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Bio-6 Amino Acid Content of the Gamma Irradiated Cotton Leaf-Worm, *Spodoptera littoralis* (Boisd.)

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ABSTRACT

The effects of gamma irradiation on amino acid content of the cotton leafworm *Spodoptera littoralis* was studied. The identified amino acids in the total body tissue of male moths were Theronine, Serine, Glutamic, Glaycine, Alanine, Valine, Cystine, Methonine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Lysine, Histidine and Arginine. The irradiation of full grown male pupae with doses 100, 200 and 300 Gy decreased the total quantity of amino acids and the amount of most individual amino acids in male moths of P_1 or F_1 generations with some exceptions for Threonine, Alanine, Glycine, Serine, Valine, Cystine and Methionine which were increased. The effect of irradiation on amino acid content of the reproductive system tissues for each male or female were also studied. The results indicated that irradiation decreased the total quantity of amino acid content of both sexes by increasing the dose and males were more radiosensitive than females. Also, irradiation decreased the amount of individual amino acids in both sexes with certain exceptions, e.g. Alanine, Methionine and Tyrosine which increased in the reproductive system of male, and Methionine which increased by more than four times as control. The amino acid content was determined as well in F_1 egg progeny, which was produced from irradiated males. Irradiation doses (100, 200 and 300 Gy) decreased the total quantity of amino acids, and all individual ones except Cystine. The greatest reduction (54.9%) was observed with Lysine at 300 Gy as compared to control.

Key Words: Gamma Irradiation / Amino Acids / Spodoptera littoralis / Lepidoptera / Biochemistry.

INTRODUCTION

The sterile insect release technique of suppressing or controlling insect populations was among the outstanding recent contributions to applied economic entomology. The success of this method of insect pest control depends, in fact, on the production of sterile males which should be active enough to compete effectively with normal males in finding their mates. The competitive moths in Lepidoterous species will result when irradiation doses are substerilizing, North[1]. Moreover, individuals treated at substerilizing doses produce offsprings which are nearly or completly sterile. This phenomenon is known as inherited or F_1 sterility. Proverbs[2] was the first to report the presence of inherited sterility in the F_1 progeny of the codling moth *Carpocapsa pomonella* (L.) when parental male moths, irradiated as pupae with 300 Gy and were mated with non-irradiated females.

The cotton leafworm *Spodoptera littorais* (Boisd.), is the most important pest of cotton in Egypt. Sterilization by irradiation as means of control was studied by Hassan[3], Sallam[4,5], Hosny et al.[6], Wakid and Hayo[7], Souka[8], El-Shall[9] and Sallam and Ibrahim[10].

The objectives of this investigation are to study the effect of irradiating male pupae on the amino acid content of total body tissues of the resulting parental male moths and F_1 male adults. Also the studies were extended to include the effect of irradiaton of pupae on the amino acids in the reproductive system tissues for parental males or female as well as in the produced eggs of F_1 generation.

MATERIALS AND METHODS

Egg-masses of the cotton leafworm, *Spodoptera littoralis* (Boisd.) originally obtained from the cotton fields of Giza Governorate, were kept in the laboratory under 25-32°C and relative humidity of 60-75%. Larvae were fed on castor oil plant leaves *Ricinus communis* and reared in clean glass jars. The full grown larvae were transferred to other jars furnished with saw dust containing 20% moisture to help in formation of pupae. The pupal sex were indentified, then the pupae were kept on a filter paper in petri dishes.

Pupal treatments with gamma rays were achieved by using the model 2 Cobalt-60 irradiation unit (Gamma cell 200). Its dosimetry was then 6.969 rad per sec. with ±5% error. Full grown male pupae were gamma irradiated with doses of 100, 200 and 300 Gy. The resulting adult males were paired with normal females to obtain F_1 eggs.

The amino acid content was determined in the total body tissue, reproductive system of adults as well as in the F_1 eggs.

1- Extraction of Total Body Tissues:

The amino acid content was determined in 3-days old adults. The treatments were :

i. P_1 male moths irradiated as full grown pupae with 10, 20 or 30 Krad.

ii. F_1 male moths irradiated as full grown male pupae with 10 or 20 Krad.

iii. Unirradiated male moths.

2- Extraction of Reproductive System Tissues:

Amino acid content was determined in the reproductive system tissues of either 3-days old male or female adults irradiated as full grown pupae with 10, 20 or 30 Krad. Also, the reproductive system tissues of unirradiated male or female were taken as a control.

3- Extraction of Eggs:

The amino acids content was determined in F_1 eggs produced from parental males irradiated as full grown pupae with 10, 20 or 30 Krad. Unirradiated eggs were used as a control.

Amino acid determination was performed by the Egyptian Danish Protein Laboratory belonging to the Egyptian Agriculure Organization according to the method of Moore *et al.*[11].

Procedure:

i. Samples of each treatment were dried in an oven at temp. 80°C till the sample reaches a constant weight.

ii. Dry samples of 400 mg were weighed in ampoules for acid hydrolysis and 7 ml of 6 N HCl was added.

iii. Ampoules were sealed under vacuum, samples were then heated in an oven at 110°C for 16 hours.

iv. The sealed ampoules were then opened and the hydrochloric acid was removed under vacuum. Sodium citrate (buffer pH 2.20) was used to dissolve samples and diluted to the required volume.

v. Filtered hydrolyzate was used for the amino acid analysis.

Beckman amino acid analyzer model 121 was used for the entire body tissues, in which a sample of 0.5 ml volume was injected. One complete analysis took approximately 4 hours. Type PA. 35 custome resin was used for basic amino acids, and type M. 82 spherical ion exchange resin was used for neutral and acidic amino acid determination. Beckman 7300 system was used for detection of the amino acids in the reproductive system tissue and eggs, in which a sample of 50 µl volume was injected.

RESULTS AND DISCUSSION

1- Total Body Tissue of Moths:

The effects of male pupae irradiation on amino acid content in the total body tissues of male moths at P_1 and F_1 progeny is shown in Table (1) and (2). Data revealed that, fifteen amino acids were indentified in normal and irradiated male at parents and F_1 progeny. Detected amino acids were Threonine, Serine, Glutamic, Glysine, Alanine, Valine, Cystine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Lysine, Histidine, and Arginine. Irradiation generally had an effect on both quality and quantity of amino acid content. The degree of effect differed mainly according to irradiation dose. All the fifteen amino acids appeared in parents and F_1 progeny moths after the irradiation of pupae with 100 Gy. However, Cystine and methionine disappeared after irradiation with 200 and 300 Gy or 200 Gy at parents and F_1 generation, respectively. A new unknown amino acid supposed to be Cystine + Methionine appeared in a position between the two acids on the chromatogram separation pattern. However, all applied doses decreased the total quantity of the detected amino acids for both parents and F_1 progeny moths.

In general, the dose of 100 and 200 Gy reduced the amount of all the amino acids detected in male parent with some exceptions for Serine and Valine which showed a slight higher level at 100 Gy (Table 1). The degree of reduction at 200 Gy was relatively more pronounced than that found at 100 Gy, especially for Tyrosine, Isolecucine, Histidine, Lysine, Leucine, Serine, Glutamic, Glysine, Threonine, Alanine and Valine. However, the effect of 300 Gy on male parents differed according to the type of detected amino acids: i.e., Serine, Threonine, Alanine, Valine and Glysine proved to be in higher amounts for irradiated moths than control in descending order. The other amino acids were decreased in irradiated moths; where the degree of reduction at this dose was lower somehow than at 100 and 200Gy.

Data in Table (2) showed that, Alanine, Methionine, Threonine, Glysine, Cystine, Valine, and Serine recorded higher level than control espectially Alanine, which showed 78% than in control at 100 Gy. Irradiation by 200 Gy reduced all amino acids except Serine, and Alanine which showed an increase by 1.4 and 23.7% than control, respectively.

2- Reproductive System Tissues:

The changes in the amino acids content in the reproductive system tissues of 3-days old adults irradiated as full grown male pupae with 100, 200 or 300 Gy are shown in Tables (3 and 4). The data indicated that irradiation generally had a decreasing effect on the total quantity of amino acid contents of both sexes. This decrease was increased by increasing irradiation dose. The rate of decrease was more pronounced with male than female where it, decreased approximately by 19, 24 and 24% than

Table (1): Amino acid content of male adults *Spodoptera littoralis* (Boisd.) irradinted as full grown pupae with gamma rays.

Amino Acids	Amino acid content							
	Control		100 Gy		200 Gy		300 Gy	
	amount	relative %	amount	relative %	amount	relative %	amount	relative %
Threonine (THR)	716.9	100	488.3	68.1	529.8	73.9	1043.1	145.4
Serine (SER)	258.0	100	270.5	104.8	161.4	62.5	576.6	233.4
Glutamic (GLU)	583.0	100	515.8	88.4	412.4	70.7	444.2	76.2
Glysine (GLY)	202.6	100	179.0	88.3	148.2	73.1	213.8	105.5
Alanine (ALA)	317.0	100	303.5	95.7	256.1	80.7	453.7	143.1
Valine (VAL)	177.5	100	184.5	103.9	152.3	85.8	202.9	114.3
Cystine (CYS)	178.5	100	69.0	30.6	0	0	0	0
Methionine(MET)	76.8	100	58.0	75.5	0	0	0	0
Isoleucine (ILE)	733.3	100	162.2	22.1	130.1	17.7	279.9	38.1
Leucine (LEU)	894.5	100	504.9	56.4	381.0	42.5	655.0	73.2
Tyrosine (TYR)	943.4	100	208.4	22.0	140.4	14.8	444.4	47.1
Phenylalanine(PHE)	740.9	100	115.3	15.5	121.7	16.4	294.7	39.7
Lysine (LYS)	657.5	100	200.4	30.4	169.8	25.8	343.8	52.2
Histidine (HIS)	612.2	100	176.0	28.7	142.9	23.3	463.4	43.0
Arginine (ARG)	676.1	100	224.3	33.1	219.0	32.3	391.2	57.8
Cystine+Methionine	0	0	0	0	15.6	0	192.0	0
Total	7768.9	100	3660.1	46.6	2980.7	38.3	5799.0	74.6

amount: mg amino acid / g dry matter.

$$\% \text{ relative} = \frac{\text{amount of A.A. in irradiated sample}}{\text{amount of A.A. in control}} \times 100$$

Table (2): Amino acid content of F_1 male adults *Spodoptera littoralis* (Boisd.) irradiated as parental full grown male pupae with gamma rays.

Amino Acids	Amino acid content					
	Control		100 Gy		200 Gy	
	amount	relative %	amount	relative %	amount	relative %
Threonine (THR)	716.9	100	916.7	127.8	386.5	53.9
Serine (SER)	258.0	100	268.1	103.8	261.6	101.4
Glutamic (GLU)	583.0	100	473.2	81.1	491.9	84.3
Glysine (GLY)	202.6	100	239.2	118.0	163.8	80.8
Alanine (ALA)	317.0	100	564.3	178.0	392.4	123.7
Valine (VAL)	177.5	100	186.7	105.1	174.8	98.4
Cystine (CYS)	178.5	100	193.2	108.2	0	0
Methionine (MET)	76.8	100	100.1	130.3	0	0
Isoleucine (ILE)	733.3	100	253.8	34.6	167.5	22.8
Leucine (LEU)	894.5	100	655.2	73.2	381.2	42.6
Tyrosine (TYR)	943.4	100	207.8	22.0	174.5	18.5
Phenylalanine (PHE)	740.9	100	143.2	19.3	91.7	12.3
Lysine (LYS)	657.5	100	317.7	48.3	192.1	29.2
Histidine (HIS)	612.2	100	216.9	35.4	173.2	28.2
Arginine (ARG)	676.1	100	359.8	53.2	234.8	34.7
Cystine+Methionine	0	0	0	0	111.0	0
Total	7768.1	100	5095.9	65.5	3397.4	43.7

amount: mg amino acid / g dry matter.

$$\% \text{ relative} = \frac{\text{amount of A.A. in irradiated sample}}{\text{amount of A.A. in control}} \text{ X } 100$$

Table (3): Amino acid content of male adults *Spodoptera littoralis* (Boisd.) reproductive system irradiated as full grown pupae with gamma rays.

Amino Acids	Amino acid content							
	Control		100 Gy		200 Gy		300 Gy	
	amount	relative %	amount	relative %	amount	relative %	amount	relative %
Asparatic (ASP)	6378.1	100	4894.2	76.7	4198.3	65.8	4141.3	64.9
Threonine (THR)	2072.3	100	1786.5	86.2	1902.1	91.7	1861.3	89.8
Serine (SER)	2963.8	100	1915.8	64.6	1822.7	61.4	1771.6	59.7
Glutamic (GLU)	9532.0	100	5101.2	53.5	5208.5	54.6	5329.2	55.9
Proline (PRO)	2375.6	100	2916.9	122.7	1976.4	83.1	1933.6	81.3
Glysine (GLY)	2463.2	100	2504.0	101.6	2122.1	86.1	2100.6	85.2
Alanine (ALA)	2391.4	100	3589.4	150.0	2701.0	112.9	2723.9	113.9
Cystine (CYS)	218.1	100	218.1	100	121.3	55.6	100.4	46.0
Valine (VAL)	2501.2	100	2402.2	96.0	2388.8	95.5	2342.0	93.6
Methionine (MET)	262.5	100	839.7	319.8	1121.1	426.9	1116.8	425.4
Isoleucine (ILE)	2120.1	100	1863.0	87.8	1923.0	90.7	1896.7	89.4
Leucine (LEU)	4246.6	100	3612.6	85.0	3557.3	83.7	3546.1	83.5
Tyrosine (TYR)	1471.3	100	2226.1	151.3	1900.0	129.1	1889.6	128.4
Phenylalanine(PHE)	2392.0	100	1600.0	66.8	1888.0	78.9	1869.1	78.1
Histidine (HIS)	1482.7	100	1293.9	87.2	1289.5	86.9	1285.1	86.6
Lysine (LYS)	3538.0	100	2660.8	75.2	2932.3	82.8	2898.9	81.9
Arginine (ARG)	5239.9	100	2463.6	47.0	2184.9	41.6	2150.1	41.0
Total	51648	100	41889.9	81.1	39237.9	75.9	38956.9	75.4

amount: mg amino acid / g dry matter.

$$\% \text{ relative} = \frac{\text{amount of A.A. in irradiated sample}}{\text{amount of A.A. in control}} \times 100$$

Table (4): Amino acid content of female adults *Spodoptera littoralis* (Boisd.) reproductive system irradiated as full grown pupae with gamma rays.

Amino Acids	Amino acid content							
	Control		100 Gy		200 Gy		300 Gy	
	amount	relative %	amount	relative %	amount	relative %	amount	relative %
Asparatic (ASP)	4844.8	100	4117.2	84.9	4198.3	86.6	4141.3	85.4
Threonine (THR)	2199.3	100	1905.6	86.6	1902.1	86.4	1953.2	88.8
Serine (SER)	2147.5	100	1797.2	83.6	2126.5	99.0	1798.7	83.7
Glutamic (GLU)	6384.1	100	5359.3	83.9	5282.9	82.7	5329.2	83.4
Proline (PRO)	2294.3	100	2025.7	88.2	2305.8	100.5	1933.6	84.2
Glysine (GLY)	2545.8	100	2192.9	86.1	2475.7	97.2	2100.6	82.5
Alanine (ALA)	3278.8	100	2800.7	85.4	2701.0	82.3	2746.8	83.7
Cystine (CYS)	101.0	100	109.0	107.0	141.4	140.0	100.4	99.4
Valine (VAL)	2872.8	100	2423.9	84.3	2388.8	83.1	2344.0	81.5
Methionine (MET)	1218.4	100	1153.8	94.6	1121.1	92.0	1116.8	91.6
Isoleucine (ILE)	2191.0	100	1985.4	90.6	1923.0	87.7	1896.7	86.5
Leucine (LEU)	4062.8	100	3699.8	91.0	3557.3	87.5	3546.1	87.2
Tyrosine (TYR)	2198.0	100	2011.3	91.5	1900.0	86.4	1722.8	78.3
Phenylalanine (PHE)	2131.0	100	1982.4	93.0	1888.0	88.5	1869.1	87.7
Histidine (HIS)	1499.3	100	1323.5	88.2	1289.2	86.0	1285.1	85.7
Lysine (LYS)	3377.2	100	3123.8	92.4	2932.3	86.8	2898.9	85.8
Arginine (ARG)	2630.4	100	2177.5	82.7	2184.9	83.0	2150.1	81.7
Total	45977	100	40189.1	87.4	40282.3	87.6	38933.4	84.6

amount: mg amino acid / g dry matter.

$$\% \text{ relative} = \frac{\text{amount of A.A. in irradiated sample}}{\text{amount of A.A. in control}} \text{ X } 100$$

Table (5): Amino acid content of *Spodoptera littoralis* (Boisd.) F_1 eggs resulting from parental males irradiated as full grown pupae.

Amino Acids	Amino acid content							
	Control		100 Gy		200 Gy		300 Gy	
	amount	relative %	amount	relative %	amount	relative %	amount	relative %
Asparatic (ASP)	5564.2	100	4722.3	84.8	4764.9	85.6	4908.7	88.7
Threonine (THR)	2320.6	100	1958.0	84.3	1953.2	84.1	2129.5	91.7
Serine (SER)	2747.8	100	2324.8	84.6	2366.8	86.1	2526.6	91.9
Glutamic (GLU)	7830.8	100	6643.0	84.8	6996.0	89.3	7478.5	95.5
Proline (PRO)	2600.1	100	2182.2	83.9	2209.9	84.9	2541.4	97.7
Glysine (GLY)	2642.7	100	2210.9	83.6	2192.9	82.9	2598.4	98.3
Alanine (ALA)	3315.8	100	2790.6	84.1	2783.4	83.9	3036.5	91.5
Cystine (CYS)	169.0	100	150.2	88.8	159.9	94.6	184.2	108.9
Valine (VAL)	3124.8	100	2426.3	77.6	2501.2	80.0	2833.8	90.6
Methionine (MET)	1268.9	100	1074.2	84.6	1092.1	86.0	1068.2	84.1
Isoleucine (ILE)	2278.9	100	1915.5	84.0	1915.5	84.0	2062.4	90.5
Leucine (LEU)	4335.3	100	3636.8	83.9	3626.3	83.6	4019.9	92.7
Tyrosine (TYR)	2111.8	100	1797.5	85.1	1716.2	81.2	1978.7	93.6
Phenylalanine (PHE)	2081.5	100	1751.1	84.1	1744.5	83.8	1903.1	91.4
Histidine (HIS)	1876.0	100	1433.1	85.5	1482.7	88.5	1588.2	94.8
Lysine (LYS)	3625.0	100	3070.2	84.6	3193.0	88.0	1666.6	45.9
Arginine (ARG)	3151.2	100	2710.5	86.0	2742.2	87.0	2912.6	92.4
Total	50824.3	100	42797.8	84.1	43440.4	85.4	45437	89.3

amount: mg amino acid / g dry matter.

$$\% \text{ relative} = \frac{\text{amount of A.A. in irradiated sample}}{\text{amount of A.A. in control}} \text{ X } 100$$

control for male at 100, 200 and 300 Gy, respectively, while in the female it was decreased by 12.5, 12.0 and 15.0% than control, respectively.

Qualitative examination of the obtained data in Tables (3 and 4) revealed that, the recorded amino acids for reproductive system tissues of both sexes were. Asparatic, Threonine, Serine, Glutamic, Proline, Glysine, Alanine, Cystine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Lysine, and Arginine.

Data in Table (3) showed that, the three irradiation doses decreased the contents of Arginine, Glutamic, Serine, Threonine, Asparatic, Phenylalanine, Tyrosine, Isoleucine, Leucine, Histidine, and Valine. The most reduction was observed in the Arginine. The rate of Asparatic, Serine, Valine, and Arginine decreased by increasing the irradiation dose. On the other hand, the tested irradiation doses caused an increase in Alanine, Methionine and Tyrosine contents. The high rate of increase was observed in case of Methionine.

The dose of 100 Gy caused an increase in the rate of Proline which it increase up to 122.7% compared to 100% at control, while the same dose did not affect the content of Cystine and had a slight effect on the content of Glysine. On the other hand, the doses of 200 and 300 Gy caused a decrease in the contents of Cystein (55.6, 46.0%) Proline (83.1, 81.3%) and Glysine (81.1, 85.2%), respectively as compared to control (100%).

Data in Table (4) indicated that irradiation caused a reduction in the total quantity of amino acid content of female reproductive system recording, 87.4, 87.6 and 84.6% as compared to the control at 100, 200 and 300 Gy, respectively. Also, the reduction was observed in the contents of all detected amino acids due to irradiation with the three doses, except for Proline and Cystine.

Table (5) showed that, irradiation doses (100, 200 and 300 Gy) decreased the total quantity of amino acid content of F_1 eggs resulting from parental males irradiated as full grown male pupae. Also, it decreased all individual amino acids except Cystine, where a slight increase was observed at 300 Gy. The greatest decrease in amino acid content was observed in case of Lysine at 300 Gy where it went-down to 45.9% of control.

In general, it could be concluded that irradiation had an effect on amino acid metabolism of the whole body male moths, reproductive system of both sexes and F_1 eggs. This agreed with Po-chedly [12] who concluded that, irradiation caused a metabolic disturbance in the amino acids of mealworm embryos. Miltin and Wiygul [13], Richardson and Myser [14], Souka [8], Mohsin[15] and Abdel-Baky [16] reached to the same conclusion.

Amino acid content of the male reproductive system was highly affected than female, i.e. Cystine and Arginine were dropped in irradiated male to less than half of its control value, while Methionine increased by more than four times as control. Hence, it could be reported that these amino acids may play a specific role in the sterility phenonmenon of the cotton leafworm, where biological data concerning *Spodoptera littolaris* revealed that, males have a main role in inherited sterility [9]. It could be seen that irradiation caused severe reduction to Lysine in the F_1 eggs which might show some relationship between this acid and the development of the egg.

REFERENCES

(1) D.T. North, Annu. Rev. Entomol., 20: 167 (1975).

(2) M.D. Proverbs, Proc. Entomol. Soc. Ongario, 29: 5 (1962).

(3) A.M. Hassan, Preliminary studies on the feasibility of utilizing radiation induced sterility technique in the control of cotton leafworm *Prodenia litura* (F.), M. Sc. Thesis, Fac. of Agric., Cairo University (1967).

(4) H.A. Sallam, Suppression of the reproductive potential of the cotton leaf worm *Prodenia litura* (F.), M.Sc. Thesis, Faculty of Agric., Ain Shams University (1969).

(5) H.A. Sallam, Studies on the effects of gamma radiation on certain cotton worms in Egypt, Ph.D. Thesis, Fac. of Agric., Ain Shams University (1976).

(6) M.M. Hosny, A.M. Wakid, E.A. El-Badry and H.A. Sallam, Z. Ang. Entomol., 72: 205 (1972).

(7) A.M. Wakid and J.M. Hayo, Z. Ang. Entomol., 67: 331 (1974).

(8) S.R. Souka, Ph.D. Thesis, The effect of gamma radiation on the cotton leafworm Spodoptera littoralis (Boisd.), Fac. of Agric., Ain Shams University (1977).

(9) S.S.A. El-Shall, Biological and biochemical studied on the gamma irradiated cotton leafworm *Spodoptera littoralis* (Boisd.) Ph.D. Thesis, Fac. of Agric., Ain Shams University (1991).

(10) H.A. Sallam and S.M. Ibrahim, 4th Conf. Nuc. Sci. Appl., Vol.2, 422 (1988).

(11) S. Moore, D.H. Spachman and W. Stein, Anal. Chem.: 30, 1185 (1958).

(12) D.S. Po-Chedley, Progress Report. TID-19463, D'youville Coll., Buffalo 6P.(1963) Abstract: Bibliographical Series, No. 15. IAEA Vienna (1965).

(13) N. Miltin and G. Wiygul, J. insect Physiol, 16: 2271 (1970).

(14) B.L. Richardson and W.C. Myser, Radiation. Res. 54: 274 (1973).

(15) A. Mohsin, Experientia, 35.1072 (1979).

(16) S.M. Abd El-Baky, Effect of gamma irradiation on some stored product insects in Egypt, Ph.D. Thesis. Fac. Agric. Ain Shams University (1986).