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Fatty and Amino acids composition of *Bactrocera zonata* as affected by substerilizing and sterilizing doses of Gamma radiation

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Abstract
Effect of gamma radiation on the whole body contents of fatty and amino acids were investigated for peach fruit fly, *Bactrocera zonata*. The statistical analysis of the data indicated that the quantities of fatty and amino acids in the whole body of the adults of *B. zonata* were affected significantly. Data obtained indicated that the free fatty acids decreased when males irradiated with substerilizing dose 70Gy while increased in the sterilizing dose 90Gy in comparing to unirradiated males. In case of female the total fatty acids were increased by increasing dose of gamma radiation, Female showed higher total fatty acids contents than males. The irradiated males and females have maintained higher free amino acids content than the unirradiated once except male irradiated with 70Gy. Amino acid content in the resulting flies from irradiated pupae is greatly affected in all acids in males and females.

Keywords: *Bactrocera zonata*, peach fruit fly, gamma radiation, fatty acids, amino acids

1. Introduction
Peach fruit fly, *Bactrocera zonata* Saunders (Diptera: Tephritidae) is a polyphagous pest and considered one of the most serious pests of most fruits and vegetables, but is particularly a destructive pest of guava, mango, peach, apricot, fig and citrus. It is a significant destructive pest in many countries [1]. In Egypt, *B. zonata* is now established, widespread and considered as a quarantine pest**.**
One of the criteria for successes of this technique is gamma-sterilized males compete favorably with normal males, the origin of the idea of using sterilization male insects is intimately related to the successful eradication of the screw worm fly, *Cochliomyia hominivorax* from the island of Curacao, U.S.A. by the release of sterilized adult males by irradiation [2].
In insects, the synthesis of fatty acids mainly takes place in fat bodies and elsewhere from amino acids, sugars and simpler fatty acids. Lipids were the major food reserves of insects, probably occurring in the form of triglycerides in which the fatty acids are combined with glycerols. Fatty acids combined in the triglycerides were usually long acid chains, both saturated and unsaturated with variable proportions [3].
Energy stored in the fat bodies of insects was in the form of triglycerides and is released upon insect demands. The degree of unsaturation of the fatty acids associated with the phospholipids was very important to regulate the fluidity of the cell membranes. In contrast, the structure of triglycerides was less important, physiologically, and so it may be more variable and influenced by diets [4].
Proteins provide a chief structural element of the muscles, glands and other tissues. The balance between protein amino acids and free forms is particularly important [5]. Amino acids, usually present as protein, are required for the production of tissues and enzymes. The absence of any one of the amino acids usually prevents growth [6].
The present work was designed to evaluate the relation between fatty and amino acids contents in the whole body of *B. zonata* in both sexes and the degree of sterility.

2. Materials and Methods
2.1 Rearing and irradiation technique: The original colony of Peach fruit fly, *B. zonata* was obtained from Department of Natural Products, National Center for Radiation Research and Technology, Cairo, Egypt.

The insect was reared under laboratory conditions of 25± 2 ºC temperature; and 60± 5% relative humidity. Five days old pupae of peach fruit fly were irradiated with substerilizing and sterilizing doses of gamma radiation (70 and 90Gy) for male and (30 and 50Gy) for female, respectively [7]. The irradiation technique was performed in 2016, using Gamma Cell-40 (Cesium-137 Irradiation Unit), at National Center for Radiation Research and Technology. The dose rate was 0.714 rad/sec. at the time of experimental research.

2.2 Fatty acid analysis: Free fatty acids were estimated using the method described by Sadasivan and Manickam [8]. Gas chromatograph (hp 6890) equipped with innowax-crosslinked polyethyelene glycol column 30m was used for deferential fatty acids analysis.

2.3 Amino acids analysis: Total amino acids were assayed by ninhydrin reagent according to the method described by Lee and Takabashi [9]. For deferential analysis, sample of 50- 100 mg of were dried, defatted and prepared according to AOCA [10] and Baxter [11]. High Performance Amino Acid Analyzer, Biochrom 20 (Auto sampler version) Pharmacia Biotch constructed at NCRRT was used for the analysis. Data analysis of chromatogram was done by EZChrom™ Chromatography Data System Tutorial and User's Guide-Version 6.7.

3. Results

3.1 Free fatty acids determination (FFA): The FFA concentration in male and female adults of *B. zonata* unirradiated and gamma irradiated as 5 days old pupae shown in Table (1). The FFA significantly decreased when males irradiated with substerilizing dose 70Gy (993.3µg), In contrast the FFA significantly increased when the sterilizing dose 90Gy was used in comparing to unirradiated males (1241.7µg).

Table 1: Free fatty acids of *Bactrocera zonata* adult irradiated as 5 days old pupae.

Male		Female	
Doses	**Free fatty acids (µg triolein/g.b.wt)**	**Doses**	**Free fatty acids (µg triolein/g.b.wt)**
Control	1241.7±20.9 [a]	Control	1352±21.6 [a]
70Gy	993.3±17.6[b]	30Gy	1459±22.7[a]
90Gy	1780±15.3[b]	50Gy	1793.7±34.5[b]

- Each value represents the mean of 5 replicates for each group ± S.E.
- Letters indicate the variance between the means (Duncan's multiple range test).
- Means with the same letters in same column were not significantly different.

Free fatty acids were significantly increased by increasing dose in female adults irradiated as 5 days old pupae. The FFA increased from 1352µg in control to 1459 and 1793.7µg in 30 and 50Gy, respectively. Female showed higher FFA contents than males except in case of sterilizing dose (50Gy); where males contained higher FFA than females.

The fatty acids contents detected in the total body of the adult of *B. zonata* irradiated as 5 days old pupae were mainly seven, whose chemical structures are presented in Table (2).

Table 2: Chemical structures of the seven fatty acids detected in the total body homogenate of in the adult of *Bactrocera zonata*.

Name	Fatty acid	Chemical Structure
C14:0	Myristic	$CH_3(CH_2)_{12}COOH$
C16:0	Palmitic	$CH_3(CH_2)_{14}COOH$
C18:0	Stearic	$CH_3(CH_2)_{16}COOH$
C22:0	Behenic	$CH_3(CH_2)_{20}COOH$
C16:1	Palmitoleic	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
C18:1	Oleic	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
C18:2	Linolic	$CH_3(CH_2)_4CH=CHCH_2CH=CH\ (CH_2)_7COOH$

Effect of gamma radiation doses on the fatty acids components of male adults of *B. zonata* that emerged from pupae irradiated as 5 days old given in Table (3). Data showed that the saturated fatty acids detected in the homogenate of the unirradiated males of *B. zonata* were 16:0 (Palmitic) and 18:0 (Stearic), while the unsaturated fatty acids were 16:1 (Palmitoleic), 18:1 (Oleic) and 18:2 (Linolic). The relative amounts of total saturated fatty acids increased from 11.02% in control to 13.36 and 11.9% in 70 and 90Gy, respectively. In contrary the relative amounts of total unsaturated fatty acids decreased from 80.17% in control to 67.37 and 75.76% in 70 and 90Gy, respectively.

It was noticed that C14:0 (Myristic) and C22:0 (Behenic) appeared in case of 90 and 70Gy with relative amounts 1.24 and 2.18%, respectively. It can be seen from the Table (3) that male adults irradiated as 5 days old pupae contain relative amounts of C16:0 less than in unirradiated males, the position was inversed in C18:0. The relative amounts of unsaturated fatty acids (C16:1, C18:1 and C18:2) in irradiated adults male of *B. zonata* decreased from the control except in case of C16:1 when males irradiated with 70Gy (31.69%) in comparing with control (24.47%).

Table 3: Relative percentages of saturated and unsaturated fatty acids detected in the adult male of *Bactrocera zonata* irradiated as 5 days old pupae.

Fatty acids / Dose	Saturated (%)				Total	Unsaturated (%)			Total
	14:0	**16:0**	**18:0**	**22:0**		**16:1**	**18:1**	**18:2**	
Control	0.0	3.17	7.85	0.0	11.02	24.47	48.41	7.29	80.17
70Gy	0.0	3.03	8.15	2.18	13.36	31.69	30.34	5.34	67.37
90Gy	1.24	2.61	8.05	0.0	11.9	22.93	45.88	6.95	75.76

Data in Table (4) showed the effect of irradiated female adults as 5 days old pupae with substerilizing and sterilizing doses on fatty acids composition. The saturated fatty acids, which found in unirradiated females of *B. zonata* were 16:0 and 18:0, while the unsaturated fatty acids were 16:1, 18:1 and 18:2. The relative amounts of total saturated fatty acids

increased from 10.27% in control to 16.48 and 18.54% in 30 and 50Gy, respectively. In contrary the relative amounts of total unsaturated fatty acids decreased from 78.07% in control to 74.70 and 72.59% in 30 and 50Gy, respectively. The fatty acid C22:0 (Behenic) was disappeared in unirradiated female adults and presented in irradiated female adults as 5 days old pupae, the relative amounts were 4.68 and 8.24% at doses 30 and 50Gy, respectively.

It can be seen from the Table (4) that female adults irradiated as 5 days old pupae contain relative amounts of C16:0 less than in unirradiated females, but in case of C18:0 the relative amounts increased from 8.75% (control) to 9.76% (30Gy) and decreased to 8.17% (50Gy). The relative amounts of unsaturated fatty acids C16:1 and C18:1 in irradiated female adults of *B. zonata* decreased from the control at the both doses. The position was inversed in C18:2, the relative amounts of this fatty acids increased from 3.78% in control to 5.01 and 4.62% at doses 30 and 50Gy, respectively.

Table 4: Relative percentages of saturated and unsaturated fatty acids detected in the adult female of *Bactrocera zonata* irradiated as 5 days old pupae.

Fatty acids / Dose	Saturated (%)			Total	Unsaturated (%)			Total
	16:0	18:0	22:0		16:1	18:1	18:2	
Control	1.52	8.75	0.0	10.27	24.62	49.67	3.78	78.07
30Gy	2.04	9.76	4.68	16.48	22.55	47.14	5.01	74.70
50Gy	2.13	8.17	8.24	18.54	23.86	44.11	4.62	72.59

3.2 Free amino acids determination (FAA): The results presented in Table (5) indicated that the FAA content of males and females was affected by exposing adults as 5 day old pupae to substerilizing and sterilizing doses of gamma radiation. In males, FAA was significantly decreased after irradiation adults as 5 day old pupae with the substerilizing dose 70Gy as compared to control. On the other hand, the sterilizing dose (90Gy) significantly increases the FAA in comparison to control. In Female, the both radiation doses significantly increased the FAA when compared to control. In general, female presented more FAA than male in control and irradiated samples.

Table 5: Free amino acids of *Bactrocera zonata* adult irradiated as 5 days old pupae.

Male		Female	
Doses	**Free amino acids (µg alanine/g.b. wt)**	**Doses**	**Free amino acids (µg alanine/g.b. wt)**
Control	4.6±0.3	Control	4.9±0.2
70Gy	3.6±0.2[a]	30Gy	8.6±0.2[b]
90Gy	9.8±0.3[b]	50Gy	11.5±0.4[b]

Legends as in table (1).

The amino acids constituents of males and females irradiated as 5 day old pupae with substerilizing and sterilizing doses are shown in Tables (6 and 7).

Table (6) shows that the effects of pupal irradiation as 5 day old at the dose levels 70 and 90Gy on the concentration percentage of amino acids in the emerged male adults, exhibited a wide range of variations. The total amino acids contents of males irradiated as 5 day old pupae with 70 and 90Gy were 11.22 and 4.33 mg/g of body weight, respectively, while it was 141.41 mg/g body weight in the unirradiated males. It is clear that the irradiated males have maintained lower protein content than the unirradiated males.

Data indicated that the normal male proteins were rich in their contents of glutamic, proline, leucine, tyrosine and arginine, the level of these amino acids were 13.43, 18.17, 11.06, 11.06 and 13.43mg/g, respectively, and extremely poor in cystine and methionine, the level of these amino acids were 0.79 and 3.95mg/g, respectively. Males irradiated as 5 day old pupae induced a decrease in the concentration of all amino acids. For example, the levels of aspartic acid were 0.99 and 0.33mg/g when males irradiated as 5 day old pupae with substerilizing and sterilizing doses, respectively, in comparing to 9.48mg/g in the control.

Table (7) shows that the effects of pupal irradiation as 5 day old at the different doses 30 and 50Gy on the concentration percentage of amino acids in the emerged adult females, exhibited a wide range of variations. The total amino acids contents of females irradiated as 5-d-old pupae with 30 and 50Gy, were 56.44 and 3.33mg/g body weight, respectively, while it was 127.4mg/g body weight in the unirradiated females. It is clear that the irradiated females have maintained lower protein content than the unirradiated females.

Table 6: Amino acids content of *Bactrocera zonata* males irradiated as 5-d-old pupae with sterilizing and substerilizing doses.

Peak No.	Amino acids	Control	70Gy	90Gy
1	Aspartic	9.48	0.99	0.33
2	Thrionine	5.53	0.44	0.2
3	Serine	5.53	0.44	0.2
4	Glutamic	13.43	1.37	0.45
5	Proline	18.17	1.10	0.3
6	Glycine	7.11	0.61	0.23
7	Alanine	9.48	0.82	0.25
8	Cystine	0.79	0.05	0.25
9	Valine	6.32	0.44	0.2
10	Methionine	3.95	0.33	1.3
11	Isoleucine	6.32	0.44	0.2
12	Leucine	11.06	0.83	0.35
13	Tyrosine	11.06	0.77	0.35
14	Phenyl alanine	7.11	0.55	0.23
15	Histedine	5.53	0.38	0.18
16	Lysine	7.11	0.61	0.28
17	Arginine	13.43	1.05	0.45
Total (mg/g)		141.41	11.22	4.33

Data indicated that the female proteins were rich in their contents of aspartic, glutamic, alanine, valine, leucine, tyrosine, lysine and arginine the level of these amino acids were 39.6, 59.6, 28.8, 27.4, 37.2, 26.4, 28.4 and 27.2 mg/g, respectively, and extremely poor in proline, cystine, methionine and histedine, the level of these amino acids were 4.55, 0.0, 8.8 and 14.4 mg/g, respectively. Females irradiated as 5 day old pupae lead to decrease in the concentration of aspartic, threonine, serine, glutamic, glycine, methionine, Isoleucine, leucine, tyrosine, phenylalanine, lysine and arginine except females irradiated as 5-d-old pupae with 30Gy, these amino acids more than in the unirradiated

females. For example, the levels of glutamic acid were 6.27 and 0.31mg/g when females irradiated as 5 day old pupae with substerilizing and sterilizing doses, respectively, in comparing to 13.65mg/g in the control.

Table 7: Amino acids content of *Bactrocera zonata* females irradiated as 5-d-old pupae with sterilizing and substerilizing doses.

Peak No.	Amino acids	Control	30Gy	50Gy
1	Aspartic	10.01	3.14	0.2
2	Thrionine	5.46	2.28	0.15
3	Serine	7.28	2.28	0.14
4	Glutamic	13.65	6.27	0.31
5	Proline	15.47	9.41	0.22
6	Glycine	4.55	2.85	0.19
7	Alanine	5.46	3.14	0.24
8	Cystine	0.91	0.29	0.02
9	Valine	6.37	2.28	0.15
10	Methionine	0.91	1.43	0.10
11	Isoleucine	5.46	2.57	0.19
12	Leucine	13.65	4.28	0.27
13	Tyrosine	7.28	3.71	0.27
14	Phenyl alanine	5.46	2.57	0.19
15	Histedine	5.46	2.0	0.15
16	Lysine	9.10	2.85	0.20
17	Arginine	10.92	5.13	0.34
Total (mg/g)		127.4	56.44	3.33

4. Discussion

Fatty acids serve various functions in insects. They are the primary energy source during periods of nonfeeding, such as diapause [12] and long migratory flights [13], and during nonfeeding stages of development [14]. Fatty acids serve as precursors in the biosynthesis of pheromones [15], waxes and eicosanoids as structural components of membranes and defensive secretions [16], and they are essential components in the function of the cuticle [17]. Because the relative importance of each of these functions varies throughout development, the rate of fatty acid biosynthesis also would be expected to fluctuate according to physiological need.

In this study, the exposure of pupae of the peach fruit fly to all doses of gamma radiation significantly decreased the free fatty acids. Same investigation was obtained by Abd Elghffar *et. al.* [18] on *Ephestia cautella*. In addition, a decrease in the concentrations percent of the relative amounts of unsaturated fatty acids (C18:1 and C18:2) in irradiated adults male of *B. zonata* was obtained when compared to the control. Claire *et al*, [19] Found that spermatozoa require a high polyunsaturated fatty acid content to provide the plasma membrane with fluidity, essential at fertilization. However, this makes spermatozoa vulnerable to attack by reactive oxygen and oxidative stress which have clear associations with reduced fertility.

The aforementioned results revealed that the relative amount of unsaturated fatty acids of normal males to normal females were in favor to males; that agreed with El- Orabi [20] on *Eumerus amoenus* and El-Orabi and Ghareib [21] on *Trogoderma granarium*. It was cleared that the irradiated males and females of *B. zonata* maintained lower amino acids content than the unirradiated. This decrease may regarded the investigation of Hamza [22]; who found that lower protein content was induced by gamma radiation in *E. amoenus*.

Due to the proline role in energy; since it is a derivative of glutamic acid and could enter the citric cycle after deamination to α-ketoglutaric acid [23], therefore, the varying concentration of proline at the different dose levels may probably due to the varying rates of utilizing the amino acid as a source of energy in the repair mechanisms (Bursell [23] on *Glossina morsitans*; Grace and Brzostowski [24] on *Antheraea eucalypti* and Landureau and Jolles [25] on *Periplaneta americana*).

Richardson and Myser [26] found that the concentration of alanine decreases after exposure of the honey bee prepupae, *Apis mellifera* and the larvae of the wax moth, *Galleria mellonella* to high doses of X- irradiation (75 Krad). They stated that the decrease in alanine is not consistent with the hypothesis X- irradiation causes a decrease in the rate of protein synthesis or increased proteolytic activity which ultimately results in an increase in free amino acid concentration. The decrease in protein synthesis could be due to that alanine serves as an alternative energy source through its conversion to pyruvate. So, the radiation induced changes could be a manifestation on (1) decreased capacity of the tissues to utilize amino acids with regard to protein synthesis, (2) the release of free amino acids due to increased protein degradation and (3) radiation induced changes in normal metabolic pathways involving amino acids. Alanine possesses a very active transaminase and plays an important role in glucose production from pyruvic acid through transamination. The glutamic acid- alanine transaminase system serves as the main pathway in both deamination of glutamic acid to ketoglutaric acid and the conversion of pyruvic acid to alanine [27]. Swenson *et al.* [28] suggested that the conversion of amino acids to glutamic acid for use in transamination reactions may be partially responsible for the survival of X- irradiated cells. Koval *et al.* [29] Stated that there was inverse relationship between glutamic acid consumption and alanine production after exposure of Trichoplusia in cells to X- ray doses. In this process, the amino group from glutamic acid is transferred to pyruvic acid to from alanine and α- Ketoglutaric acid, which may then be utilized in the tricarboxylic acid cycle for ATP production.

Buscarlet *et al.* [30] Studied the effect of gamma irradiation and starvation on the free amino acid content in *Tribolium confusum* male. They found that restoration of free amino acid after starvation was possible, but after irradiation, restoration of amino acids is much lower especially at high dose levels (63-504 Gy). Also, the results confirm the inhibitory effect due to damage caused in mid- gut epithelium and to the consequent disturbances of cell renewal, nutrient absorption, secretion of digestive enzymes and other possible processes in intermediary metabolism.

The observed decrement of free fatty acid and free amino acids explained by El-Naggar [31]; who proclaimed that gamma radiation induced biochemical alteration directly by breaking down the bonds of the target molecules or by radiolysis of water which is present in animal body. This generates free radicals such as OH^-, H^+ that attacks a lipid molecule is adjacent to the double bonds.

Eventually, the sterile males provide a successful method for pest control. Many attentions are needed to discover a bioindicators or chemo prints for sterility. The aforementioned results revealed that gamma radiation has significantly affected the differential free amino acid and free fatty acids.

5. Conclusion

Ultimately, it could be concluded that the differential free amino acid or free fatty acids could be used as a bioindicators or chemo prints for detecting the substerile or sterile male in the released field.

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