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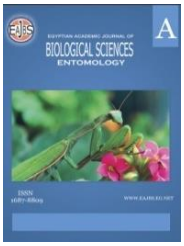

# Ultrastructure and Histopathological Alteration in the Ovaries of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) Induced by X-ray Radiation

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## ABSTRACT

*Ceratitis capitata* is considered one of the world's most destructive pests. Medflies can infest over 300 species of fruit plants, adapt to a variety of climate zones and have a high invasive potential. Medfly can target economically valuable fruit, resulting in significant economic losses estimated to be in the billions of dollars per year. This study aimed to investigate the effect of X-ray irradiation on the female ovary of *Ceratitis capitata* (resulting from irradiated pupae) in comparison to a normal female ovary of the same age. The structure of normal and treated female ovaries of *Ceratitis capitata* was studied using light and transmission electron microscopy. The female reproductive system of *Ceratitis capitata* is composed of paired lateral ovaries. Each ovary consists of polytrophic meroistic ovarioles formed by a terminal filament, a germarium and a vitellarium. The female ovaries were greatly affected by X-ray irradiation because of the appearance of abnormal shape and size of egg chambers, degeneration of nurse cells and their nucleus, the nucleus of follicular epithelium appear devoid of chromatin materials, presence of lysosomes and myeloid bodies, the oocytes appear devoid of yolk granules and its nucleus. The results of this study show that X-ray radiation technology has a clear effect on *C. capitata* and could be considered an effective and safe method to control medflies populations.

## INTRODUCTION

*Ceratitis capitata* (Wiedemann) is regarded as one of the most damaging pests in the world. Over 300 varieties of fruit plants can be infested by medflies, which can also adapt to different climate zones and have strong invasive potential (Morales *et al.*, 2004; Meats and Smallridge, 2007). Medfly has the ability to target commercially valued fruit, causing enormous economic losses that are thought to be in the billions of dollars per year (Malavasi *et al.*, 2014).

The sterile Insect Technique is a non-polluting method and a species-specific of control of the insect population that relies on the release of sterile insects and mass rearing (Knipling, 1955; Krafsur, 1998). Under natural circumstances, the target population will be managed or even locally eradicated if enough sterile insects can be released for a long

period of time (Knipling, 1998). SIT is most successful in controlling low-level medfly populations when a high ratio of sterile to wild flies is attained (Anonymous, 1999). SIT has the advantage that the resultant effects may persist in the population for several generations (Wall and Howard, 1994). SITs are eco-friendly species-specific pest control strategies used in pest management. Because sterile insects are incapable of self-replication and of establishing wild populations in the environment, this approach has a lower negative environmental impact (Jun *et al.*, 2022; Du *et al.*, 2019). Released sterile insects mate with wild insects in the target region and prevent them from reproducing, which lowers the levels of infestation in the offspring (Jun *et al.*, 2022). Ionizing radiation is frequently used as a treatment to make insects sterile in SITs (Mastrangelo *et al.*, 2018).

Because of its many features, including discontinuous radiation emission, no radioactive waste, reduced transport costs, easy operation, and excellent penetration depth, X-ray irradiation-based SITs have been used to treat a variety of pest infestations (Yun *et al.*, 2016). For various insect species, X-ray radiation dose varies in the SIT (Jun *et al.*, 2022). For sterilizing, *Epiphyas postvittana* required a 400 Gy X-ray dose (Follettt and Snook, 2012), but in *Aedes albopictus*, a smaller dose of 40 Gy radiation might be used to induce 100% sterility (Yamada *et al.*, 2014). Bahari (1994) described how choosing the right radiation dose is essential for effective pest control, whether by causing sterility or mortality.

The objective of this study is to determine the suitable doses of X-rays irradiation to control the female medfly *Ceratitis capitata* without affecting the viability of treated insects to compare with normal wild insects, as well as the impact of X-rays on the internal structure of the medfly ovary to prevent reproduction and generate it sterile when compared to normal wild insects.

## MATERIALS AND METHODS

### Insect Rearing Technique:

*Ceratitis capitata* was obtained from the laboratory colony reared in Horticulture Pest Department, Plant Protection Research Institute, Dokki, Egypt. Insects were maintained under lab conditions (25 ± 2 °C and 60–70% RH). Adults were reared in a wooden cage with metal screen sides of 30x30x40 cm. Adult insects were fed sugar and protein hydrolyzate (3:1) in the cage. As a water source for the reared insects, a tiny bottle was placed in the cage. Larvae were fed artificial food when they were young (500ml water, 3g citric acid, 3g sodium benzoate, 84.5 yeast, 84.5 sugar, and 330g wheat bran).

### X-ray Irradiation Process:

X-ray irradiation treatment was performed using an electron beam accelerator (3MeV, ICT, VIVARAD, Co., France) equipped with an X/e convertor, located at the NCRRT, Nasr City, Cairo, Egypt. The seven-day-old pupae were placed in plastic boxes (5 x 5 x 3 cm) with small holes punched for ventilation. The GafchromicTM HD-V2 Film Dosimetry (dynamic dose ranged from 10 to 500 Gy; lot #: 01091801) was used.

### X-ray Irradiation of The Pupae Stage of *C. capitata*:

Seven-day–old pupae of the Medfly were exposed to 50 and 100 Gy doses of X-ray radiation. Each treatment was replicated three times with 300 pupae in each sample tube. The emerging flies were transferred to small treatment cages (15x15x15 cm). Cages were supplied with sugar and protein hydrolyzate for feeding insects. Cages with normal flies were also set against treatment cages.

### Histopathological and Ultrastructure Alterations in Female Ovaries Induced by X-Ray Radiation of *C. capitata:*

The normal and irradiated ten-day-old females that resulted from 50 and 100 Gy

irradiated as pupae were anesthetized with ether and dissected microscopically. Tiny forceps were used to remove the ovaries. A saline solution for insects (0.1 M NaCl, 0.1 M KCl) was utilized, to keep the interior structures from drying up.

The ovaries of three groups of ten-day-old medfly females were dissected, and the ovaries were fixed in 3% phosphate-buffered glutaraldehyde (pH 7.4 at 4 °C for two hours). Semi-thin sections are stained with toluidine blue (1%) and then viewed using a Cambridge light microscope. Ultrathin slices were mounted, put on copper grids, and ready for examination by transmission electron microscope TEM, JEOL (JEM-1400 TEM). The work was done in TEM lab FA-CURP, Faculty of Agriculture, Cairo University Research Park.

## RESULTS

### Light and Ultrastructure Examination of The Normal Female Ovary of *C. capitata:*

The female reproductive system of *Ceratitis capitata* is composed of paired lateral ovaries. Each ovary consists of polytrophic meroistic ovarioles formed by a terminal filament, a germarium and a vitellarium. Each ovariole is separate and contains its own stem cells and egg chambers at different development stages. In the germarium region, the egg chamber is surrounded by a somatic cell layer containing nurse cells and oocytes (Figs. 1a and 1b). The nurse cell nuclei which are adjacent to the developing oocyte are larger in size than the anterior ones (Fig. 1a). The nurse cell nucleus is spherical in shape as shown in Figures (1a, 2a, 2b and 2c). Cytoplasmic junctions (ring canals) directly connect oocytes to adjacent nurse cells (Figs. 1a, 2a, 2b and 2c). In the vitellarium region, the egg chamber completes its development (vitellogenesis and choriogenesis). The egg chamber occupied by nurse cells differs in their size according to the degree of development of the previous egg chamber. The entire egg chamber and mature oocyte are surrounded by the follicular epithelium layer as shown in Figures (1a, 1b, 2a, 2c, 2d and 2e). The follicular cells contain oval nuclei as shown in Figures (1a, 1b, 2c, 2e and 2f). Each mature oocyte contains yolk granules and possesses its own nucleus as shown in Figures (1b, 2d and 2e).

### Histopathological and Ultrastructure Alterations in Female Ovaries Induced by X-ray Radiation of *C. capitata*:

Seven-day-old pupae of *C. capitata* were exposed to 50 and 100 Gy of X-ray doses. The examined ovaries of irradiated females with dose levels of 50 and 100 Gy (10 days after emergence) showed various forms of cell degeneration, where many structural abnormalities were apparent. The abnormal shape and size of the egg chambers, where the follicular epithelial cells that surround the egg chamber disappears, its membrane lyses and degeneration of nurse cells occur and cytoplasmic junctions break between cells and disappear at the dose level 50 and 100 Gy as shown in Figures (1c, 1e, 3a, 3b, 3c, 4a, 4c and 4d). The number of nurse cells decreases in the egg chamber at the 50 and 100 Gy dose levels (Figs. 1c, 1e. 3a, 3b, 4a and 4d). At the dose level 50 the appearance of deformed, irregular shape nuclei and some nuclei are empty from chromatin materials as shown in Figures (3a, 3c and 3d). The appearance of a large number of lysosome inside egg chambers especially the nurse cells in both 50 and 100 Gy dose levels is apparent in Figures (1e, 3c, 3d, 4a, 4b and 4c). The dose level of 50 Gy led to the appearance of myeloid bodies as a result of phagolysosomes and phagocytic activity inside the nurse cells as shown in Figure (3d). The mature oocytes are devoid of yolk granules and the follicular cells epithelium has nuclei empty from chromatin materials at the dose levels 50 and 100 Gy are shown in Figures (1d, 1f, 3e and 4e).

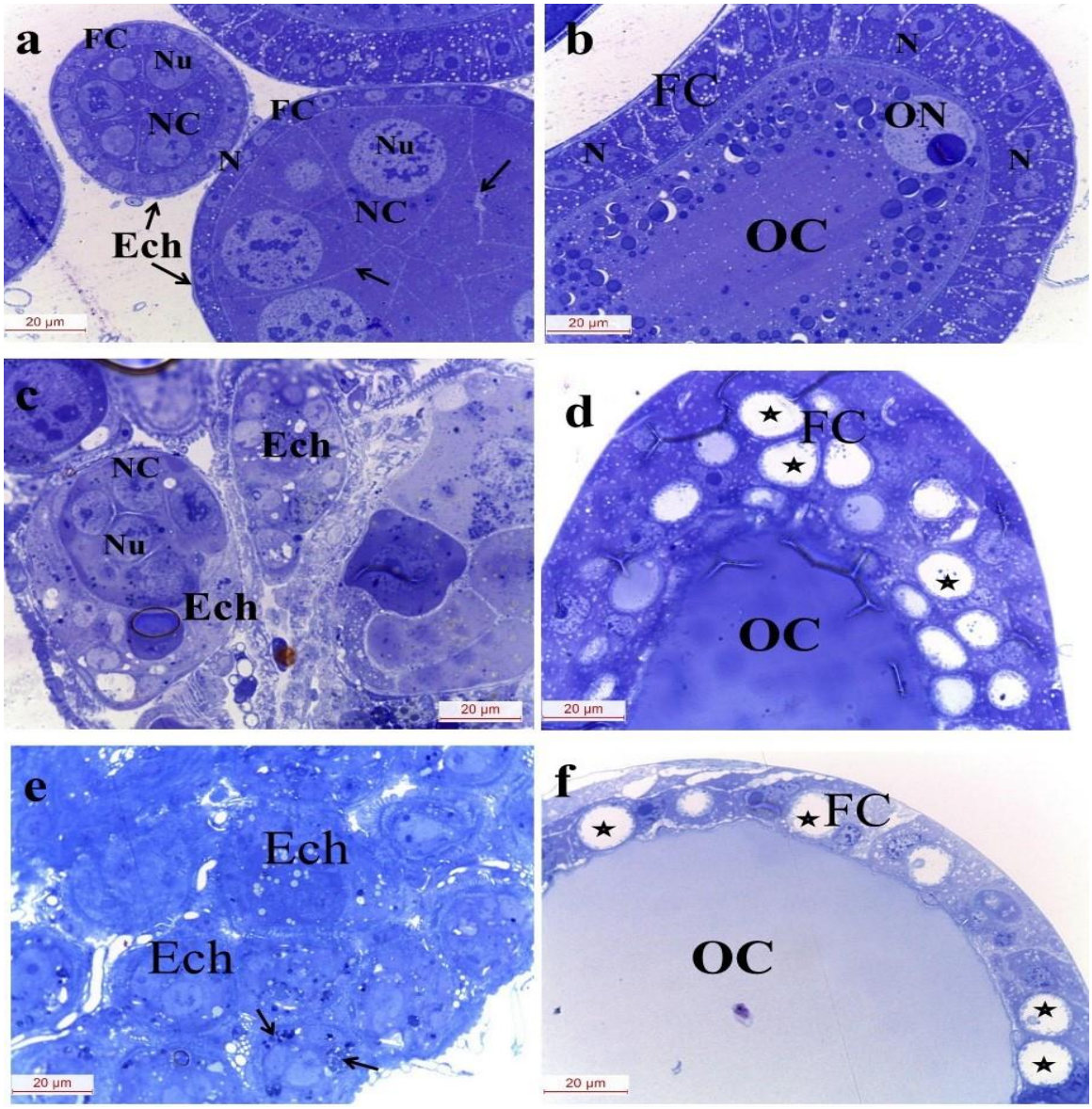


**Fig. 1(a-f) Semi-thin photograph of *Ceratitis capitata* ovary**. **a) and b) Normal female ovary showing a)** Egg chamber (Ech) containing nurse cells (NC) with its rounded nucleus (Nu) and is surrounded by follicular cell epithelium (FC), each nurse cell attached with its neighbor cell and oocyte by cytoplasmic junctions (arrows), **b)** Mature oocyte (OC) and its nucleus (ON) surrounded by follicular cell epithelium (FC) with oval nucleus (N). **c) and d) Irradiated female ovary with dose 50 Gy showing c)** Abnormal shape of egg chamber (Ech), nurse cells (NC) and its nucleus (Nu) are attached with each other without breaks and cytoplasmic junctions, **d)** Mature oocyte (OC) empty from yolk granules and surrounded with follicular cell epithelium (FC) without breaks between them, its nucleus (stars) devoid of chromatin materials. **e) and f) Irradiated female ovary with dose 100 Gy showing e)** Abnormal shape and size of immature egg chambers (Ech) and presence of lysosomes (arrows) inside each egg chamber, **f)** Mature oocyte (OC) empty from yolk granules and surrounded with follicular cell epithelium (FC) without breaks between them, its nucleus (stars) devoid of chromatin materials.

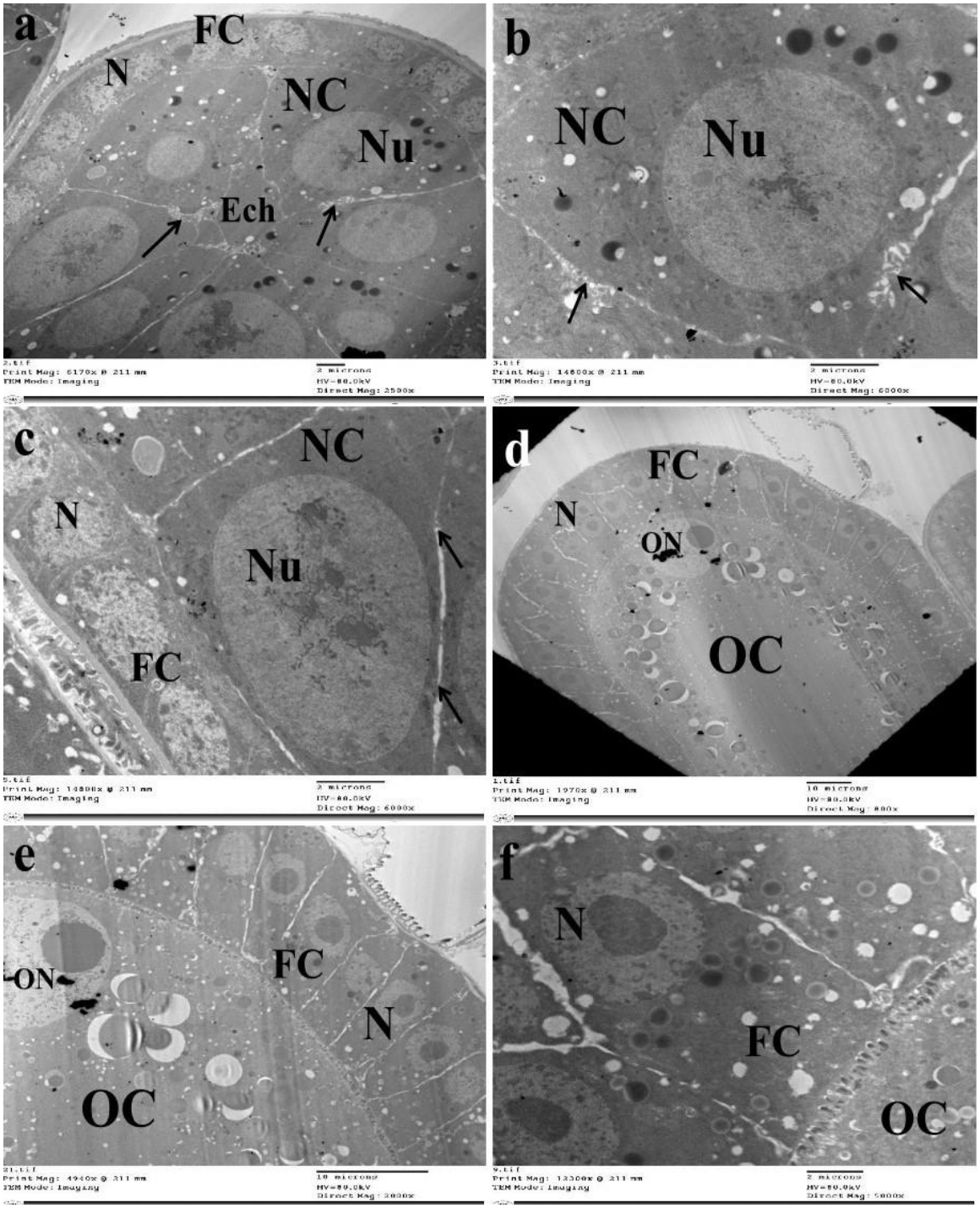


**Fig. 2(a-f) TEM micrographs of the normal female ovary of *Ceratitis capitata*. a)** showing normal egg chamber (Ech), nurse cell nucleus (Nu), cytoplasmic junctions (arrows) between nurse cells (NC), follicular epithelium (FC) and its nucleus (N). **b)** and **c)** showing higher magnification of cytoplasmic junctions (arrows) between nurse cells (NC), nurse cell nucleus (Nu), follicular epithelium (FC) and its nucleus (N). **d)** showing mature oocyte (OC), oocyte nucleus (ON), follicular cell epithelium (FC) and its nucleus (N). **e)** and **f)** showing higher magnification of oocyte (OC), oocyte nucleus (ON), follicular cell epithelium (FC) and its nucleus (N).

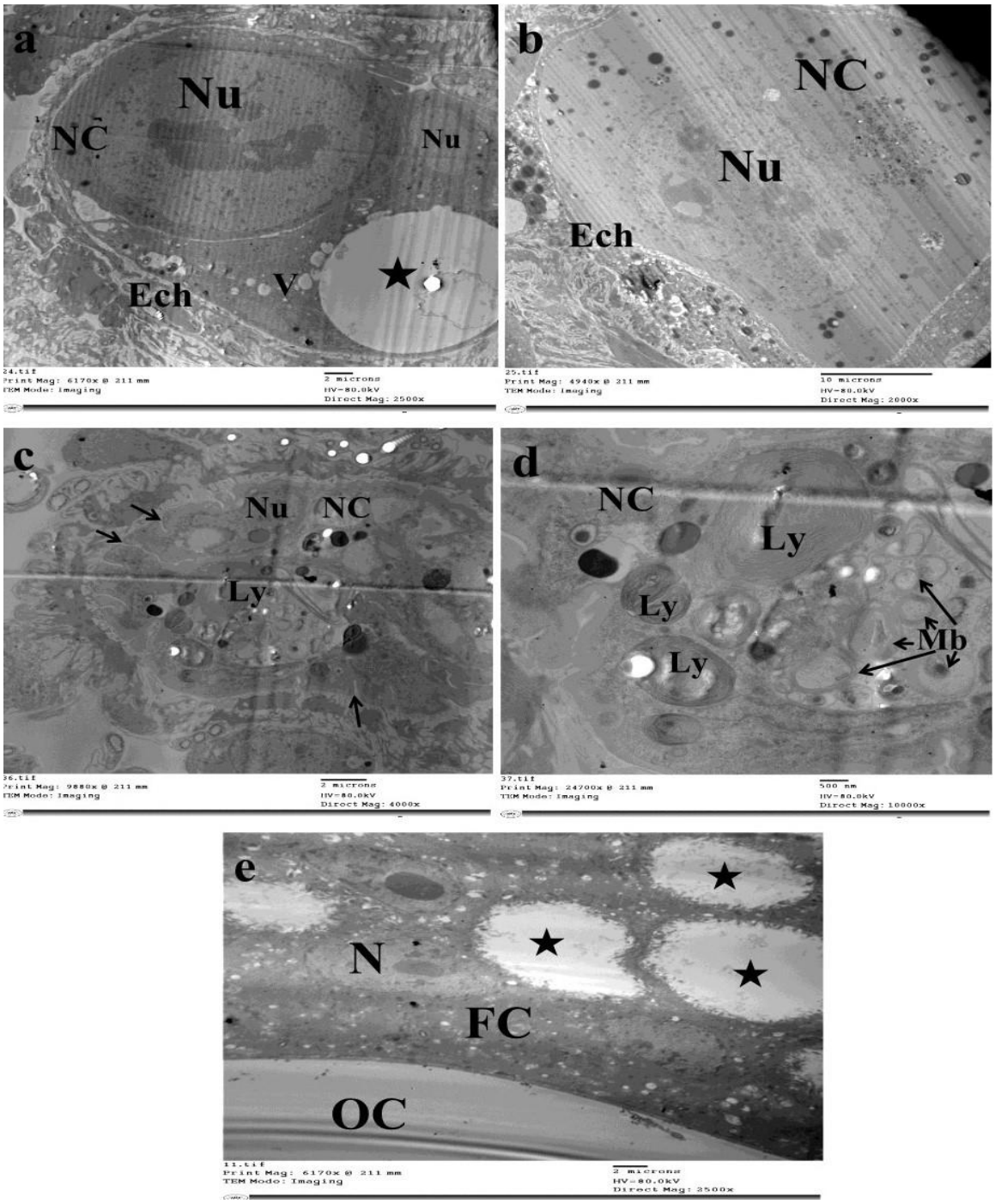


**Fig. 3(a-e) TEM micrograph of irradiated *Ceratitis capitata* ovary with dose 50 Gy**. **a)** and **b)** Abnormal shape and size of egg chambers (Ech) showing degenerated nurse cells (NC), the irregular shape of the nurse cells nuclei (Nu), empty NC nucleus (star) and presence of many vacuoles (V). **c)** Degenerated egg chamber (Ech) showing clear lyses of egg chamber membrane (arrows), degenerated nurse cell (NC) with degenerated nucleus (Nu) and presence of large amount of lysosomes (Ly). **d)** High magnification of nurse cell (NC) showing the presence of large amount of lysosomes (Ly) and myeloid bodies (Mb, arrows). **e)** High magnification of oocyte (OC) showing follicular cell epithelium (FC) with empty nuclei (stars) and the oocyte devoid of yolk granules.

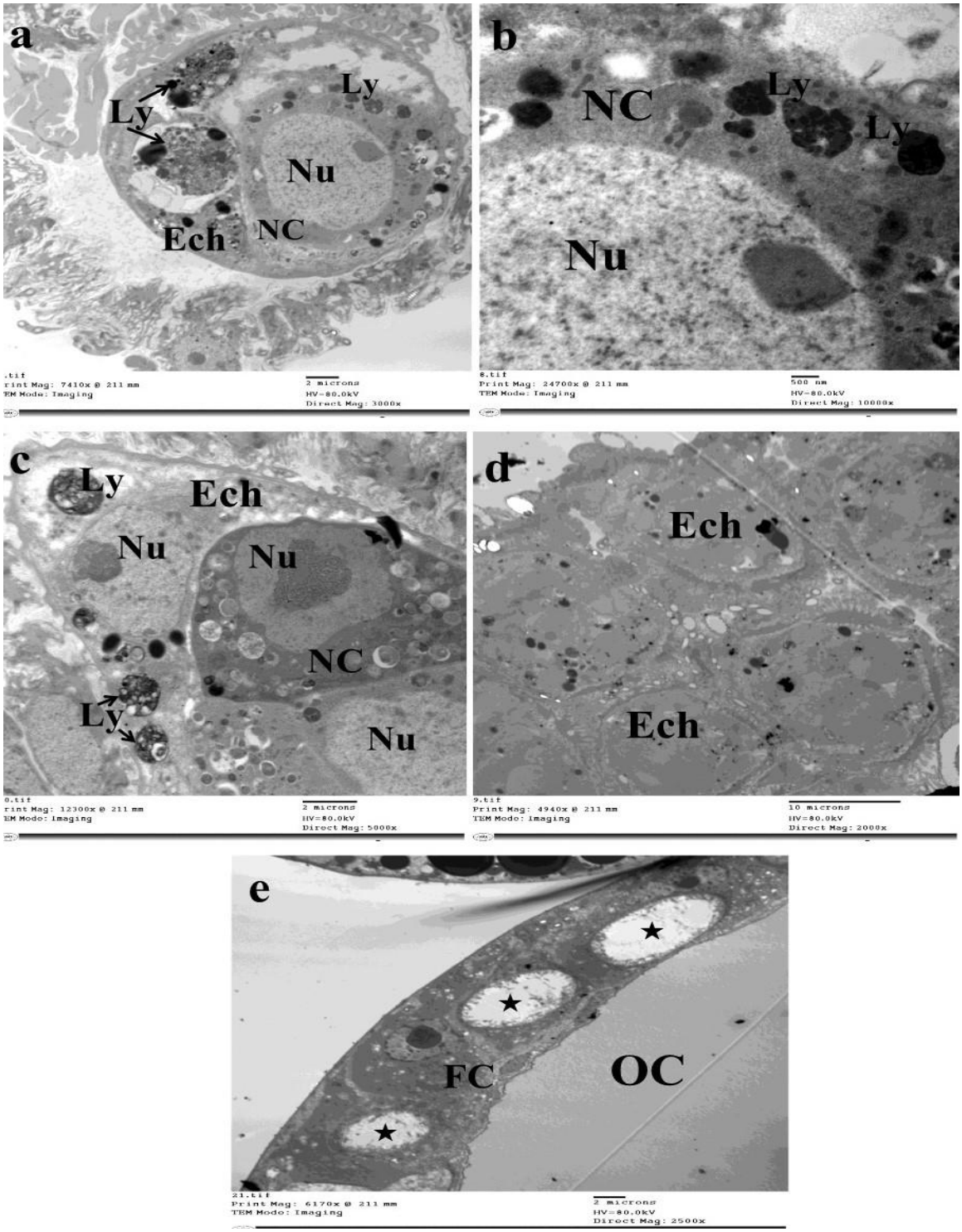


**Fig. 4(a-e) TEM micrograph of irradiated *Ceratitis capitata* ovary with dose 100 Gy. a) and c)** Degenerated egg chamber (Ech) showing abnormal shape of nurse cells (NC) and its nucleus (Nu), presence of large number of lysosomes (Ly). **b)** High magnification of degenerated nurse cell (NC) showing abnormal nurse cell nucleus (Nu) and presence of large amount of lysosomes (Ly). **d)** Showing abnormal shape and size of immature egg chambers (Ech). **e)** High magnification of oocyte (OC) showing follicular cell epithelium (FC) with empty nuclei (stars) and the oocyte devoid of yolk granules.

## DISCUSSION

The adult females of *Ceratitis capitata* cause severe damage to fruits. Shehata *et al.* (2011) reported that adult females of *Bactrocera zonata* insert their eggs by piercing fruits with their ovipositors causing severe damage to them. Insect fruit pests cause severe economic losses, therefore it is recommended to use safe methods to control these pests other than the use of insecticides.

The ovaries of *C. capitata* females belong to the polytrophic-meriostic type (Chapman, 1998; Heba Elelimy, 2022). Koch and King (1966) stated that there is a stem-line oogonium. Each stem line divides mitotically into two cells. One is an oogonium and the other is a cystoblast. The oogonium divides to form an oocyte, while the remaining cells become nurse cells (Gross, 1903). The stem cells of follicle cells give birth to follicle cells (Margolis, 1995). The egg chamber is formed by the follicle cells epithelium surrounding the cyst (Roth *et al.* 1995). Each egg chamber contains one oocyte and several nurse cells (King *et al*, 1968).

Pair of stem cell niches that are laterally located on either side of the germarium give rise to the somatic follicle cells of the egg chamber (Nystul and Spradling, 2007). Oocytes are surrounded by follicle epithelium, which determined the shape and size of oocytes and initiates the intercellular spaces (Raikhel *et al.,* 2005). The majority of follicular cells transform into columnar epithelium around the oocyte. The follicle cells cover the oocyte with the vitelline membrane and eggshell. When oocyte maturation is complete, nurse and follicle cells undergo apoptosis (Cooley *et al.,* 1992).

Cell migratory and morphogenetic events are required for the creation of a complex structure like the egg chamber; at various developmental stages, various cell populations inside the egg chamber contribute to the mechanisms required for egg chamber development. Border cells move far during this migratory process in the mid-to-late stages of oogenesis. Border cells are specified from a small population of terminal follicle cells at the egg chamber anterior, via secretion by adjacent polar cells of the unpaired ligand (Yoon *et al*., 2011). Then, this population of 6–8 cells separate from the nearby follicular cells, migrates between nurse cells in the direction of the posterior of the egg chamber, and inserts into the dorsal side of the oocyte to make the micropyle (He *et al.*, 2011).

The nucleus of the nurse cell contains chromatin material dissociated and randomly fills the nuclear volume, a sign indicating the completion of the endomitotic process (Shehata *et al.,* 2011). Shehata *et al.* (2011) observed that the nurse cell nucleus is spherical, located in the oöplasm and its size differs according to the developmental stage of the egg chamber. The X-ray irradiation doses at the beginning of the experiment ranged from 50 to 500 Gy helped to determine the suitable doses which will not affect the viability of irradiated fruit fly *C. capitata* compared with the normal wild one. The doses 50 and 100 Gy were selected because the insect failed from emerging from pupae exposed to higher doses. The advantage of irradiating the pupal stage is due to the easier transport and handling than fragile eggs, active larvae, and adults, according to research on *Aedes albopictus* (Oliva *et al.*, 2013), *Bactrocera dorsalis* (Chang *et al.*, 2015) and *Ephestia elutella* (Jun *et al.*, 2022).

The adult female ovarioles of *Ceratitis capitata* resulting from irradiated pupae with dose levels 50 Gy and 100 Gy of X-ray induced degeneration of ovarian sheath, and its' separation of it from the follicular epithelial cells. Also, lysis of follicular cell epithelium, the component of the nurse cell nucleus seemed to be unclear, vacuoles were dispersed in the cytoplasm, and the nurse cells were small in size with the reduction in its number. The oocyte showed lysis, the disappearance of its components and shrinkage of its nucleus. More deterioration of egg chamber components was observed at 100 Gy dose

level than at 50 Gy of X-ray. The results obtained are consistent with those previously reported by King (1957), who observed two general effects of radiation: i) an abnormal distribution of the developmental stages of oogenesis leading to a decrease in the rate of oogenesis generally, and ii) the inhibition of cell division particularly in oogonial cells. Similar findings are provided by Hasan (1995), who found that irradiation in *Tigriopus brevicornis* not only retarded ovarian growth but also caused malformations in the morphology of the developing follicles and nurse cells nuclei. Additionally, treatment with these doses was sufficient to totally stop oogenesis in adult *Tigriopus brevicornis*. Also, Mostafa *et al.* (2019) observed the same result in the ovary of *Musca domestica* females after exposure to gamma radiation. In the cytoplasm, the presence of lytic areas could be due to the activity of lysosomal hydrolase (Dalia and Lamia, 2019). The results of the present study showed that a large number of lysosomes appear inside the egg chamber and nurse cells of *Ceratitis capitata* irradiated with dose levels 50 and 100 Gy of X-ray. The deterioration of follicular cell epithelium is a noticeable effect of the ovarian cells in the polluted region. This could delay oocyte maturity and result in uneven yolk deposition (Osman and Shonouda, 2017). Our findings agreed with Osman and Shonouda (2017), who worked on the ovarian structure of *Blaps polycresta*.

Millions of sterile flies must be released into the wild population as part of the sterile insect technique (SIT) in order for there to be a high probability of sterile males mating with wild females (Gilmore, 1989).

It is clear from the result of the present work that the female ovary of *Ceratitis capitata* was strongly affected by 50 Gy and 100 Gy X-ray dose levels and this was clearly observed in the electron micrographs of the ovarioles. This may prove that the sterilization of adult female *Ceratitis capitata* induced by X-ray irradiation may be a safe method to control this pest.

## CONCLUSIONS

It can be concluded that the normal female ovary of *C. capitata* consists of polytrophic meroistic ovarioles. Each ovariole is separate and contains its own stem cells and egg chambers at different development stages. The egg chamber is surrounded by a somatic cell layer that contains nurse cells and oocytes. Seven days old pupae were exposed to 50 Gy and 100 Gy of X-ray doses and in comparison, to the normal female ovary, the emerged adult female's ovaries were examined in detail using light and transmission electron microscopy. The resulting effect of X-rays on the ovaries of *C. capitata* led to the following: (i) The abnormal shape and size of the egg chambers. (ii) Disappearance and lyses of egg chamber membrane. (iii) Decrease in the number and degeneration of nurse cells and their nuclei led to the disappearance of cytoplasmic junctions and separation between cells. (iv) The appearance of a large number of lysosomes inside egg chambers especially the nurse cells. (v) The appearance of myeloid bodies as a result of phagolysosomes and phagocytic activity inside the nurse cells. (vi) The mature oocytes are devoid of yolk granules and the follicular cells epithelium has nuclei empty from chromatin materials. In conclusion, X-ray radiation technology has a clear effect on *C. capitata* and is considered an effective and safe method. Future studies can compare the effects of ionizing radiation of X-ray and gamma irradiation for controlling medflies populations.

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## ARABIC SUMMARY

### التركيب الدقيق والتغيرات النسيجية لحشرة *سيراتيتس كابيتاتا* الناتجة عن المعاملة بالأشعة السينية

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*سيراتيتس كابيتاتا* تعتبر من اكثر الأفات تدميرا فى العالم. ذبابة فاكهة البحر الابيض المتوسط قادرة على غزو اكثر من 300 نوع من نباتات الفاكهة، والتكيف مع مجموعة متنوعة من المناطق المناخية، ولديها امكانية غزو عالية. يمكن ان تستهدف ذبابة فاكهة البحر الابيض المتوسط الفاكهة ذات القيمة الاقتصادية، مما يؤدى الى خسائر اقتصادية كبيرة تقدر بمليارات الدولارات سنويا. هدفت هذه الدراسة الى معرفة تأثير الاشعة السينية على المبيض الانثوى *لسيراتيتس كابيتاتا* (الناتج عن الخوادر المشععة) مقارنة بالمبيض الانثوى الطبيعى فى نفس العمر. تمت دراسة تركيب المبيضين الانثوى الطبيعى والمعالج فى *السيراتيتس كابيتاتا* بأستخدام المجهر الالكترونى والميكرسكوب الضوئى. يتكون الجهاز التناسلى الانثوى *للسيراتيتس كابيتاتا* من زوج من المبايض الجانبية. يتكون كل مبيض من مبيضات مرورية متعددة التغذية مكونة من خيوط طرفية وجيرماريم وفيتلاريم. تأثر المبيضين الانثوى بشكل كبير بالتشعيع بالاشعة السينية بسبب ظهور شكل وحجم غير طبيعى لغرف البويضة، وتدهور فى الخلايا الممرضة ونواتها، وظهور نواة الفوليكلر ابيثليم خالية من الكروماتين، ووجود الليزوزومات واجسام ميلويد، وتظهر البويضات خالية من حبيبات الصفار ونواتها. اظهرت النتائج هذة الدراسة ان تقنية الاشعة السينية لها تأثير واضح على *السيراتيتس كابيتاتا* وتعتبر طريقة فعالة وأمنة للقضاء على تجمعات ذباب فاكهة البحر الابيض المتوسط.