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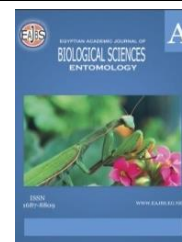
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Histopathological Changes in The Embryonic Development of *Bactrocera Zonata* (Saund, 1841) (Diptera: Tephritidae) Induced by Gamma Irradiation

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ABSTRACT

Five days old *B. zonata* pupae were subjected to gamma irradiation at doses of 25, 30, 35 and 50 Gray (Gy), from a Cesium cell-137. The resulting males were crossed with untreated females. The mean total number of eggs laid by the female (fecundity) is significantly decreased ($P < 0.05$) with the dose increase. The viability (hatching percent) of deposited eggs at all three doses showed a significant decrease ($P < 0.05$). Laid eggs by females mated with males resulted from pupae treated with gamma radiation with doses 25,30,35 and 50 Gy were processed for histological studies. First cleavage nuclei were observed about 2 hours pop and the blastoderm formation occur about 4 hours pop in control. At 8 hours pop, the blastoderm thickness forming the germ band. In about 24 hours pop brain appeared. The germ band Segmentation and formation of different organs started about 36hrs POP. Doses of 25 and 30 caused a failure of cleavage nuclei to migrate toward the periphery to form the blastoderm. While other embryos were blocked at germ band formation. In doses of 35 and 50 Gy, massive cellular and tissue damage to the embryos was reported.

INTRODUCTION

Bactrocera Zonata (Saund, 1841) (Diptera: Tephritidae) is a serious pest in Egypt that resulted in huge losses in the production of fruits in many parts of tropical and subtropical regions (El-Eryan *et al.*, 2018). The first official identification of the peach fruit fly in Egypt was in 1998 (El-Minshawy *et al.*, 1999). The annual losses in Egypt, as a result of this pest infestation, were about 190 million L.E. (Eppo 2005). Chemical insecticides are nowadays one of the main methods used for insect control. However, the serious health and ecological disadvantage of this method are obvious, there is a need for alternative control measures, which should be active against the pest, safe for humans, and eco-friendly (Radwan *et al.*, 2012). One of the promising ways is through the use of radiation. The sterile insect technique is a promising eco-friendly method for controlling or suppressing the population density of a number of fruit flies (Dyck *et al.*, 2005).

Embryonic development of insects became an attractive subject about 130 years ago, and several studies have been issued, which enhanced our knowledge of morphological and histological aspects of insect embryonic development (Uljanin, 1875, 1876; Anderson, 1973; Yu liu *et al.*, 2010).

This work is an attempt to investigate gamma irradiation effects on *B. zonata* embryogenesis when applied early during embryonic development using the histological technique.

MATERIALS AND METHODS

Insect Rearing:

B. zonata colony was obtained from Plant Protection, National Research Center, Dokki, Egypt. The insect was held in the laboratory at $25\pm 2^{\circ}\text{C}$ and $60\pm 5\%$ RH (Gabarty *et al.* 2020). Adult maintained in a wooden cage measuring 60x40x40cm (length: width: height) of which one side was covered by a removable muslin screened frame and the upper side was made of wire mesh. The rearing cages were provided with water and food for the flies which consisted of sugar and yeast hydrolyzed in a ratio of 3:1, respectively. As an oviposition site for the flies, the rearing cages were provided with perforated plastic mandarin models which were filled with water at the lower 1/3 non-perforated portion to prevent egg desiccation. The plastic mandarin models were investigated daily and any oviposited eggs were collected in water and scattered evenly on the surface of a larval artificial diet medium. This media was suggested by (Mahmoud, 2004) and composed of 100 g wheat bran, 25g yeast, 30g sucrose, 0.5 g sodium benzoate, 0.1 ml HCl (conc.70%) and 80 ml distilled water. The constituents of the diet media were mixed together until fully homogeneous, then spread into rectangular plastic trays measuring 15x15x20 cm (length: width: height). The collected eggs were distributed on the surface of larval media, approximately 1 ml eggs in water/1 kilogram of media. The trays were covered with a white muslin cloth and closed tightly with a rubber band to ensure the maintenance of suitable humidity. As a pupation site, after 5 days, the larval trays were placed upon larger trays containing a thin layer of sand to receive the fully developed larvae ready for pupation. Following pupation, the pupae were transferred to the adult rearing cages for emergence.

Irradiation Process:

Five days old *B. zonata* pupae, approximately 2 days pre-adult emergence, were subjected to gamma irradiation at doses of 25, 30, 35 and 50 Gray (Gy), from a Cesium cell-137 installed at National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt. The dose rate of the radiation was 0.663 rad sec.

Biological Studies:

The irradiated pupae were maintained up to adult emergence. Upon adult emergence, subsequently

1-Irradiated females were excluded from all cages before mating.

2-fifty irradiated males and fifty unirradiated females were caged together in a rearing cage measuring 30 x 15 x15 cm.

3-Unirradiated males x unirradiated females (control).

As previously mentioned, each cage was provided with a punctured plastic mandarin as an oviposition site. Male fertility and egg viability were determined by counting the number of deposited eggs, subsequently, eggs were arranged in rows on a black moistened piece of cloth resting on moistened cotton in a petri- dish, up to egg hatching. Hatched eggs were counted, and percentage hatchability was estimated.

The percentage of hatched eggs was calculated by using the following equation:

$$\text{Egg-hatchability \%} = A / B \times 100$$

Where:(A) represent the Total No. of hatched eggs, and (B) is the Total No. of eggs laid. The sterility percentage as a result of irradiation was calculated (Tappozada *et al.*, 1966):

$$\text{Percentage Sterility} = 100 - ((a \times b / A \times B) \times 100)$$

Where,

(a) represent No. of eggs/female in treatment.

(b) = percent hatchability in treatment.

(A) = No. of eggs/females in control.

(B) = percent hatchability in control.

Four replicates were performed for each cross.

Histological Study:

For the histological study, newly laid eggs (immediately after oviposition) for control and affected eggs resulted from untreated females crossed with males resulting from irradiation of five days old *B. zonata* pupae were placed on a moistened piece of cotton in a Petri dish and were kept at 27 ± 2 °C and 70-75% R.H. Five eggs were taken at different intervals of time (0, 4, 8, 18, 24, 36, 72) hours. Eggs were prepared for microscopic examination as follows:

Eggs were dechorionated in 6 % sodium hypochlorite for 4 min. then washed three times for twenty mins with distilled water. (West *et al.*, 1968). Placed in bouin's solution for 24-48 hours depending on the stage of development and hydrated in consecutive baths 30, 50, 70, 85, 95, and 100% ethanol. Then clear in Xylene, embedded in paraffin and sectioning 5-6 µm. Thick sections using a modified method of Harris, hematoxylin stain. (Kalifa, 1957).

Statistical Analysis:

The obtained data were manipulated statistically by one-way ANOVA using (SPSS/PC) computer program calculated at a 5% level.

RESULTS

1-Effect of Gamma Irradiation on Fecundity and Eggs Viability:

The result in (Table 1) shows that the total mean number of eggs per female (fecundity) significantly decreased ($P < 0.05$) as the dose of gamma irradiation increased. The effect was most prominent at 50 Gy where the mean number of eggs laid was 3.25 ± 1.9 compared with 279.5 ± 2.1 in the control group.

The result in (Table 1) shows that gamma radiation caused a significant decrease ($P < 0.05$) in the viability (hatching percent) of deposited eggs at all three doses used. Where the number of hatched eggs reached 93.2 ± 1.3 , 31.7 ± 1.18 , 2.2 ± 0.75 , and 0.00 ± 0.00 for 25, 30, 35, and 50 Gy respectively compared with 272.7 ± 3.17 for control.

Table 1: Effect of gamma radiation on fecundity and eggs viability.

Dose (Gy)	Mean no. of egg/female ± S.E.	No. of hatched eggs		No. of un-hatched eggs		Sterility Index (S. I.)
		Mean ± S.E.	%	Mean ± S.E.	%	
C	$279.5 \pm 2.1^*$	$272.7 \pm 3.17^*$	97.5	$6.75 \pm 1.18^*$	2.4	-
25	$121 \pm 0.91^*$	$93.2 \pm 1.3^*$	77	$27.7 \pm 0.62^*$	22	65
30	$94.7 \pm 1.7^*$	$31.7 \pm 1.18^*$	33.4	$63 \pm 1.7^*$	66	88
35	$21 \pm 0.7^*$	$2.2 \pm 0.75^*$	10.4	$18.7 \pm 1.37^*$	89	99
50	$3.25 \pm 1.9^*$	$0.00 \pm 0.00^*$	0	$3.25 \pm 1.9^*$	100	100

2-Histological Studies:

The egg of *B. zonata* has the typical dipteran appearance. Elongated, elliptical, whitish and 1.0 to 1.2 mm (0.04 to 0.05 in.) long, rounded at the posterior end, slightly pointed anteriorly. The egg tapers anteriorly from its blunt posterior end. A strong chorion enwrapped the oocyte from the outside followed by an inner vitelline membrane. The oocyte is characterized by having an abundance of yolk at the ooplasm. The cytoplasm forms a peripheral layer; the periplasm and an inner cytoplasmic reticulum within the yolk granules are held (Fig.1).

Cleavage and blastoderm formation

Cleavage started 2 hours postoviposition (POP). After 4 hours (POP) cleavage nuclei increased in number. The cleavage nuclei migrate away from the center to become distributed in the yolk. Some the cleavage nuclei migrate back into the yolk as secondary vitellophages. The nuclei of the primary vitellophages aren't enclosed by the cell membrane. Nucleus migration continued and each nucleus was surrounded by a halo of cytoplasm until the blastoderm formed. Blastoderm is formed from one layer of a cell arranged in a complete ring enclosed by the yolk and surrounded by a cytoplasmic membrane (Fig. 2). The parts of the blastoderm in the midventral thicken to form a germ band which will be developed into the future embryo (Fig.3).

Gastrulation:

Gastrulation is the process by which the mesoderm is invaginated within the ectoderm. In 8 hours, pop, the blastoderm thickens forming the germ band. The cell of blastoderm that doesn't take part in the germ band formation become flattened to form the serosa and amnion (the embryonic envelopes), which begin to appear with differentiation of the inner layer. The germ band at this stage consists of multilayer strips of cells that are differentiated into two layers ectoderm and mesoderm (Fig.3).

Segmentation and Organogenesis:

In 18 hour (POP) inner layer give rise to the head, thorax and abdomen (Fig.4). The foregut develops as stomodeal invagination near the cephalic end of the embryo (Fig. 3). The hindgut develops as proctodeal invagination in a manner like the stomodeal invagination but at the caudal end of the body (Fig. 3). About 24 hours pop brain appears as two large interconnected cerebral lobes enclosed by a neurilemma sheath (Fig.5).

By 36 hours (POP) the crop posterior part of the esophagus dilates to form a thick hard conical structure, the proventriculus (Fig. 6). The proventriculus formed from the posterior end of the esophagus projecting into the anterior region of the midgut. By this time the midgut now is a closed tube completely surrounded by the yolk mass (Fig. 6). By 36 hours trachea appears as an ectodermal invagination. By this time the heart is formed from cardio blasts. About 72 hours (POP) showing fully formed embryo and all organs were formed. (Fig.7)

Histopathological studies:

Eggs from females mated with males resulting from pupae treated with gamma radiation with doses 25,30,35 and 50 Gy were processed for histopathological studies. Doses of 25 and 30 caused failure of cleavage nuclei to migrate toward the periphery to form the blastoderm (Fig 2B, C). While other embryos were blocked at germ band formation (Fig 3C, D). Also, there was vacuolation of cytoplasm, and lysis of yolk (Fig 2B, 3D). The brain becomes compressed and didn't differentiate into know basic structures (Fig 5B, C). Dose of 35 and 50 Gy showed signs of deterioration. The eggs had abnormal shapes, lysis of yolk granules and disintegration of egg content (Fig 3E, F). The brain loses its morphic nature and appeared compressed and lysed. (Fig 5D, E). Lysed of midgut yolk, the crop appeared atrophied in the size (Fig 6E, F). In some embryos, it was difficult to distinguish the different structures of the hindgut. (Fig 7E, D).

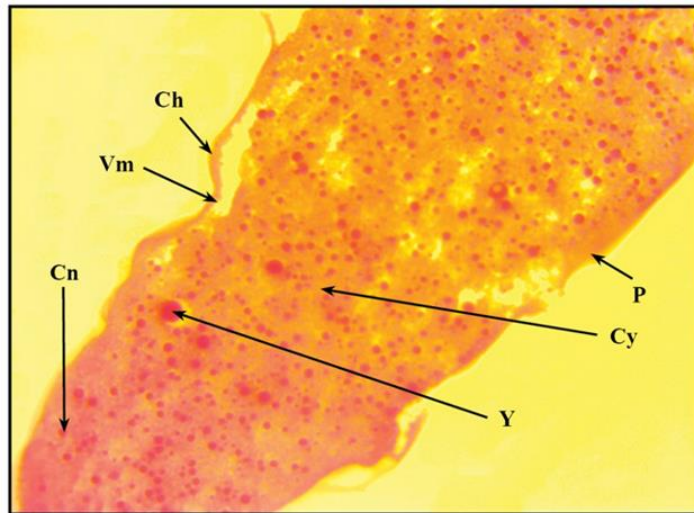


Fig.1: Longitudinal section of one hour-old egg of *Bactrocera zonata* showing Chorion (Ch), Cleavage nuclei (Cn), Vitelline membrane (Vm), Yolk (Y), Cytoplasm (CY), Periplasm (P).

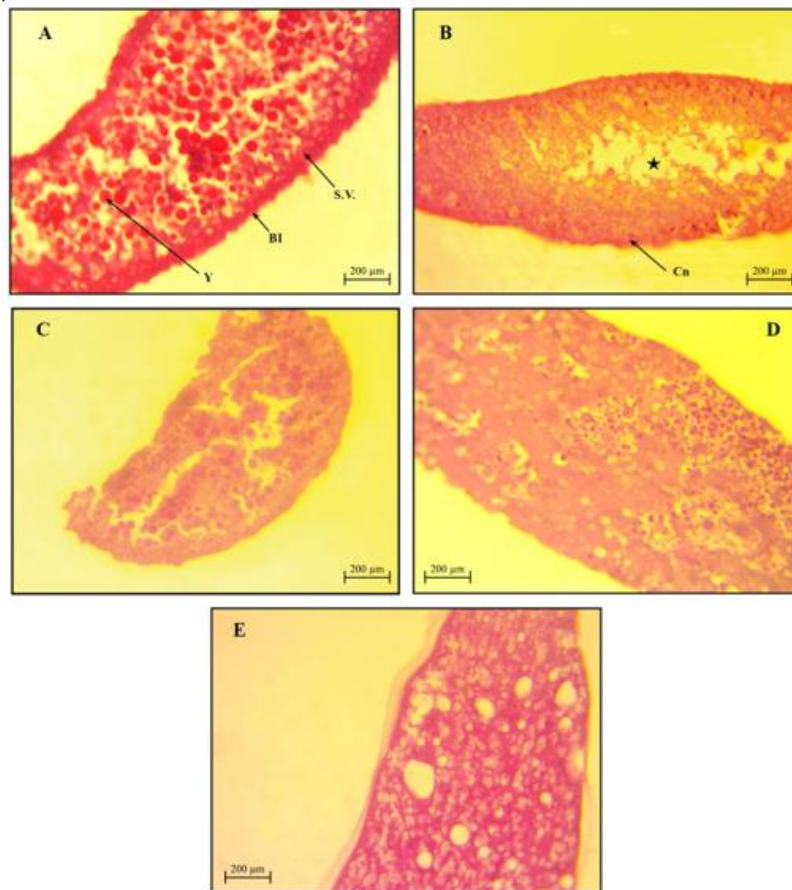


Fig.2: Longitudinal section of 4-hour-old egg of *Bactrocera zonata*, (A) control showing Secondary vitellophages (S.V.), Blastoderm (Bl), Yolk (Y), (B) 25 Gy showing, vacuolation of cytoplasm represented by a star, irregular distribution of cleavage nuclei (Cn), (C) 30 Gy, (D) 35 Gy and (E) 50 Gy showing vacuolation of cytoplasm and absence of cleavage nuclei.

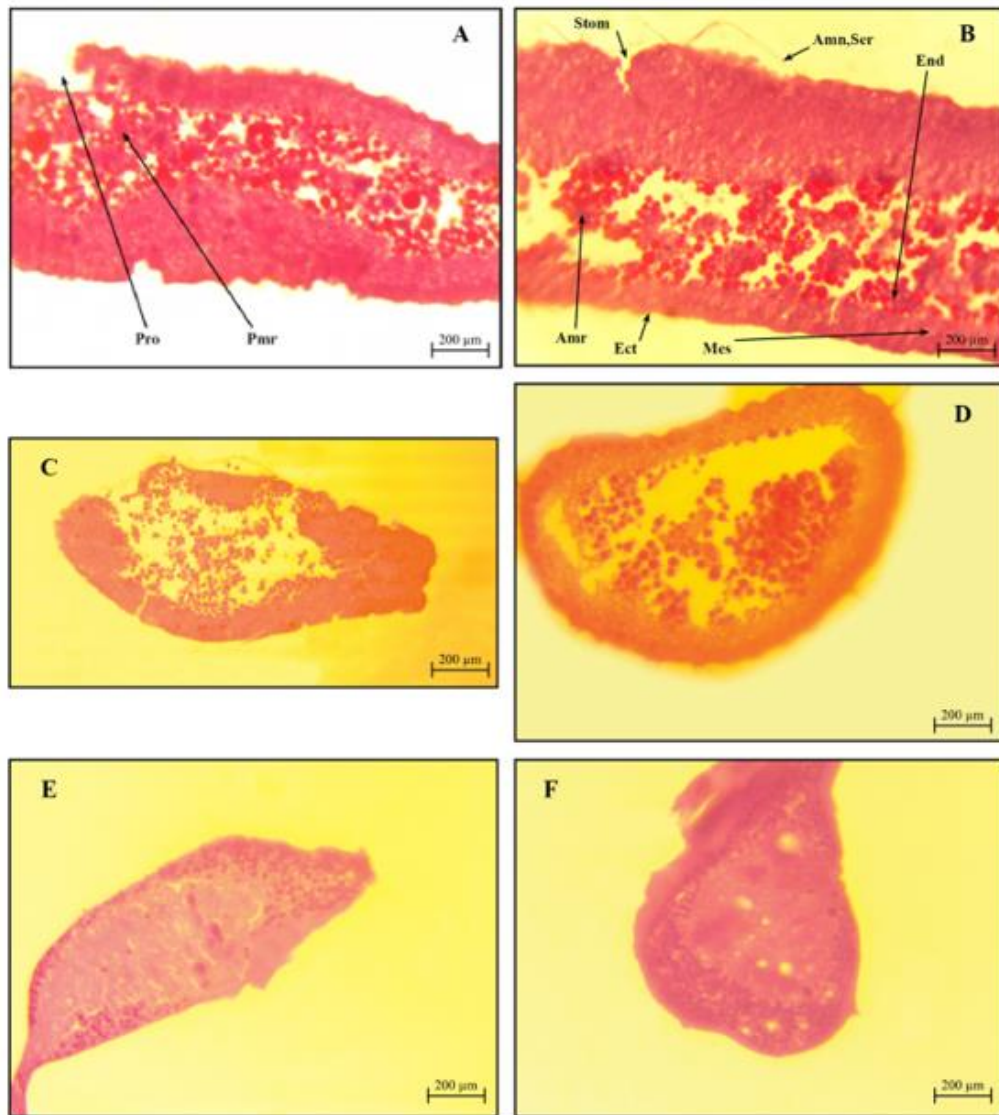


Fig.3: Longitudinal section of an 8-hour-old egg of *Bactrocera zonata*, (A, B) control showing, Anterior midgut rudiment (Amr), Stomodaeum (Stom), Ectoderm (Ect), Amnion serosa (Amn, Ser), Mesoderm (Mes), Endoderm (End), proctodeum (pro) posterior midgut rudiment (pmr). (C) 25 Gy, (D) 30 Gy showed cleavage nuclei at the egg centre (E) 35 Gy and (F) 50 Gy complete lysis of egg content.

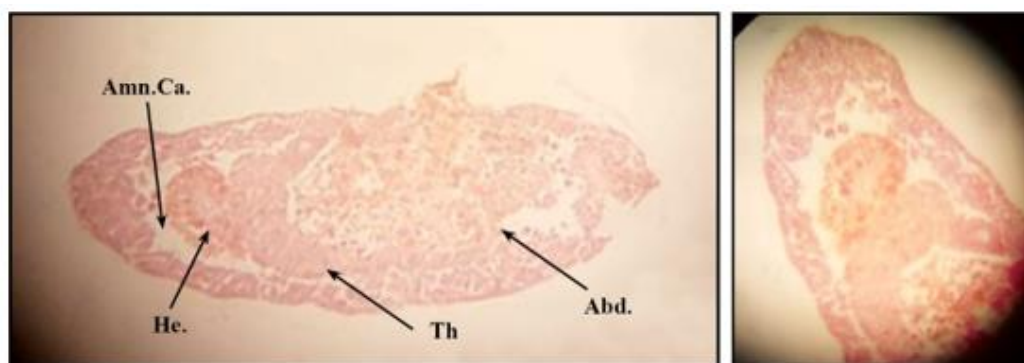


Fig. 4: Longitudinal section of an 18-hour-old egg of *Bactrocera zonata* showing Head (He), Thorax (Th), Abdomen (Abd), and Amniotic cavity (Amn. Ca.).

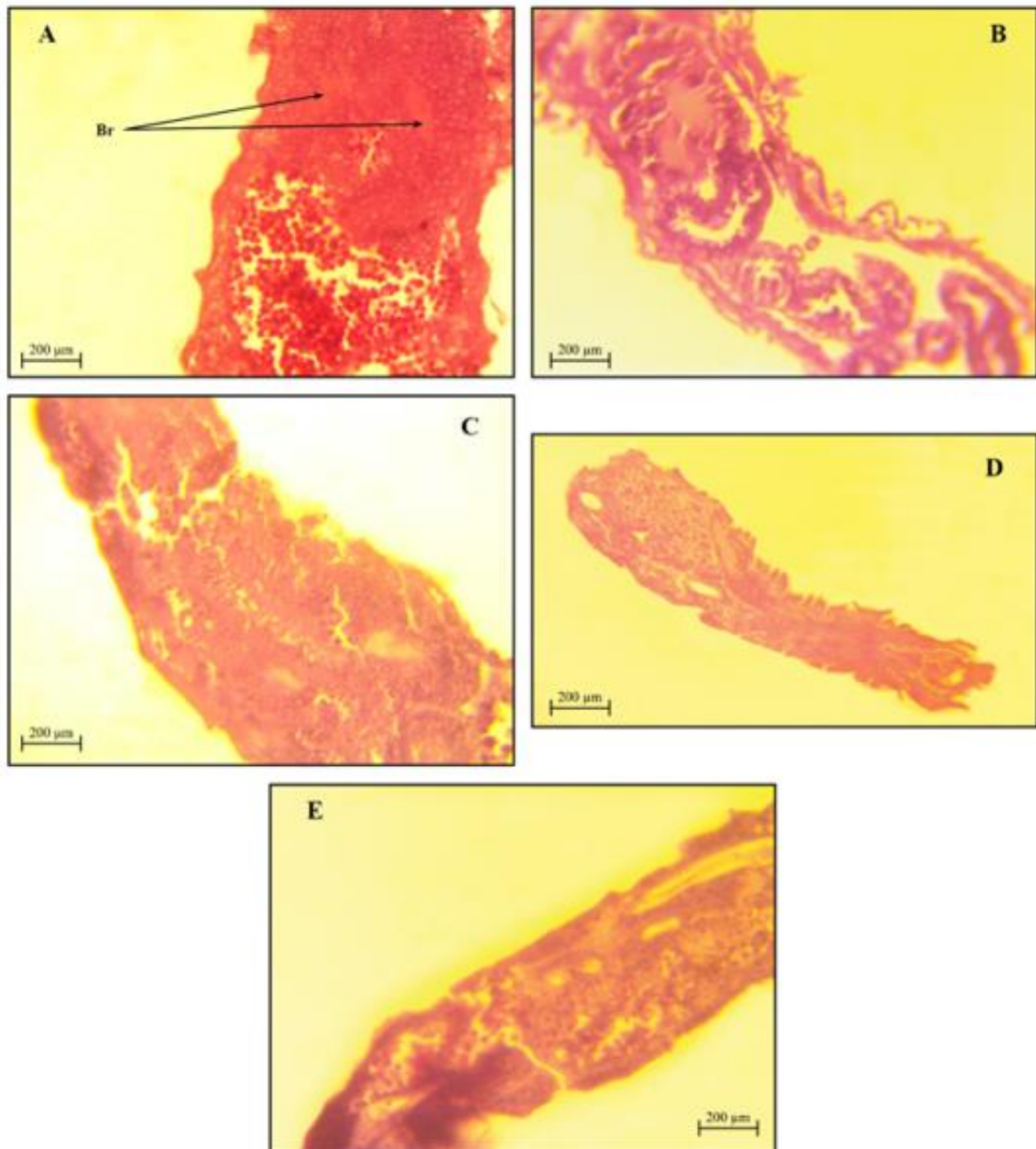


Fig.5: Longitudinal section of a 24-hour-old egg of *Bactrocera zonata*, (A) Control showing, Brain (Br), (B) 25 Gy, (C) 30 Gy, (D) 35 Gy and (E) 50 Gy showing an abnormal outline of the egg and lysis of the brain.

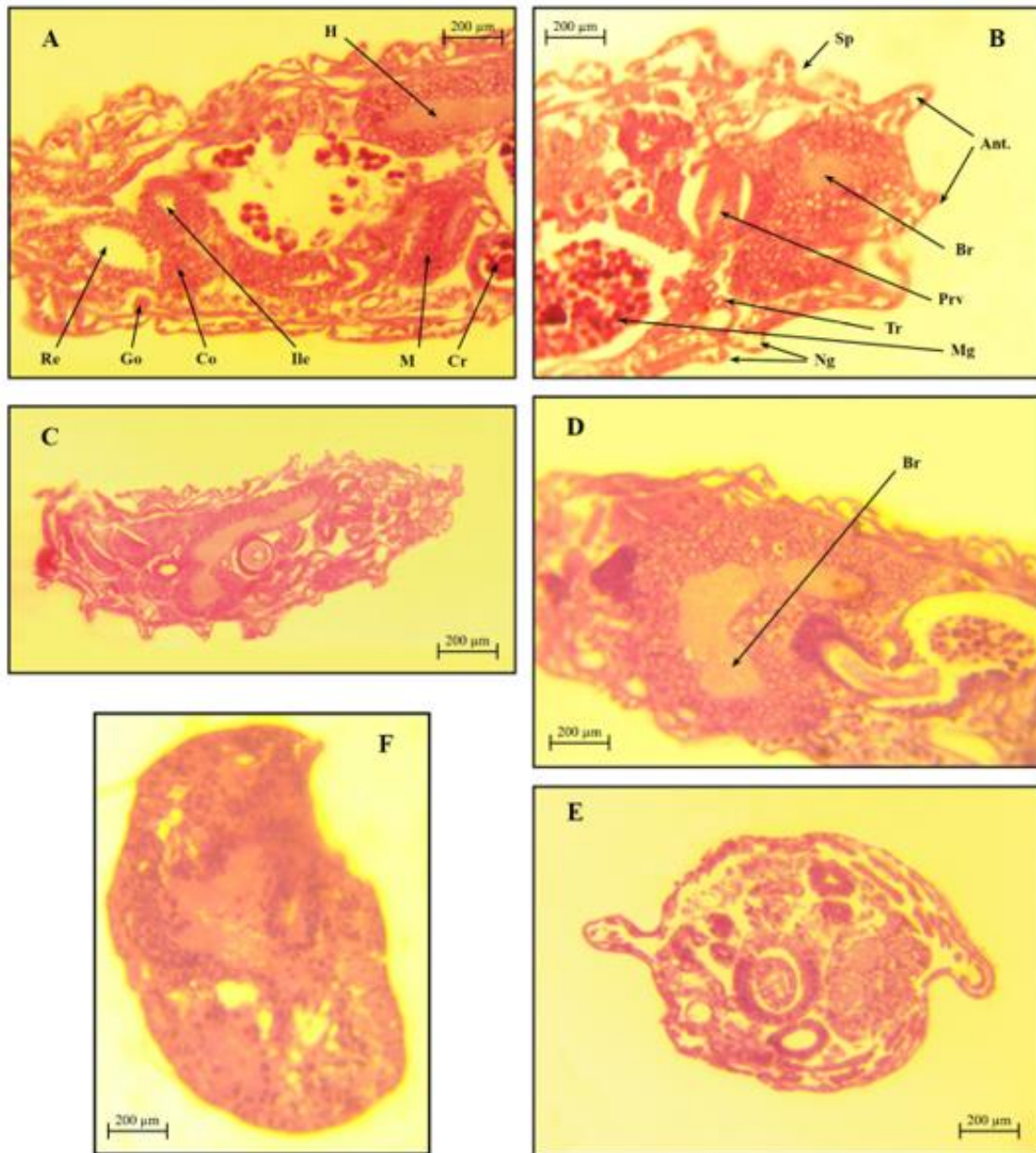


Fig.6: Photograph of longitudinal section of a 36-hour-old egg of *Bactrocera zonata*, (A, B) control showing, Heart (H), Rectum (Re), Colon (Co), Gonad (Go), Ileum (Ile), Muscle (M.), (C) 25 Gy, (D) 30 Gy Showing the asymmetrical shape of the brain (E) 35 Gy abnormal structure of the formed organs and (F) 50 Gy disintegration of the egg content.

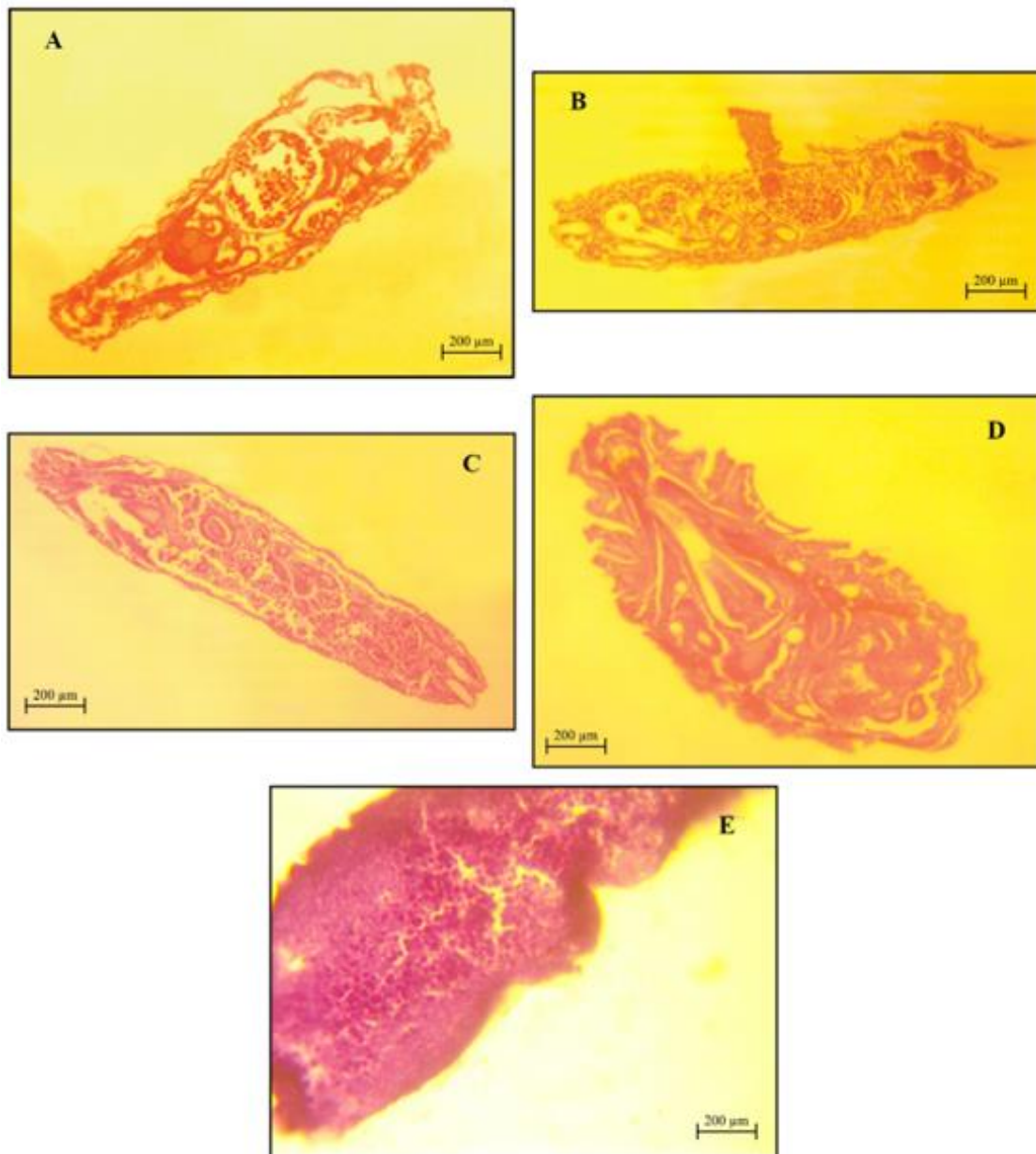


Fig.7: Photograph of longitudinal section of a 72-hour-old egg of *Bactrocera zonata*, (A) control showing Fully formed embryo. (B) 25 Gy, (C) 30 Gy, (D)35 Gy and (E) 50 Gy showing complete destruction of the egg contents.

DISCUSSION

1-Biological Study:

The study showed that the number of laid eggs by females crossed with males resulted from pupae irradiated with different doses of gamma radiation decreased as the dose increased. The highest reduction in the number of eggs laid was at high doses of (50 Gy). These results are also true with (Zahran *et al.*,2013), where they observed that no eggs were deposited when *Bactrocera zonata* irradiated with 50, 70, or 90 Gy received from a Cesium cell-137. Also, (Draz *et al.* 2016) demonstrated that a dose of 70 Gy for males and 50 Gy for females or both sexes were sufficient to decrease the daily egg-laying of mated females when *Bactrocera zonata* were irradiated with cobalt-60. While (Khan and Islam,2006) reported that gamma irradiation of (0-10 Gy) increased oviposition in *Musca*

domestica. Also, (Mansour, 1987), observed that irradiation of pupae of *Musca autumnalis* using gamma radiation has no effect on female fecundity. Moreover, (Sayed *et al.*, 2020) observed a decrease in percent hatchability as the dose of gamma radiation increased and it was 71.33 at the dose level of 50 Gy and reached 0 at 90 Gy. (Muhammad *et al.*, 2015) reported that radiation caused a prolonged action of pupal stage duration and hatching is reduced by applying radiation on *Bactrocera zonata*.

The present study has shown that the sterility index of *B. zonata* males resulting from irradiated pupae was increased as the dosage of gamma radiation increased. Complete sterility (100%) occurred at 50 Gy when un-irradiated females crossed with irradiated males. Where (Zahran and Hegazy *et al.*, 2013) found that doses ranging from 8-10 K-rad (=100 Gy) induced high levels of sterility. Puanmanee (2010) also reported an increase in the sterility of *Bactrocera correcta* when treated males crossed with untreated females as the gamma dosage increased. El-Akhdar and Afia (2009) reported that gamma irradiation of (90 Gy) on the peach fruit fly *Bactrocera zonata* (Saunders), leads to the failure of males to succeed in the courtship with females. Ogaugwu *et al.* (2012) reported that a gamma radiation dose of 75 Gy resulted in complete male sterility in *B. invadens*, while doses of 25 and 50 Gy induced partial sterility in the males. Tuncbilek *et al.* (2012) showed that gamma radiation was more pronounced than UV radiation. An increase in gamma radiation dose and in time of exposure to UV rays caused a gradual decrease in the number of hatching eggs.

2-Histological and Histopathological Studies:

Longitudinal sections of eggs were examined as early as (0-1h) “newly laid eggs” to describe the structure and cleavage of zygote. In the present study, no mitotic and meiotic stages can be observed, even as early as 0 h post oviposition. This agrees with the finding of (Tawfik, 1975), where no meiotic figures were observed in *A. panteles glomeratus* and in *Musca domestica* (Radwan *et al.*, 1993).

The first cleavage nuclei were observed for about 2 hours POP (about 28% development). While in *Musca domestica* (Radwan *et al.* 2012) the onset of cleavage was 15 min POP. Similar observations have been reported in *Stomoxys calcitrans* (Aji dagba *et al.* 1983).

About 4 hours POP, cleavage nuclei have increased in number and migrated to the periphery of the egg but some migrate back into the yolk as secondary vitellophages, Primary vitellophage observed in the center and delimiting cell furrows are clear. Formation of secondary vitellophages is also described in *Stomoxys calcitrans* (Aji dagba *et al.* 1983), *phlebotomus papatsi* (Abbassy *et al.*, 1995 a), and *Musca domestica* (Radwan *et al.*, 2012). While in *Lucilia sericata* cells from the anterior and posterior midgut enter the rudiment and enter the yolk as tertiary vitellophages were observed during embryonic development (Davis *et al.*, 1966).

The blastoderm formation in *B. zonata* occurs about 4 hours POP. In the present study, at 8 hours POP, the blastoderm thickness along the mid-ventral line formed a germ band, and the cells on the dorsal and lateral sides of the blastoderm become flattened to form serosa. This is the case in *Musca domestica* (Radwan *et al.*, 2012), *phlebotomus papatsi* (Abbassy *et al.*, 1995 a).

In *Mandusa sexta*, serosa is formed 12 h POP (Lamer and Dorn, 2001) The germ band consists of multilayer strips of cells that are differentiated into two layers ectoderm and mesoderm.

In the present study, germ band differentiation and segmentation are observed at 18 hours POP. Segmentation of the inner layer forming thorax, and head segments were observed 3-day POP in *Shistocerca gregaria* (Mahdy *et al.*, 2019). In the present study, the stomodaeum didn't invaginate before presumptive anterior midgut rudiment cells but they

nearly invagination at the same time. This is also true in *Culex aegypti* (Davis, 1966), and *Aedes aegypti* (Raminani and Cupp, 1978).

In the present study about 24 hours, the brain appeared as two ganglion masses. By 36 hours POP trachea appears as an ectodermal invagination. Brain formation started 108 hours in the posterior region of blastocephalon in *Phlebotomus papatasi* (Abbassy *et al.*, 1995 a). The brain was completely developed in 75 hours in *Aedes aegypti* (Raminani and Cupp, 1978). In the present study doses of 25 and 30 caused failure of cleavage nuclei to migrate toward the periphery to form the blastoderm. While other embryos were blocked at germ band formation. Also, there was vacuolation of cytoplasm, and lysis of yolk. Dose of 35 and 50 Gy showed signs of deterioration. The eggs had abnormal shapes, lysis of yolk granules and disintegration of egg content. The brain loses its morphic nature and appeared compressed and lysed. Lysed of midgut yolk, the crop appeared atrophied in the size. In some embryos, it was difficult to distinguish the different structures of the hindgut.

Lecis (1969) exposed 1, 2 and 3-day embryonated eggs of *Anopheles maculipennis* to 500, 1000, 2000 and 4000 rad of γ -rays. Doses of 500 and 1000 rad didn't cause apparent disturbances of development, but 2000 and 4000 rad caused mortality in 1-day eggs in both embryonic and larval stages. None of the doses had any apparent effects on the 2 and 3-day eggs.

Irradiation of biological material resulted in the breakage of molecular bonds, creation of ions, and the formation of free radicals. These free radicals attack more molecular bonds and causes damage in DNA that led to the formation of fatal mutation in the germ cells (Curtis 1971, Lachance 1967). Damage to somatic cells also occurs, especially in cells undergoing mitosis. This can explain the histopathological effect of gamma radiation on the embryonic development of *B. zonata* during the present study.

Ethical Approval: This research paper was approved by the research ethics committee from Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2022/11/1).

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ARABIC SUMMARY

التغيرات النسيجية للتطور الجنيني لحشرة باكتروسييرا زوناتا الناتجة عن المعاملة بأشعة جاما

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تم تعريض العذارى لحشرة باكتروسييرا زوناتا البالغة من العمر خمسة أيام لإشعاع جاما بجرعات 25 و30 و35 و50 جراي من سيزيوم 137. تم عمل تزاوجات بين الذكور المشعة والاناث الطبيعية بدون تشعيع. انخفض متوسط إجمالي عدد البيض الذي تضعه الأنثى (الخصوبة) بشكل ملحوظ مع زيادة جرعة إشعاع جاما. كما تم ملاحظة انخفاض كبير في نسبة فقس البيض تدريجياً بزيادة الجرعات. تمت دراسة التطور الجنيني لحشرة باكتروسييرا زوناتا خلال المراحل المختلفة من عمر الجنين. وقد تبين ان مرحلة الانقسام تبدأ بعد ساعتين من وضع البيض حتى مرحلة تكوين طبقة البلاستودم عند 4 ساعات من وضع البيض. وتتكون الطبقة الجرثومية عند عمر 8 ساعات حيث تتركب من عدد من الطبقات الخلوية التي تتميز الى طبقة الاكتودرم والميزودرم. ويمكن ملاحظة المخ عند 24 ساعة. بعد 36 ساعة تبدأ عملية تكوين القلب والتعضى حتى اكتمال النمو عند 72 ساعة. تم عمل تزاوجات بين الاناث الطبيعية والذكور المعالجة بالجرعات المذكورة سابقاً وعمل دراسات لانسجة البيض الناتج من التزاوج. في جرعات 25 و30 تسبب في فشل في عملية التفالج وفي هجرة الخلايا المتكونة ناحية الاطراف. بينما في بعض الاجنة توقفت عملية التطور الجنيني عند تكون الطبقة الجرثومية. في جرعات 35 و50 Gy لوحظ تلف كبير في الخلايا والأنسجة للأجنة.