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THE RADIOPROTECTIVE ROLE OF SELENIUM IN THE IMPROVEMENT OF SOME BIOLOGICAL AND BIOCHEMICAL ASPECTS OF GAMMA IRRADIATED MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* (WIED.)

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Key words: Gamma irradiation, Selenium, Ceratitis capitata, Biology, Biochemical changes.

الدور الواقى للسيلينيوم فى تحسين بعض النواحى البيولوجية والبيوكيميائية لذبابة فاكهة البحر المتوسط المشعة بأشعة جاما سيراتيتس كابيتاتا

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خلاصة

تهدف هذه الدراسة إلى تقدير التأثير الواقى للسيلينيوم بتركيزات مختلفة (10^{-4}, 5×10^{-5}, 10^{-5}, 5×10^{-6}, 10^{-6}) M فى الوسط الغذائى ليرقات ذبابة فاكهة البحر المتوسط وتعريض العذارى الناتجة للجرعة الإشعاعية (90 جراى) على بعض النواحى البيولوجية والبيوكيميائية.

وقد أوضحت النتائج عدم تأثير النسبة الجنسية (عدد الذكور إلى الإناث) لذبابة فاكهة البحر المتوسط باستخدام السيلينيوم ولكن حدث انخفاضا معنويا فى نسبة خروج الحشرة فى التركيزات المنخفضة عنها فى التركيات العالية. ولقد أظهرت النتائج انخفاضا معنويا عاليا فى نسبة فقس البيض للجرعة المعقمة منفردا أو مع التركيزات المختلفة من السيلينيوم وأيضاً حدث نقص معنوى فى خصوبة الأنثى بالتركيزات المختلفة (10^{-4}, 5×10^{-5}, 10^{-5}) M وفى الجرعة المعقمة. ولقد أوضحت النتائج أن معاملة الحشرات بالسيلينيوم منفردة أو مجتمعة مع الجرعة المعقمة للإشعاع أظهرت زيادة معنوية فى المحتوى البروتينى والدهنى أما بالنسبة للمحتوى الكربوهيدراتى فقد أوضحت النتائج زيادة مع كل تركيزات السيلينيوم منفردا ولكن التركيز المنخفض ((10^{-6} M مع الجرعة المعقمة (90 جراي) اظهر نقصا معنويا عنة فى التركيزات العليا الاخرى للسيلينيوم. وأظهرت النتائج أيضاً زيادة معنوية فى مستوى الجلوكوز فى التركيزات المنخفضة للسيلينيوم عنها

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فى التركيزات العالية منفردة أو مجتمعة مع الجرعة المعقمة . ولكن لوحظ انخفاضا فى المحتوى البروتينى فى اليرقات وزيادة فى المحتوى الدهنى والكربوهيدراتى ولم يحدث تأثير فى مستوى الجلوكوز فى اليرقات نتيجة المعاملة بالسيلينيوم. نلخص من هذا البحث لإلقاء الضوء على استخدام المعاملة المشتركة من السيلينيوم والجرعة المعقمة كطريقة ناجحة لتقليل الآثار الضارة للإشعاع واستخدامها فى طرق المكافحة المتكاملة لذبابة فاكهة البحر المتوسط.

ABSTRACT

This study aimed to evaluate the protective effect of sodium selenite (SS) at different concentrations (10^{-6}; 5×10^{-6}; 10^{-5}; 5×10^{-5}; 10^{-4}M) added to larval diet of irradiated Ceratitis capitata on some biological and biochemical parameters. The resulting pupae were exposed to the sterilizing dose of gamma irradiation (90Gy). The percent of adult emergence showed a significant decrease with low concentrations. A significant decrease was in the percent of hatchability at 90Gy alone or combined with different SS concentrations. The combined effect of SS with the concentrations (10^{-4}; 5×10^{-5}; 10^{-5}) and gamma irradiation significantly decreased the number of eggs laid daily after mating with SS treated males, while the concentration 10^{-4} increased the number of eggs laid daily after mating normal males with SS treated females. The combined effect of SS with gamma irradiation showed an increase in total protein and total carbohydrate levels while total lipid levels decreased significantly in irradiated pupae. A significant increase was observed in total carbohydrates at concentrations 5×10^{-6}, 10^{-5}, 5×10^{-5} and 10^{-4}M of SS. However, the combined effect of 10^{-6} SS with gamma irradiation showed a significant decrease in total carbohydrate levels compared to irradiated pupae. The glucose levels were significantly increased at the concentrations 5×10^{-6}, 10^{-5}, 5×10^{-5}, 10^{-4} M of SS combined with the sterilizing dose. Generally, the combined treatment of selenium and gamma irradiation could be used successfully in order to improve performance of the irradiated adults and could subsequently be applied in the integrated pest management program (IPM) to help in the success of the sterile insect technique (SIT) of med fly.

INTRODUCTION

Tephritid fruit flies are worldwide economically significant pests and their importance has been steadily increased in recent years. The Mediterranean fruit fly (med fly), *Ceratitis capitata* (Wied.), is one of the world's most destructive fruit pests which has the ability to tolerate cooler climates better than most of the other species of tropical fruit flies and is highly polyphagous. *C. capitata* larvae feed and develop on many deciduous, subtropical, and tropical fruits and some vegetables (Thomas et al., 2013)**.** Its control mainly relies on the sterile insect technique (SIT) which is a biological, eco-friendly method of pest suppression or elimination that fits well into the wide approach of preventive insect pest management (IPM) (Knipling, 1955)**.** Cho et al. (2013) illustrated that the most successful method to control Tephritidae was SIT and this requires continuous mass-rearing, sterilization of adult flies, and release of sterilized males into the fields to compete with wild males for mating with wild females, and so these female will not produce offspring. Repeated release of sterile males can effectively suppress the population of *C. capitata*. Meanwhile, it has been proved that gamma irradiation results in tissue damage in cellular organelles in midgut and its microbiota and was responsible for the inferiority of sterile males compared with wild males (Hamden et al., 2013). Hence, radiation-induced dominant lethal mutations, which result in early embryonic death, could also provoke damage to somatic cells leading to reduc competitiveness of the males (Calkins & Parker, 2005).

All insects respond to stressful changes of the environment in amazingly different ways. Gamma ionizing irradiation generated an antioxidative defense mechanism to overcome the toxic effects of reactive oxygen species (ROS). The generated ROS react with proteins, polysaccharides, nucleic acids, and polyunsaturated fatty acids and cause alteration of their structure and functions (Natural Holistic Health Report, 2010).

Selenium is an essential and mineral micronutrient element incorporated into seleno-proteins. Selenium deficiency is known to cause significant health problems such as immune deficiency and cancer (Sheck et al., 2010). It is also required for sperm maturation and sperm motility and reduces sperm abnormalities induced by chemicals (Fahmy et al., 2008). The radioprotective action of sodium selenite (SS) was demonstrated by Rafferty et al. (1998). Little is known about the use of selenium to reduce the adverse effects of gamma irradiation in insects. Thus, the aim of the present study was to evaluate the radioprotective effect of SS in larval diet of *C. capitata* to ameliorate the biological and biochemical changes induced by gamma irradiation in an attempt to improve the SIT by overcoming the induced damage of the somatic cells.

MATERIALS AND METHODS

Rearing technique:

The med fly *C. capitata* was reared in the insectary of the Atomic Energy Authority, Inshas Egypt. The larval rearing technique was described by Tanaka et al. (1969). The insects were reared at 25 ± 2ºC and 60-70% RH. The diet offered to the larvae was recommended by Wakid et al. (1998). Adults were fed on sugar and yeast hydrolyzate in a ratio of 3:1 by weight as reported by Hooper (1987).

Irradiation technique:

The full grown pupae of *C. capitata* were irradiated 1-2 days before emergence with the sterilizing dose (90 Gy). Irradiation was achieved by using a gamma cell ^{60}Co unit installed at the Cyclotron Department, Nuclear Research Center, Atomic Energy Authority, Inshas. The dose rate of the applied source ranged from 1.17643 KGy /h to 0.998 KGy/h.

Biological studies:

Sodium selenite (SS) was obtained from Sigma Aldrich Company, Saint Louis, USA. The following biological parameters were measured: the larval and pupal duration, the percentage of pupation, the pupal size, the pupal weight, the total number of pupae, the percentage of adult emergence and sex ratio after applying different concentrations of SS; 10^{-1}, 10^{-2}, 10^{-3}, 5×10^{-4}, 10^{-4}, 5×10^{-5}, 10^{-5}, 5×10^{-6}, 10^{-6}, 10^{-7} and 10^{-8} M (Molar), which were mixed with the larval diet. The most effective five concentrations of SS; 10^{-4}, 5×10^{-5}, 10^{-5}, 5×10^{-6} and 10^{-6} M, were selected from the preliminary test for estimating the number of eggs/female/day and the percentage of egg hatchability after applying irradiation. The control groups were prepared without adding SS to each biological parameter.

Biochemical studies:

Experimental design:

The selected SS concentrations 10^{-4}, 5×10^{-5}, 10^{-5}, 5×10^{-6} and 10^{-6} M were mixed with the larval diet. Hence, total protein, total lipids, total carbohydrates and glucose levels were determined in the 3rd larval instar and the full grown pupae (8-9 days old) before and after irradiation.

Estimation of total protein, total carbohydrates and glucose levels:

The 3rd larval instar tissue, 0.5 g, or the full grown pupae tissue, 0.5 g, was homogenized in 5 ml potassium phosphate buffer pH 7.5, then centrifuged at 4000 rpm for 15 min. at 4°C (Beckman J2-HS). The supernatant was collected and stored at -80°C. The obtained supernatant was used to determine total protein with the Folin-Ciocalteu technique of Lowry et al.(1951)**,** total carbohydrates by the phenol-sulfuric acid method of Dubois (1956), and glucose by the enzymatic colorimetric method of Trinder (1969) using kit No. GL 13-20 (Biodiagnostic, Egypt). Measurements were performed according to the manufacturer's instructions.

Estimation of total lipids:

The 3rd larval instar tissue, 0.25 g, or the full grown pupae tissue, 0.25 g, was homogenized in chloroform: methanol (2:1). The homogenization was conducted in an ice bath to prevent evaporation and vortexes followed by centrifugation at 3000 rpm for 15 min, the supernatant was collected for the total lipid assay. The total lipids were determined by sulpho-phospho-vanilin method of Knight et al. (1972) and Zollner and Kirsch (1962) using kit No. TL 20-10 (Biodiagnostic, Egypt). Measurements were performed according to the manufacturer's instructions.

Statistical analysis:

The data were expressed as means ± S.E.M. and were subjected to statistical analysis using analysis of variance (ANOVA). All statistical analyses were done using SAS program and the means were analyzed using Duncan's multiple range test (Steel and Torrie, 1980). The significance was set at $P< 0.05$.

RESULTS

Biological study:

Tables (1 and 2) showed that the use of SS at 10^{-4}, 10^{-6}, $10^{-7}$and 10^{-8} M concentrations affected the total number of pupae popped from 300 well-selected eggs and the reduction in the percentage of pupation recorded 8.1%, 10.6%, 9.3% and 9.4%, respectively. Moreover, the pupal size showed a significant increase by 8% at the SS concentration of 10^{-7} M. There was no obvious effect on the sex ratio of the med fly at different concentrations of SS (table 3), while a significant decrease in the percent of adult emergence occurred after the use of the lower concentrations, 10^{-8} and 10^{-7} M of SS recording 5.3% and 5.6%, respectively. Furthermore, the data in table (4) indicated that the number of eggs laid daily per female that mate with SS-treated irradiated males was significantly decreased by 18%, 26.9% and 25.9% at SS concentrations of 10^{-5}, $5\times10^{-5}$and 10^{-4} M, respectively. Moreover, the percentage of egg hatchability was significantly decreased by 97.5%, 97.7%, 98.6%, 99.3% and 98.8% when the full grown SS-treated pupae were irradiated at SS concentrations of 10^{-6}, $5x10^{-6}$, 10^{-5}, 5×10^{-5} and 10^{-4} M, respectively. While significant decreases were observed in the number of eggs laid daily per female that mate with irradiated (90Gy) and in the percentage of egg hatchability. Table (5) showed that the number of eggs laid daily per female resulting from SS-treated females matted with normal males (N♂×T♀) was significantly decreased by 26.9%, 16.1% and 17.2% at SS concentrations of 5×10^{-6}; 10^{-5} and 5×10^{-5} M, respectively.

Table (1): Effect of different concentrations of sodium selenite (SS) added to the larval media of C. capitata on total number of pupae and percent of pupation.

Sodium selenite	Total no. of	%	Pupation	%

concentrations (M)	pupae	Difference	(%)	Difference
Control	217.5 ± 5.5[a]	-	72.5 ± 1.8[a]	-
10^{-8}	197 ± 1.2[b]	-9.4	65.7 ± 0.4[b]	-9.4
10^{-7}	197.3 ± 2.7[b]	-9.3	65.8 ± 0.9[b]	-9.2
10^{-6}	194.5 ± 3.5[b]	-10.6	64.8 ± 1.2[b]	-10.6
5×10^{-6}	212 ± 1.0[a]	-2.5	70.7 ± 0.3[a]	-2.5
10^{-5}	216 ± 1.0[a]	-0.7	72 ± 0.3[a]	-0.7
5×10^{-5}	223 ± 5.0[a]	2.5	74.3 ± 1.7[a]	2.5
10^{-4}	200 ± 6.0[b]	-8.1	66.7 ± 2.0[b]	-8

Means ± S.E. in the same column with different letters are considered statistically significant at P<0.05.

Table (2): Effect of different concentrations of sodium selenite (SS) added to the larval media of C. capitata on pupal size and pupal weight.

Sodium selenite concentrations (M)	Pupal size/ml	% Difference	Pupal weight (g)/100 pupae	% Difference
Control	46±0[b]	-	1.09±0.01[a]	-
10^{-8}	47.3±0.6[b]	2.8	1.08±0.03[a]	-0.9
10^{-7}	49.7±0.3[a]	8	1.06±0[a]	-2.8
10^{-6}	46±1.5[b]	0	1.09±0.01[a]	0
5×10^{-6}	46±0.7[b]	0	1.07±0.01[a]	-1.8
10^{-5}	47±1.0[b]	2.2	1.08±0.01[a]	-0.9
5×10^{-5}	46±1.0[b]	0	1.08±0.01[a]	-0.9
10^{-4}	47±0.7[b]	2.2	1.06±0.01[a]	-2.8

Means ± S.E. in the same column with different letters are considered statistically significant at P<0.05.

Table (3): Effect of different concentrations of sodium selenite added to the larval media of C. capitata on the percentage of adult emergence and sex ratio.

Sodium selenite concentrations (M)	Adult emergence (%)	% Difference	Sex ratio		% Difference
			♂	♀	
Control	97.5±1.8[a]	-	0.98±0.04[a]	1	-
10^{-8}	92.3±2.[b]	-5.3	1.0±0.2[a]	1	2
10^{-7}	92±1.7[b]	-5.6	1.06±0.1[a]	1	8.2
10^{-6}	99.3±0.7[a]	1.9	1.08±0.2[a]	1	10.2
5×10^{-6}	98.3±0.3[a]	0.9	1.14±0.2[a]	1	16.3
10^{-5}	99.3±0.3[a]	1.9	0.96±0.2[a]	1	-2.04
5×10^{-5}	98.3±0.3[a]	0.9	1.16±0.1[a]	1	18.4
10^{-4}	98±0.6[a]	0.5	1.02±0.04[a]	1	4.1

Means ± S.E. in the same column with different letters are considered statistically significant at P<0.05

Table (4): Effect of different concentrations of sodium selenite added to the larval media on the number of eggs/female/day and the percentage of egg hatchability of normal and irradiated male C. capitata.

Sodium selenite concentrations (M)	No. of eggs/female/day	% Difference	Egg hatchability (%)	% Difference
Control	48.3±0.9[a]	-	95.3±1.5[ab]	-
10^{-6}	37.4±3.3[bcd]	-22.6	96.8±1[a]	1.6
5×10^{-6}	33.7±1.3[d]	-30.2	95.3±1.8[ab]	0
10^{-5}	38.1±0.2[bcd]	-21.1	94.3±0.8[ab]	-1.1
5×10^{-5}	41.8±0.6[abc]	-13.5	93.1±1.1[b]	-2.3
10^{-4}	40.9±2.9[bcd]	-15.3	96.3±1.2[a]	1.1
90Gy	39.3±1.1[bcd]	-18.6	2.1±0.1[c]	-97.8
10^{-6} +90Gy	44.4±2[ab]	-8.1	2.4±0.3[c]	-97.5
5×10^{-6} +90Gy	41.9±1[abc]	-14.3	2.2±0.4[c]	-97.7
10^{-5} +90Gy	39.6±2[bcd]	-18	1.3±0.1[c]	-98.6
5×10^{-5}+90Gy	35.3±3.8[cd]	-26.9	0.7±0.1[c]	-99.3
10^{-4} +90Gy	35.8±2.8[cd]	-25.9	1.1±0.1[c]	-98.8

Means ± S.E. in the same column with different letters are considered statistically significant at P<0.05.

Table (5): Effect of different concentrations of sodium selenite on the number of eggs/female/day and the percentage of egg hatchability of normal female C. capitata.

Sodium selenite concentrations (M)	No. of eggs/female/day	% Difference	Egg hatchability (%)	% Difference
Control	48.3±0.9^{a}	-	95.3±1.5^{a}	-
10^{-6}	45.1±2.8^{ab}	-6.6	96.4±1.8^{a}	1.2
5×10^{-6}	35.3±0.4^{c}	-26.9	95.2±0.7^{a}	-0.1
10^{-5}	40.5±2.1^{bc}	-16.1	95.1±0.9^{a}	-0.2
5×10^{-5}	40±3.9^{bc}	-17.2	95.6±1.2^{a}	0.3
10^{-4}	52.3±2.6^{a}	8.3	97.1±1.1^{a}	1.9

Means ± S.E. in the same column with different letters are considered statistically significant at $P<0.05$

Biochemical study:

Table (6) showed that the total protein were significantly decreased by 14% and 11% in the tissue of the 3rd instar larvae treated with SS concentrations, 10^{-5} M and 5×10^{-5} M, respectively. The total lipids increased significantly by 12.2% in larval tissue treated with SS at concentration of 10^{-5}M. The total carbohydrate level also recorded a significant increase by 5.6% and 4.7% in the larval tissue treated with SS at concentrations of 10^{-6} M and 5×10^{-5} M, respectively (Table 7). However, a significant decrease (4.8%) was observed in the larval tissue treated with SS concentration of 10^{-5} M.

Table (8) revealed that the total protein were significantly increased by 30.7% in the pupal tissue resulting from larvae treated with SS at a concentration of 5×10^{-6} M. In addition, a significant increase in the total lipids by 25.6% was recorded at SS concentration 10^{-5} M in treated pupal tissue. However, the gamma irradiation (90 Gy) resulted in a significant decrease in the levels of total protein and total lipids in pupal tissue recording 22% and 56%, respectively. On the other hand, the combined effect of SS with gamma irradiation (90 Gy) showed a significant increase in total protein and lipid levels in the pupal tissue in all tested SS concentrations compared to gamma-irradiated group. A significant increase in the total carbohydrate levels by 11.5% was recorded in pupal tissue treated with SS concentration of 10^{-5} M (Table 9). In addition, the combined effect of SS at concentrations of 5×10^{-6}, 10^{-5}, 5×10^{-5} and 10^{-4} M with gamma irradiation (90 Gy) showed a significant increase in total carbohydrate levels in the pupal tissue by 11%, 12.7%, 10.6% and 15.5%, respectively.

Table (6): Effect of different concentrations of sodium selenite added to the C. capitata larval media on total protein and total lipids in 3rd larval instar.

Sodium selenite concentrations (M)	Total protein × 10^2 (µg/ml)	% Difference	Total lipids (mg/dl)	% Difference
Control	116.6±0.6^{a}	-	493.5±19.4^{bc}	-
10^{-6}	122.9±1.9^{a}	5.4	528±10.8^{ab}	7
5×10^{-6}	122.9±3.1^{a}	5.4	459.1±6.5^{c}	-7
10^{-5}	100.4±6.9^{b}	-14	553.9±2.2^{a}	12.2
5×10^{-5}	103.5±3.8^{b}	-11	521.6±4.3^{ab}	5.7
10^{-4}	128.5±1.3^{a}	10.2	491.4±17.2^{bc}	-0.4

Means ± S.E. in the same column with different letters are considered statistically significant at P<0.05.

Table (7): Effect of different concentrations of sodium selenite mixed with the C. capitata larval media on total carbohydrates and glucose in 3rd larval instar.

Sodium selenite concentrations (M)	Total carbohydrates (mg/ml)	% Difference	Glucose (mg/dl)	% Difference
Control	13.7±0.2^{b}	-	75.4±1.8^{ab}	-
10^{-6}	14.4±0.02^{a}	5.6	81.1±1.7^{a}	7.6
5×10^{-6}	13.2±0.02^{cb}	-3.3	81.1±2.2^{a}	7.6
10^{-5}	13.02±0.14^{c}	-4.8	74.1±0.7^{b}	-1.7
5×10^{-5}	14.3±0.04^{a}	4.7	77.1±1.1^{ab}	2.3
10^{-4}	13.6±0.24^{b}	-0.5	81.5±2.9^{a}	8.1

Means ± S.E. in the same column with different letters are considered statistically significant at P<0.05

A significant increase in the glucose level was obtained in pupal tissue treated with SS concentrations 5×10^{-6}M and 5×10^{-5}M recording 22% and 15.1%, respectively. Similar to the combined effect of SS at concentrations of 5×10^{-6}, 10^{-5}, 5×10^{-5} and 10^{-4}M with gamma irradiation (90 Gy), glucose level showed a significant increase by 21.9%, 43.1%, 15.8% and 15.8%, respectively.

Table (8): Effect of different concentrations of sodium selenite added to the C. capitata larval media on total protein and total lipids of normal and irradiated (90Gy) full grown pupae.

Sodium selenite concentrations (M)	Total protein × 10^2 (µg/ml)	% Difference	Total lipids (mg/dl)	% Difference
Control	87.3 ± 5^{dc}	-	300.3 ± 11.8^{bc}	-
10^{-6}	90.4 ± 0.6^{bdc}	3.6	314.7 ± 15.1^{ab}	4.8
5×10^{-6}	114.1 ± 0.6^{a}	30.7	288.8 ± 17.4^{bc}	-3.8
10^{-5}	86 ± 7.5^{dc}	-1.5	377.2 ± 10.8^{a}	25.6
5×10^{-5}	82.3 ± 1.3^{d}	-5.7	230.6 ± 6.5^{cd}	-23
10^{-4}	79.1 ± 3.1^{de}	-9.4	239.9 ± 11.2^{cd}	-20
90Gy	67.9 ± 3.1^{e}	-22	131.5 ± 28^{e}	-56
10^{-6} + 90Gy	90.4 ± 5.6^{bdc}	3.6	285.9 ± 35.9^{bc}	-4.8
5×10^{-6} + 90Gy	102.9 ± 1.9^{ab}	17.9	312.5 ± 40.9^{ab}	4.1
10^{-5} + 90Gy	101 ± 5^{abc}	15.7	232.7 ± 8.6^{cd}	-23
5×10^{-5} + 90Gy	94.1 ± 8.1^{bdc}	7.8	284.5 ± 30.2^{bc}	-5.3
10^{-4} + 90Gy	86 ± 2.5^{dc}	-1.5	212.6 ± 6.3^{d}	-29

Means ± S.E. in the same column with different letters are considered statistically significant at P<0.05.

Table (9): Effect of different concentrations of sodium selenite added to the C. capitata larval media on total carbohydrates and glucose of normal and irradiated (90Gy) full grown pupae.

Sodium selenite concentrations (M)	Total carbohydrates (mg/ml)	% Difference	Glucose (mg/dl)	% Difference
Control	7.8 ± 0.1^{de}	-	63.4 ± 1.8^{ed}	-
10^{-6}	8 ± 0.3^{cde}	2.8	70.7 ± 2.9^{cbd}	11.5
5×10^{-6}	8.4 ± 0.2^{adcd}	7.7	77.3 ± 2.6^{b}	22
10^{-5}	8.7 ± 0.3^{abc}	11.5	68.5 ± 0.3^{cd}	8
5×10^{-5}	7.9 ± 0.04^{de}	0.9	73 ± 0.3^{cb}	15.1
10^{-4}	8.1 ± 0.1^{bcde}	3.3	57.7 ± 0.6^{e}	-9
90Gy	8.5 ± 0.2^{abcd}	8.3	70.6 ± 3.6^{cbd}	11.4
10^{-6} + 90Gy	7.4 ± 0.3^{e}	-5	69.8 ± 1.6^{cbd}	10.1
5×10^{-6} + 90Gy	8.7 ± 0.3^{abc}	11	77.3 ± 2.3^{b}	21.9
10^{-5} + 90Gy	8.8 ± 0.3^{ab}	12.7	90.7 ± 2.1^{a}	43.1
5×10^{-5} + 90Gy	8.6 ± 0.3^{abc}	10.6	73.4 ± 2.6^{cb}	15.8
10^{-4} + 90Gy	9 ± 0.2^{a}	15.5	73.4 ± 4.4^{cb}	15.8

Means ± S.E. in the same column with different letters are considered statistically significant at P<0.05.

DISCUSSION

Biological study:

El-Kholy (2008) demonstrated the effect of sylimarin as an antioxidant on the increase of the med fly percentage pupation compared to control group. In addition, there was no effect of melatonin added to the med fly larval diet on the pupal weight as reported by Shoman and Mahmoud (2010), but the percentage of adult recovery after irradiation was increased. They also illustrated that the male sterility was not affected when med fly males were treated with melatonin before irradiation. Similar results were reported by El-Kholy (2009) who studied the biological effects of vitamin C applied to larval diet on the normal and irradiated med fly, also the study extended to illustrate the increase in the irradiated males' competitiveness values by increasing the vitamin concentration combined with gamma irradiation. Sylimarin also increased the competitiveness values of the med fly at 70 and 90 Gy (El-Kholy, 2008). Martin-Romero et al. (2001) revealed that the number of eggs laid increased twice when both male and female flies of *Drosophila* were maintained on selenium supplemented diet. However, the E-Selen, 0.5 g, supplemented to larval diet decreased the number of eggs laid daily per female that mate with E-Selen treated males and the larval diet treatment with 1.5 g E- Selen increased the percent of adult emergence but the sex ratio was not affected (El-Akhdar et al., 2012). The E-selen addition to the larval diet as antioxidant showed a relatively increased of irradiated adult males survival due to increment of the Linoleic acid concentration of the irradiated pupae and consequently increment of plasma membrane fluidity which it is essential to cell to accomplish its biological functions (Zaghloul et al., 2011).

It may be suggested that the observed sterility was due to the fact that irradiation of a given cell induced lipid peroxidation as the free radicals attack the cell membrane. Also the irradiation at 90Gy affects the polyunsaturated fatty acids that contain multiple double bounds and hence spermatozoa required high polyunsaturated fatty acid content to provide the plasma membrane with the fluidity essential at fertilization. Thus, the spermatozoa will be vulnerable to reactive oxygen species attack and oxidative stress which have clear associations with reduced fertility (Garg and Mahajan, 2006; Claire et al., 2007).

Biochemical study:

El-Kholy (2009) recorded that the supplementation of vitamin C at concentrations of 1000 and 2000 mg/kg in the larval rearing media of med fly has no significant effect on the levels of total lipids, total carbohydrates and total protein of both med fly males and females compared to the control group. The authors found a significant decrease in total carbohydrates and total protein in the irradiated groups and concluded that the combined effect of vitamin C with gamma irradiation had an obvious protective effect at the high concentration (2000 mg/kg) of vitamin C.

The disappearance of the proteins may be attributed to the presence of free radicals which could increase protease activity, leading to protein hydrolysis in the

irradiated flies. Moreover, Nestel et al. (2003) found that the lipid content was decreased significantly from the onset of metamorphosis until the adult emergence of *C. capitata* while the glycogen content dropped to a low level at the time of adult ecdysis. Meanwhile, the protein levels were reduced during the pupal stage and increased slightly at the end of this stage.

However, from the above data in the present study, protein and lipid contents indicated a significant fluctuation in their concentrations, which increased or decrease significantly. This indicated that the larvae or pupae can compensate for any change in proteins and lipids during the its life span activity.

The obtained results on the effect of different SS concentrations on the total protein, total lipids, total carbohydrates and glucose of the 3rd larval instar tissue of *C. capitata* was in accordance with the results of Lipinski et al. (2008) and Schmolz et al. (2005), due to the storing of carbohydrates by the late larval instars for further development. Also the same authors stated that the carbohydrate content was decreased in late pupae of *Apis mellifera.* Meanwhile, Cavalcante and Cruz-landim (2004) recorded an increase of different proteins from larvae to pupae of *Apis mellifera* but a significant decrease during pupation. The obtained results also agree with the results of Amin et al. (1996) who recorded a significant reduction in total protein and total lipids with the increase in gamma dose from 40 to 60 to 80 Gy in newly emerged male and female of *Culex pipiens.* The same authors found that the total carbohydrate levels at 80 Gy showed a significant increase in males but a significant decrease in female. So, it may be suggested that these changes may be due to the change in the feeding behavior. Gabarty and Mahmoud (2015) reported a significant decrease in the total protein and total lipids in males of *Rhynchophorus ferrugineus* when full grown pupae were irradiated with Gamma Cell-40 (Cesium -137) at doses of 10, 15, and 20 Gy. They also reported a clear significant increase in the total carbohydrates at doses of 10 Gy and 15 Gy followed by an obvious reduction at the dose of 20 Gy. They suggested that the main effect of gamma irradiation on proteins was represented by the decrease in protein synthesis, the change in protein chemical structure or affecting the translation process of proteins. They also concluded that gamma irradiation reduced the absorption of fats and fat soluble vitamins.

CONCLUSION

The SIT is a biological eco-environmental friendly method of med fly suppression or elimination into the area-wide leads to that leaves no residual effect in the environment. A better understanding of med fly biological and biochemical aspects introduces a new vision in pest management among the IPM program. It may also be concluded that the addition of SS at the concentrations of 5×10^{-6}, 10^{-5}, 5×10^{-5} M as antioxidant to the larval rearing media of the *C. capitata* could improve the biological, biochemical, and even the cytological disturbances occurred by gamma irradiation, hence improving the adult performance and the activities of sterile males in the SIT programs.

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