged from its components (Figure 4).

4. Discussion

4.1. Latent effect of gamma irradiation on some biological aspects *M. domestica*

The present study revealed that, the number of eggs laid by females resulted from pupae irradiated with different doses of gamma radiation was found to be dose dependent i.e. decreased as the dose of the gamma irradiation used increased. In addition, the non-hatched eggs percentage increases as the dose of the gamma irradiation used increased. These results are in disagreement with those previously mentioned by Khan and Islam (2006), where they observed that, gamma radiation dose level up to 10Gy significantly increased oviposition in *M. domestica*, however the obtained results are in agreement with Resilva, Obra, Zamora, and Gaitan (2007) who reported that, irradiation of *Bactrocera philippinensis* pupae with doses lower than 67Gy did not prevent egg hatching. Also, Draz, El-Aw, Hashem, and El-Gendy (2008) reported that, irradiation of *Bactrocera zonata* 7-day-old male pupae with 10, 30, and 50Gy of gamma radiation increased the non-hatched egg percent. Where, the non-hatched egg percent recorded 30.6–47.2% for females mated with males irradiated with 10Gy, 94.34–99.96% for females mated with males irradiated with 30Gy and 96.57–100.0% for females mated with males irradiated with 50Gy, respectively. Similar observations were recorded by Gabarty (2011) for the greasy cutworm *Agrotis ipsilon* where, the percentage of non-hatching eggs resulted from irradiated full-grown male pupae was increased as the dose of the gamma irradiation increased and the highest percent of non-hatching eggs was caused by 240Gy. Meanwhile, the lowest dose 40Gy caused...
Figure 1. (a-d): photomicrograph of *M. domestica* control vitellarium region: (O) oocyte, (NC) nurse cells, (N) nucleus, (NM) nuclear membrane, (V) vacuole, (OSH) ovarian sheath, (FEC) follicular epithelium cells, (CH) chromatin, (GC) Golgi complex and (RC) ring canal.

Figure 2. (a,b): photomicrograph in vitellarium region of *M. domestica* irradiated with lethal dose of gamma radiation (LD$_{25}$): (FEC) follicular epithelium cells, (O) oocyte, (N) nucleus(V) vacuole, and (OSH) ovarian sheath.
Figure 3. (a-c): photomicrograph in vitellarium region of *M. domestica* irradiated with lethal dose of gamma radiation (LD$_{50}$): (O) oocyte, (N) nucleus, (NM) nuclear membrane, (RC) ring canal, (NC) nurse cells and (DNC) degenerated nurse cell.

Figure 4. (a,b): photomicrograph in vitellarium region of *M. domestica* irradiated with lethal dose of gamma radiation (LD$_{75}$): (N) nucleus and (NM) nuclear membrane.
the lowest percentage. Also, the obtained results revealed that, gamma radiation lethal doses (LD$_{25}$, LD$_{50}$, and LD$_{75}$) affect the fecundity and hatchability % of the house fly, *Musca domestica* where, the number of eggs laid by irradiated females mated with non-irradiated males was significantly (p < 0.001) decreased as compared with those of control groups. In addition, the sterility index of *M. domestica* females resulted from irradiated pupae was increased as the dose level of gamma radiation increased compared with the sterility percent in control group (0.0%). That means exposure to gamma radiation resulted in a progressive increase in the sterility % of all different treatments. These results are in consistent with those obtained by Mansour (1987) where, the sterility in *Musca autumnalis* treated males and females increased as radiation dosage increased, and Puanmanee, Wongpiyasatid, Sutantawong, and Hormchan (2010) where, there was an increase in the sterility of *Bactrocera correcta* when treated males crossed with untreated females and the sterility percent was increased as the gamma dosage increased. Similar results were obtained by Mohmoud and Barta (2011) who recorded that, the hatchability of *Bactrocera zonata* eggs reduced when non-irradiated females mated with males resulted from pupae irradiated with 10Gy.

The obtained results showed that, a variable effect of gamma irradiation on different biological parameters of the house fly, was occurred in first generation (F1). Where, gamma radiation lethai doses of LD$_{25}$ and LD$_{50}$ increased larval and pupal mortality, larval and pupal durations, as well as reduce on the percentage of adults emerging and growth index. These results are in agreement withSaleh, Gaaboub, Rawash, and El-Sawaf (1981) who recorded a decline in pupation and adult emergence percent in UV-irradiated *Culex pipens* and *Aedes aegypti*, El-Naggar, Megahed, Sallam, and Ibrahim (1984) who found, the mortality among *Agrotis ipsilon* larvae of the first generation was high and increased as the dose of gamma radiation increased, Pransopon, Sutantawong, Hormchan, and Wongpiyasatid (2000) who reported that, the survival of *Helicoverpa armigera* immature stages decreased with the increasing of gamma radiation dose level, Sallam, El-Shall, and Mohamed (2000) who recorded that, the spiny bollworm *Earias insulana* larvae reaching the adult stage decreased in number as the irradiation dose increased and Gabart (2011) who recorded that gamma irradiation LD$_{50}$ and LD$_{95}$ doses affected the biological parameters of the greasy cut-worm *A. ipsilon*. Similar observations were recorded by Hassan, Amer, Hammad, Gabarty, and Selim (2017) who found that, gamma irradiation affected the biological activity of *C. pipiens* during the first and second generations, also the larval, pupal mortality and duration were increased, as well as the percentage of adults emergence and growth index were decreased as the doses increased.

### 4.2. Histopathological and ultrastructure alteration induced by lethal doses of gamma irradiation on female ovary of *M. domestica*

Exposure of pupae to LD$_{25}$ of gamma radiation induced degeneration of ovarian sheath, distortion, and irregularities in the outlines of follicular epithelial cells, separation of the ovarian sheath from the follicular epithelial cells, lysis of the cytoplasm in oocyte, the components of the nucleus seemed to be unclear and vacuoles were dispersed around the cytoplasm. In addition, the nurse cells were small in size with reduction in its number. At LD$_{50}$, the oocyte showed lysis, disappearance of its components and shrinkage of the nucleus. Also, the nurse cells in the vitellaria region degenerated, appearance of irregularity and shrinkage in the shape of ring canals was observed. Meanwhile, LD$_{75}$ caused full faded oocytes and nurse cells. The obtained results are in agreement with previously reported by King (1957) who 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. Studies reported by LaChance and Burns (1963) and LaChance and Leverich (1968) on female *Cochliomyia hominivorax* indicated that, gamma radiation not only showed down the rate of ovarian growth but also caused cytopathological changes in developing egg follicles. Similar evidence comes from Hasan (1995) who noticed that, irradiation not only delayed ovarian growth, but also produced deformities in the shape of the developing follicles and nurse cell nuclei in *Tigriopus brevicornis*. In addition, treatment with these doses was sufficient to stop the process of oogenesis in *T. brevicornis* adults completely. The present histopathological study on the house fly, *M. domestica* ovary could provide important information for designing a sterile female technique for this medically important pest.

### 5. Conclusion

Gamma irradiation has a latent effect on different biological aspects of the house fly, *Musca domestica*. Also, the structure of irradiated females affected the ovaries by the tested doses of gamma radiation (LD$_{25}$, LD$_{50}$, and LD$_{75}$). So, these radiation doses can be used in the SIT programs against *M. domestica*. Further, in near future we need to focus on SIT strategies against different insect species in order to avoid insecticides hazards.
Disclosure statement
No potential conflict of interest was reported by the authors.

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