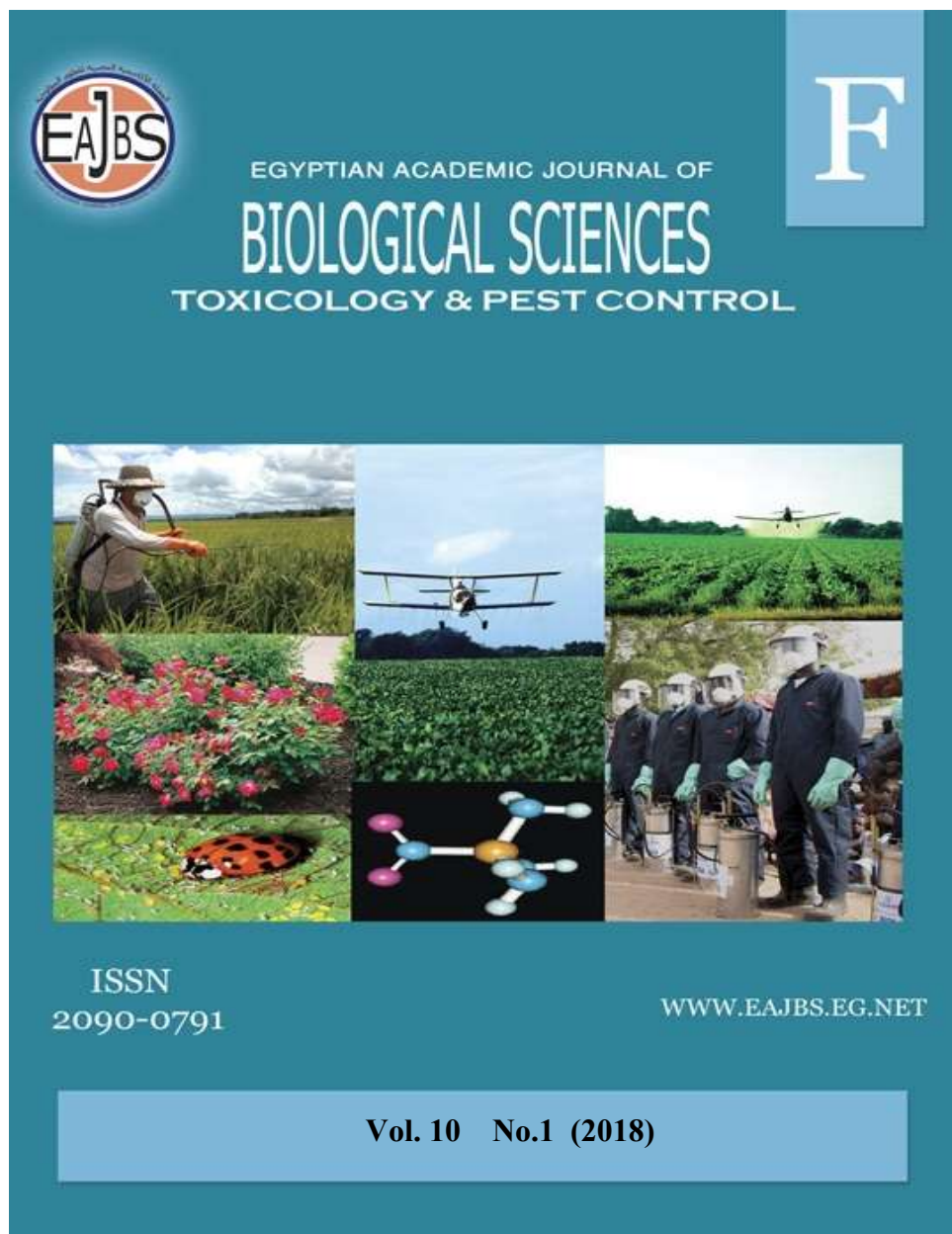


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Evaluation of Using Silymarin as A Radio-Protective Agent of the Peach Fruit Fly, *Bactrocera zonata* Irradiated with Gamma Radiation

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ABSTRACT

The performance of sterile males is one of the important topics for the successful use of the sterile insect technique. Silymarin (Sm) as antioxidant could play an effective role in ameliorating the physiological damage of *B. zonata* cells that may be induced during rearing the colony and/or irradiation treatment. Different Sm concentrations (0, 10, 30, 50, 80 and 100 mg/kg) were added to the larval diet to study its impact on the reproductive biology and fitness of *B. zonata*. Also, the radio-protective activity of Sm was firstly tested through studying the quality control parameters of the adult flies irradiated with the sterilizing dose levels (0, 70, 90, 100 and 110 Gy) that previously fed on Sm in larval stage. Secondary, the protective effect of Sm were evaluated through ultrastructure studies of the male testes. The results indicated that additives of 80 and 100 mg/kg Sm concentrations increased the pupal and adult recovery, they were significantly increased to (77.2 and 76.2%) and (69.7 and 68.2%), respectively as compared to (72.2%) and (63.3%) in the control treatment, respectively. The results also revealed that in 80 mg/kg Sm concentration, the percentages of hatched eggs were increased either alone or in combined with the different sterilizing doses. Moreover, the adult flight ability and emergence percentages were increased in the all combination treatments. The ultrastructure study showed an improvement of the testis irradiated with 90 Gy when combined with 80 mg/kg as compared to the radiation treatment alone.

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), distributed in many regions over the world that attacks over 50 cultivated and wild plants (Duyck *et al.*, 2004). In Egypt, it is recorded for the first time in 1998 (El-Minshawy *et al.*, 1999). Recently, it becomes a serious pest that caused a great loss of fruit productions (Khan *et al.*, 2005). Sterile insect technique (SIT) is considered one of the successful control methods against Tephritidae species in many regions. The insect produces in large number, exposed to sterilizing doses of gamma ray and

released in the field in order to suppress the population (Knipling 1955, Dyck *et al.* 2005 and Bakri *et al.*, 2005). The continuous reared insect for several generations may cause production fluctuates in quantity and quality of the colony. On the other hand, the sterilizing doses of gamma ray could provoke damage to a somatic cells resulting in negative impact on the performance of sterile males such as the emergence, flight ability and longevity (Barry *et al.*, 2003a, Robinson 2005 and Lance and McInnis, 2005). Furthermore, most of the cell damage of irradiated insects are caused in particular the reproductive organs that are radiosensitive because of their active division (Tilton, E.W. and Brower 1983, Banu and Salam 2009). Antioxidant additives could protect the insect cell by their role in the body defense system against reactive oxygen species (ROS) which is produced by irradiation treatments. Moreover, antioxidant components lead to save the insect life span by decreasing the metabolic rate and strengthen the mechanisms of antioxidative defenses (Zaghloul *et al.*, 2011 and Mortimer *et al.*, 2012)

In this respect, a number of studies had been attempted to ameliorate the biological and physiological damage induced by irradiation using antioxidant additives to diets (El-kholy, 2008; Shoman & Mahmoud, 2010; and Mikhael, *et al.*, 2011). However, the impact of certain antioxidant additives on the biological and physiological activates of peach fruit fly *B. zonata* targeted with SIT are required. Silymarin is natural antioxidant extracted from the *Silybum marianum* plant containing various flavonolignans and it is considered one of the important protective ingredients due to its strong antioxidant and tissue regenerative properties (Dehmlow *et al.*, 1996 and Zholobenko and Modriansky, 2014). The mode of action of Silymarin is considered to be responsible for its protective actions by several mechanisms; the first is the direct scavenging free radicals and chelating free Fe and Cu (Milic *et al.*, 2013). the second is preventing free radical formation by inhibiting specific ROS-producing enzymes (Varga *et al.*, 2006), the third is improving an integrity of mitochondria in stress conditions (Surai, 2002), the fourth, is maintaining an optimal redox balance in the cell by activating a range of antioxidant enzymes and non-enzymatic antioxidants (Vargas-Mendoza *et al.*, 2014), the fifth is Activating vitagenes, responsible for synthesis of protective molecules, including heat shock proteins (HSPs) (Cristofalo *et al.*, 2013).

The present investigation has been suggested to evaluate the role of Silymarin as antioxidant additives for improving the *B. zonata* colony, furthermore, studying its role as radio-protective agent for improving the performance of sterile male flies .

MATERIALS AND METHODS

Insect Rearing:

Pupae of Peach fruit fly *B. zonata* were collected from samples of infested fruits collected from Giza governorate. Adults were maintained in laboratory conditions, at 22 ± 2 C, 60–70% Rh. and 12: 12 h. photoperiod. The artificial diet 3: 1 by weight from sugar: yeast hydrolyzed, respectively was used for adult feeding. While, the larvae were reared on the brewer's yeast-based diet (Tanaka *et al.*, 1969). The adult flies were transferred to the adult rearing cages (60 x 60 x 60 cm) with wooden frames, supplied with mesh screen from all sides. The eggs were collected by yellow plastic vials with the whole in their sides to allow the adult females laid their eggs inside this vial which provided with water in its base.

Bioassay Studies:

One day Eggs were collected, counted and transferred to a plastic tray (12.5 x 7 x 3cm) containing 120 gm of larval artificial diet provided with the Silymarin concentration levels 10, 30, 50, 80 and 100 mg/kg alongside the control treatment (0.0) without any additives. The Silymarin was obtained from Sigma Aldrich Company, Saint Louis, USA. Each tray was placed in another larger plastic tray (20 x 20 x 10cm) contained a thin layer of clean sand and covered with muslin cloth. The sand was sieved for the collection of formed pupae. Each treatment was conducted with 5 replicates. The Pupae were daily counted and placed inside plastic Petri dishes (10, 1.5cm) until adult emergence. The emerged adults were placed inside the adult experimental cage (20 x 20 x 20). The sterility percentages were calculated according to Toppozada et al., (1966) while, the bioassay parameters (the egg laid by female per day, egg hatch, emergence, pupal recovery, adult recovery, longevity and adult flight ability) were carried out following the International Quality Control Manual (FAO/ IAEA/ USDA 2003).

Irradiation Technique:

Full grown pupae of *B. zonata* 24 hours before emergence were irradiated with different doses (0 , 70, 90 and 100 Gy) using Cobalt-60 gamma cell. The emerged male and female flies were separated using anesthetization by diethyl ether before transferred to the adult experimental cages. The male flies that emerged from irradiated full grown larvae were mated with un-irradiated female flies. Three replicates with one hundred pairs per replicate were used for each dose level. The bioassay parameters were conducted as we mentioned before according (FAO/ IAEA/ USDA 2003). The source of gamma radiation is located at the cyclotron project, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt. The dose rate of the source was 7.0 Gy/min.

Electron Microscopic Analysis:

The experimental testes of *B. zonata* were dissected and fixed in 3% phosphate buffered gluteraldehyde pH 7.4 at 4°C for two hours. The specimens were dissected and transferred through different chemical treatments for preparing the specimens blocks according to (Luft ,1961). Ultra-thin sections (50 nm thick) were obtained from the selected blocks and mounted on copper grids and prepared for examination following (Reynolds,1963). The sections were viewed and photographed on transmission electron microscope (TEM) (Joel Ltd., 1200 ExII, Japan) in Electron Microscope Unite, Faculty of Agriculture, Cairo University, Egypt.

Statistical Analysis

Data were subjected to statistical analysis using analysis of variance (ANOVA) technique; the means were separated using Duncan's multiple range test (P= 0.05) (Steel and Torrie, 1960).

RESULTS**Bioassay Studies:**

The impact of Silymarin concentration levels on the performance of *B. zonata* colony was presented in Table (1). The results revealed that the percentages of pupal recovery were relatively similar (72.2, 72.9 and 72.1 and 73.7 %) with the four concentration levels 0, 10, 30 and 50 mg/kg, respectively; however, the percentages were significantly increased to (77.2 and 76.2%) in the case of 80 and 100 Gy, respectively. A similar trend was observed in the percentage of adult recovery where, the percentages were significantly higher in 80 and 100 mg/kg Sm concentrations

than the other concentration levels 0.0, 10, 30 and 50 mg/kg (Table 1). The number of formed pupae per milliliter had a similar number among the Sm concentration levels. Also, the data in Table (1) presented that the sex ratio of female flies was significantly higher than the ratio of male flies, however the ratios were not varied changed among all concentration levels.

Table (1). Certain biological parameters of the peach fruit fly, *B. zonata* from larvae reared on larval media containing different Silymarin (Sm) concentrations.

Sm Con. (mg/kg)	(%) Pupal recovery	(%) Adult recovery	(Avg.) no. pupae / ml	(Avg.) Sex ratio Male : Female	(Avg.) eggs/fem/ day	(%) egg hatch
0	72.2±1.3a	63.7±1.9a	43.1±0.3	0.39 : 0.61	21.2±0.5	80.2±1.2a
10	72.9±1.5a	63.5±1.3a	42.6±0.8	0.40 : 0.60	20.8±0.7	81.5±1.5a
30	72.1±1.2a	64.1±1.1a	42.8±0.5	0.37 : 0.63	21.5±0.4	82.1±0.4a
50	73.7±0.6a	64.4±0.6a	43.9±0.1	0.39 : 0.61	21.9±0.3	83.4±0.8b
80	77.2±1.3b	69.7±0.9b	43.5±0.1	0.38 : 0.62	22.5±0.7	87.1±0.6c
100	76.2±0.8b	68.2±0.5b	42.6±0.3	0.41 : 0.59	20.3±0.9	86.3±0.9c

Means designated with the same letter in the same column are not significantly different ($P \geq 0.05$)

On the other hand, the data of Table (1) showed that there were insignificant differences in the average number of eggs laid by female per day as compared to the control treatment. While, the egg hatch in the 50, 80 and 100 mg/kg concentrations were significantly increased to (84, 87.1 and 87.0 %), respectively and insignificantly increased to (81.5 and 82.1 %) at the low concentration levels 10 and 30 mg/kg, respectively as compared to (80.2%) in the control treatment (0 %).

Obtained data in Table (2) clarified the impact of (80 mg/kg) Sm concentration followed by irradiating male pupae to 0, 70, 90, 100 and 110 Gy on the performance of irradiated insect as a radio-protective agent. The data showed that the adult flies were emerged in a high percentage in the case of irradiation pupae with 0, 70, 90, 100 and 110 Gy that fed on 80 mg/kg Sm concentration as larvae as compared to only irradiated ones. A similar trend was observed in the longevity averages (Table 2) where the adult flies were lived longer in the combination treatments (gamma irradiation dose levels and 80mg/kg Sm concentration) than those only irradiated ones.

Table (2). Effect of gamma irradiation on certain quality control parameters of *B. zonata* produced from larvae reared on larval media containing 80 mg/kg Silymarin (Sm) concentration.

Treatments*	(%) Emergence	(Av.) Longevity (day)	(%) Flight ability	(%) Egg hatch	(%) Sterility
0 Gy	83.3±1.20	74±1.06	80.2±1.0	81.8±1.3	-
Sm.+ 0 Gy	85.4±1.80	75±1.21	83.1±1.08	84.5±1.5	-
70 Gy	80.2±0.85	65±0.86	81.2±1.23	20.5±0.4	74.9
Sm.+ 70 Gy	83.3±1.03	70±1.22	84.3±1.56	25.5±0.1	68.8
90 Gy	78.1±1.20	68±0.56	79.4±1.46	0.2±0.03	99.8
Sm.+ 90 Gy	80.9±1.02	71±1.32	81.5±1.05	0.3±0.01	99.6
100 Gy	74.8±0.87	65±1.16	78.5±0.99	0.0	100
Sm.+ 100 Gy	77.2±0.65	68±1.30	81.9±1.20	0.1±0.01	99.9
110 Gy	70.5±1.20	63±0.56	75.8±1.33	0.0	100
Sm.+ 110 Gy	74.5±0.395	64±2.81	77.6±1.02	0.0	100

Also, the data of Table (2) revealed that the flight ability of irradiated flies with 0, 70, 90, 100 and 110 Gy with 80mg/kg Sm concentration were higher than the doses without Sm.

On the other hand, The percentages of egg hatch resulted from mated female

with irradiated male previously fed on 80 mg/kg of Sm as larvae were presented in the Table (2), in the 80 mg/kg Sm concentration, the egg hatching was increased in the case of 0, 70, 90 and 100 Gy as compared with only irradiated ones, while, the percentage was recorded (0) in the case of 110 Gy alone or with Sm treatment. A similar trend was noticed when the sterility was calculated in the percentages of hatched eggs based (Table 2).

Ultrastructure Studies:

Normal Testis:

Like most of Tephritidae species, the mature testes of the male (17 days old) of peach fruit fly *B. zonata* appeared a yellow color and oval-shaped. Each testis consists of a testis wall and along saclike follicle filled with the male germ cells in several stages of development (Fig. 1A). The testis wall contained a peritoneal sheath, a layer of muscles, a basement membrane, follicular epithelium and net of tracheoles (Fig. 1A and C). The peritoneal sheath is the outer structure of the testis contained a great number of grain with different shapes and levels of compactness (Fig. 1B).

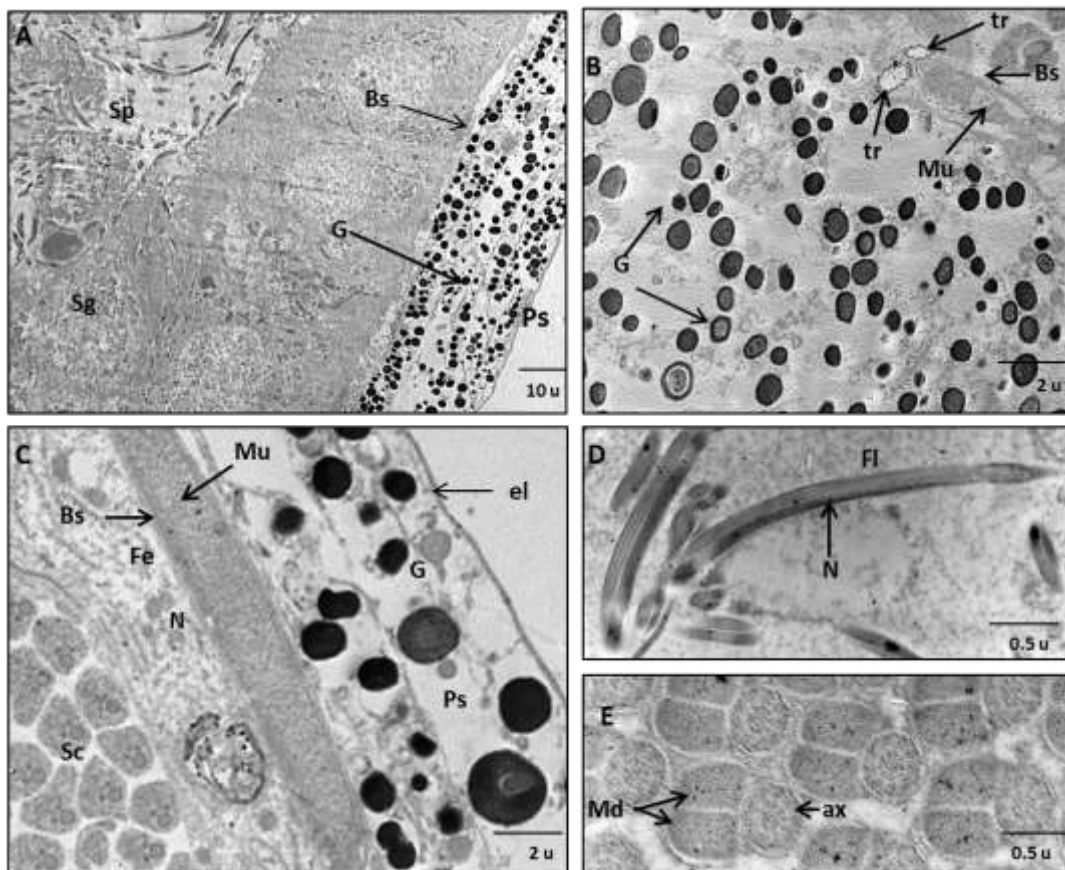


Fig. (1): Transmission electron micrograph of *B. zonata* testes : (A) 2000X, testis wall peritoneal sheath, , development of germ cells and free germ cells. (B) 4000X, the grains in the peritoneal sheath with different sizes and electron densities. (C) 5000X, follicular epithelium, muscular layer and development of spermatocytes. (D) 10000X spermatozoa. (E) 40000X, the spermatids with axoneme and two mitochondrial derivatives . Axoneme (ax) basement membrane (Bm); external layer (ex); follicular epithelium (Fe); flagellum (Fl); grain (G); mitochondria (Md); muscle (Mu); nucleus (N); peritoneal sheath (Ps); spermatocyte (Sc); spermatogonia (Sg); tracheole (tr).

Some of the grains appeared wrapped by a membrane, with light material inside, other are formed by the fibrous dense material. (Fig. 1B and C). In addition, a muscle tissue appeared surrounding the peritoneal sheath (Fig. 1C). On the other hand, the long saclike follicle is filled several stages of development from the spermatogonia to the mature spermatozoa (Fig 1A and C). The mature sperm appeared in a head and a flagellum, the flagellum of the spermatozoa contained two mitochondrial derivatives that extended to the posterior during sperm formation (early spermatid) and the axoneme that further elongated from the centriole (nucleus) (Fig. 1D and E). The mitochondrial derivatives and the axoneme were found at the opposite side of the nucleus (Fig. 1D).

Irradiation Treatment:

The ultrastructure examination of the *B. zonata* testis was pointed out many alterations induced by 90 Gy sterilizing dose. In the wall of the testis, the matrix of peritoneal sheath appeared irregular, the external layer appeared rupture in some part (Fig. 2A and B), the vacuoles were obviously found in the cytoplasm and the muscle layer appeared vacuolated and degenerated mitochondria (Fig 2A and C)

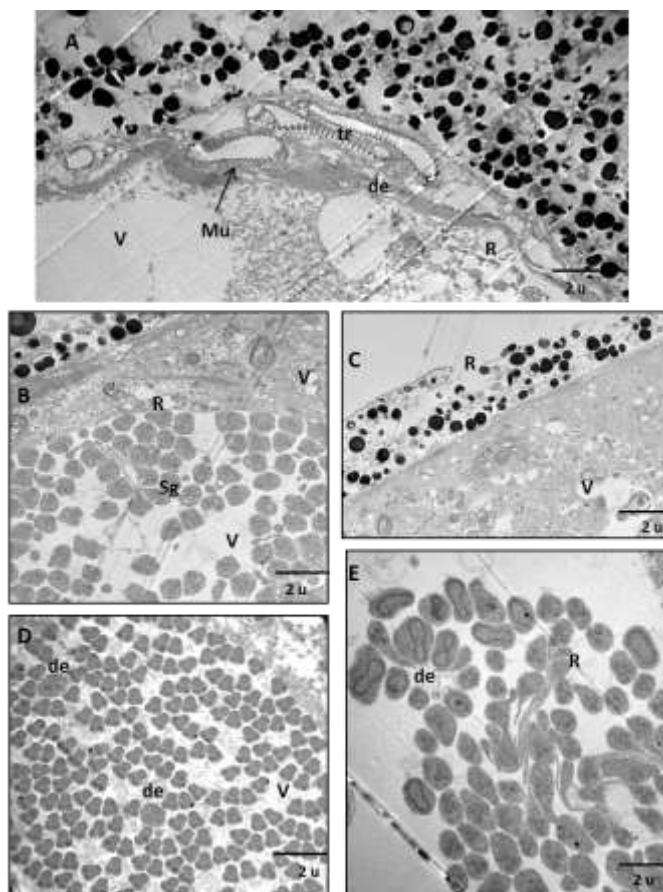


Fig.(2) :Transmission electron micrograph of irradiated *B. zonata* testes showing : (A) 3000X, the peritoneal sheath, muscle layer and basement membrane were affected, the cytoplasm of epithelial follicle was filled with great number of vacuoles . (B) 5000X, rupture in the linear that surround the spermatocyte and vacuolation in the cytoplasm of spermatogonia. (C) 4000X, the peritoneal sheath appeared thick, rupture in the external layer of peritoneal sheath, vacuoles and cytoplasm of germ cells. (D) 12000X, degeneration of some spermatids. (E) 5000X,

Moreover, many vacuoles have appeared in the cytoplasm of the germ cells, also the reduction in number of the spermatocytes and spermatids were very apparent than the control (Fig. 2A, B and C), the membranes that surrounded the sperm bundles were ruptured (Fig. 2C and D) and degeneration on the spermatocytes and spermatids were observed (Fig. 2D and E) moreover, some regular spermatocytes and spermatids were observed (Fig. 2E). These abnormalities could produce the production of non-functional sperms or decreased in sperms production Furthermore, the alteration in the mature spermatophore in particular the axoneme and mitochondrial derivatives hadn't been shown.

Irradiated and Silymarin Treatments:

The examined testes of irradiated males of *B. zonata* which produced from larvae fed on 80 mg/kg Sm concentration in the larval diet showed various forms of cell degeneration. These aberrations appeared relatively similar with those shown in irradiated alone, the testis wall appeared in various deteriorations where irregular shape of the peritoneal sheath, the vacuoles were showed in the cytoplasm and the muscle layer appeared vacuolated (Fig 3) . However, the peritoneal sheath, muscle layer and basement membrane were less affected than the irradiated ones. Moreover, vacuoles were noticed in the cytoplasm of the germ cells, the spermatogenesis exhibited much more gross abnormal structures.

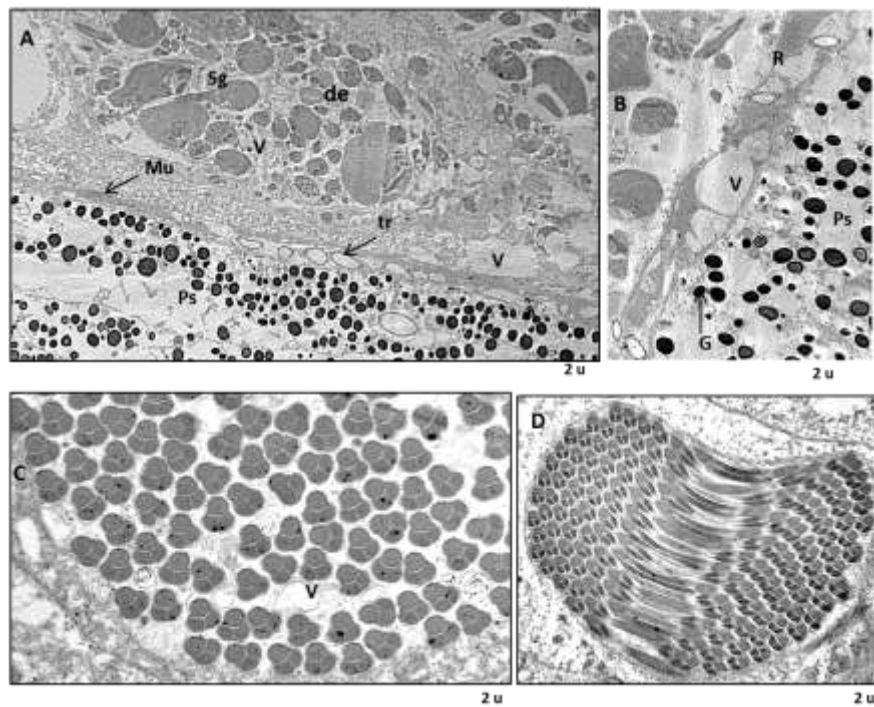


Fig. (3) :Transmission electron micrograph of irradiated *B. zonata* male testes resulted from larvae fed on 80gm/gm Sm concentration showing : (A)2500X, the cytoplasm of epithelial follicle was filled with great number of vacuoles and deformed in the spermatogenesis. (B) 5000X, rupture in the muscle layer and basement membrane of the peritoneal sheath.(C) 15000X, Vacuolation in the cytoplasm of spermatids (D) 5000X malformed of some developmental sperm. Degeneration (de); peritoneal sheath (Ps); spermatogonia (Sg); rupture (R) and vacuoles (V).

DISCUSSION

Bioassay Studies:

The management of insect colony to produce the adult flies in high quantity and quality is one of the important tools for the successful use of SIT against tephritidae pests.

The present study indicated that Sm as an additive to the larval diet of *B. zonata* improved certain reproductive biological aspects as egg hatch and recovery of pupae and adults, in the contrary, the fecundity, pupal size and sex ratio were as the same of untreated one. Also, the results indicated that the 80 mg/kg Sm concentration of were more effective for improving the insect fitness than the other concentrations. These results are coincident with those reported by, Mohamed, (2012) who used vitamin E to the larval rearing media of *Galleria mellonella*, El-Akhdar *et al.*, (2012) who added E-selenium to larval diet of *C. capitata* and El-Kholy (2008) who revealed that the addition of Silymarin to the *C. capitata* larval media at different concentrations caused insignificant changes in the sex ratio and insect survival. Our results contradict that of Martin-Romero *et al.*, (2001) who noticed an increase in the number of eggs laid per female of *Drosophila melanogaster* that reared on selenium-supplemented diet, and also Showman and Mahmoud (2010) who noticed that the pupal size was increased by using the melatonin as additives to the larval diet of *C. capitata*.

It is worth to mention that, the sterilizing doses of gamma radiation may cause damage to both genetic and somatic cells subsequently induce sterility of the adult stage and consequently reduce the quality of released sterile males. In the present study, using 80 mg/kg Sm concentration as a radio-protective agent in the *B. zonata* larval diet improved emergence, survival and adult flight of irradiated insect at all doses applied. It was reported that antioxidant agent could protect the insect cell from the deleterious effect of the gamma irradiation sterilizing doses (Barry *et al.*, 2003b, El-Kholy *et al.*, 2008 Mikhaiel *et al.*, 2011 and El-kholy, *et al.*, 2016). Also, El-Akhdar *et al.*, (2012) reported that the E-selen as antioxidant to larval diet of *C. capitata* increased the sterility and the mating competitiveness value of irradiated males, moreover, the Melatonin as antioxidant agent was evaluated on the irradiated male of *C. capitata* after adding to the larval diet, the pupal recovery, adult survival, flight ability and the competitiveness value were increased (Shoman and Mahmoud, 2010). Furthermore, the radio protective effect of Selinum as another antioxidant agent was studied by Fadi, (2016) on the irradiated males of *C. capitata*, where the flies performance and competitiveness were increased significantly in the combination treatments.

Our results revealed that the 80 mg/kg Silymarin concentration less reduced the sterility of irradiated insects at all dose levels. The results indicated that the dose of 90 Gy is the effective dose for SIT application to *B. zonata*. In spite of the sterility of irradiated *B. zonata* adults was reduced by Silymarin additives, the sterility induction by the dose of 90 Gy alone or with Silymarin are similar to the levels of sterility that are suggested for SIT programmes [*e.g.*, > 99.5% sterility is recommended for Tephritidae].(FAO/IAEA/USDA 2003). These findings may help for improvement of the SIT by increasing the insect performance in the sterilizing doses applied.

Ultrastructure Studies:

The testis structure of *B. zonata* appeared the same of those previously studied on Tephritidae species by Bairati (1967) on *Drosophila melanogaster*, Valdez (2001)

on *Anastrepha ludens*, Hassan *et al.*, (2017) on *Culex pipiens*. The result cleared that the testis of *B. zonata* contained an external wall surrounded the germ cells. The wall consists of a peritoneal sheath containing a great number of pigmented vesicles and muscular layer. The pigmented vesicles are responsible for forming an outer physical barrier which protects the testis. (Bao and Dolder, 1991), According to Chapman (1998), the muscular layer may contribute to the displacement of the spermatid bundle in order to free spermatozoa into the testes and toward the posterior duct to lead out of the testis. This structure not only described in tephritidae, the same aspects of testicular organization were described in other species such as on *Culex pipiens* (Hassan *et al.*, 2017) and on *Blaps sulcata* (Kheirallah *et al.* 2017). Moreover, the present study confirmed the organizational pattern of mitochondrial derivatives with axoneme that was observed in the flagellate spermatozoa. The structure of mature spermatozoa of *B. zonata* appeared as those in Dipteran insects which showed by Shehata *et al.* (2006) on *B. zonata* and Fadi (2016) on *C. capitata*, and also other insect species such as *Tribolium castaneum* which showed by Dias *et al.* (2015), it consisted of the head and flagellum which having an axoneme arranged in a wheel-like pattern and two mitochondrial derivatives.

In the present ultrastructure study of the irradiated *B. zonata* testis, the malformation of the peritoneal sheath was observed and the cytoplasm was vacuolated, also rupture, degeneration and vacuoles in the testicular wall and the germ cell were observed in both 90 Gy gamma irradiation dose alone and with its combination with 80 mg/kg Sm concentration. However, the alterations caused by the combination treatment were less than the irradiated one. These results are relatively similar with those reported on *C. capitata* by Mahmoud and Shoman (2009) who showed a large amount of degeneration in the male testes when the pupae irradiated with 90 Gy. These deteriorations were recovered in organelles, moreover the sperms which succeed in completing their growth were more active after the addition of melatonin as antioxidants. The same effect upon the pretreatment of the irradiated *C. capitata* with Selenium as an antioxidant was recorded by Fadi (2016). Furthermore, rupture, necrosis, degeneration and vacuoles in the testicular wall in the testes of *Culex pipiens* were observed at the LD 25, LD 50 and LD 75 of gamma ray applied (Hassan *et al.*, 2017). Later, Shen and Berryman (1967) observed an increasing the vacuoles in the testis and dispersion of spermatocytes of *Rhyacionia buoliana* irradiated by gamma rays. In the present study, the gamma ray alterations were not observed on the mature spermatozoa of *B. zonata* by 90 Gy sterilizing dose. These results contradict those of Fadi (2016), Kheirallah *et al.* (2017) and Hassan *et al.* (2017). While, they agree with that obtained by Paoli *et al.* (2014) on *Rhynchophorus ferrugineus* who couldn't find any differences between the irradiated and non-irradiated sperm at the 80 Gy of gamma ray. And later with Hodges (1983) who reported that abnormalities in spermatids and sperm of *Dermestes frischii* males were observed only in as primary or secondary spermatocytes cells by sterilizing dose 3.0 Krad. The present study suggested that, Silymarin (Sm) additives could use for enhancing the biological aspects of reared *B. zonata*, and also improving the performance of sterile male flies. The 80 mg/kg of Sm was the effective concentration that could add in the larval media of *B. zonata* as antioxidant additive.

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ARABIC SUMMERY

تقييم استخدام السيليمارين للحماية من تشيع ذبابة الخوخ باشعة جاما

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تعتبر فاعلية الذكور العقيمة أحد الامور الهامه للاستخدام الناجح لتقنية اطلاق الحشرات العقيمة. يمكن ان يقوم السيليمارين المضاد للاكسدة بدورا فعالا لتحسين الأضرار الفسيولوجية التي يمكن ان تحدث خلال التربية المستمرة لمستعمرة حشرات ذبابة الخوخ او نتيجة للتعرض للجرعات الاشعاعية. وقد أجريت تجارب لدراسة تأثير إضافة تركيزات مختلفة من سليمارين (0 ، 10 ، 30 ، 50 ، 80 و 100 ملغم / كغ) إلى بيئة تربية اليرقات لدراسة تأثيره على حيوية حشرات ذبابة الخوخ . أيضا تم اختبار التأثير الحامي للسليمارين كمضاد للاكسدة عن طريق أولاً من خلال دراسة عوامل ضبط الجودة للذباب الكامل المشع بمستويات مختلفة من الجرعات المعقمة لاشعة جاما (0،70 و 90 و 100 و 110 غراي) التي كانت تتغذى سابقاً علي السليمارين في مرحلة اليرقات ، ثانيا تم تقييم تأثير الحماية للسليمارين من خلال دراسة التركيب الدقيق لخصي الذكور. أوضحت النتائج أن اضافة السليمارين بتركيزات 80 و 100 ملغم / كغ زادت من نسبة طور العذراء وايضا الاطوار الكاملة ، حيث زادت بشكل كبير إلى (77.2 و 76.2٪) و (69.7 و 68.2٪) على التوالي مقارنة بـ (72.2٪) و (63.3٪) في المعاملات القياسية على التوالي. وكشفت النتائج أيضا أنه في تركيز 80 ملغم / كغم من السليمارين ، زاد عدد البيض الفاقس في كلا من إما المعاملة المنفردة أو بالاقتران مع الجرعات المعقمة من اشعة جاما. علاوة على ذلك ، زادت نسبة الطيران للذباب وايضا نسبة خروجه من طور العذراء . وقد أظهرت دراسة التركيب الدقيق تحسنا في الخُصي المشعّة بـ 90 غراي باستخدام السليمارين بتركيز 80 مجم / كجم مقارنة بالمعالجة الإشعاعية وحدها.