# EFFECT OF LOW DOSAGES OF GAMMA IRRADIATION ON NUCLEIC ACIDS AND TOTAL PROTEINS CONTENT OF EPHESTIA CAUTELLA (WALKER) MOTHS AND THEIR GENERATIONS

### AMIN T.R. AND SALWA A. BOSHRA

- 1 Pest Physiology Department, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt.
- 2 Biological Applications Department, Nuclear Research Centre, Atomic Energy Authority, Cairo, Egypt

(Manuscript received Aug. 2001)

#### Abstract

Irradiation of *Ephestia cautella* (Walk.) full grown male pupae with substerilizing doses (150 and 250 GY) reduced deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content in male parents (P) and also in males and females of F1 and F2 progenies. Irradiation of full grown male pupae with 250 GY increased total protein content in parents, while decreased it in their F1 and F2 progenies. The reduction of both nucleic acids and total protein was higher in F2 progenies than F1 progenies than P.

Irradiation of full grown pupae with 150 and 25 GY induced inherited sterility, F1 males were more affected than F1 females. The possibility of correlation between the reduced number of progenies and the reduction of nucleic acids and total protein have been discussed.

# INTRODUCTION

The almond moth, *Ephestia cautella* (Walk.) is one of the serious pests of stored grain products. The control of lepidopteran pests by the sterile insect release technique (SIRT) has received much attention, and some results appear promising (Brower and Tilton, 1975). In Lepidoptera, the F1 progeny of an irradiated male with substerilizing doses have a greater degree of sterility than treated parents and more competitive with native moths than sterilized ones (El-Shall *et al.*, 1997).

In insects, coded genetic information is contained in deoxyribonucleic acid (DNA) molecule, it carries them from cell to cell and from generation to another. It is known that DNA molecule does not participate directly in the process of protein synthesis or

formation of genetic characters including the ability to reproduce, but employs different types of non-genetic ribonucleic acid (RNA) molecules for carrying its genetic information from the nucleus to the site of protein synthesis, i.e., ribosomes (Verma and Agrawal, 1985).

The present study was, therefore, conducted to determine the changes in the content of DNA, RNA and total protein in male parents and their progenies after treatment of *E. cautella* pupae with substerilizing doses (150 and 250 GY) of gamma irradiation. This might explain the biological damage produced by irradiation as represented by reduced number of progeny and apparent sterility inherited to filial generations.

#### MATERIALS AND METHODS

The test insects were taken from a laboratory culture of *E. cautella* that were maintained at 27±2°C and 65±5% R.H. and reared on a diet composed of 1 kg crushed wheat and 16 ml glycerin. The last instar larvae ready to pupate were collected, sexed and introduced into pupation rolls composed of corrugated cardboards. Full grown male pupae were irradiated with 150 and 250 GY in a 60Co gamma cell at the Technology Atomic Energy Authority using a dose rate of 2.3 Gray/min. Newly emerged male adults were allowed to mate with untreated females and oviposit in inverted jars with screen bottoms. Eggs which fell were collected daily. Five replicates were used for each dose level and the control (three pairs for each replicate). Samples of 100 eggs were placed in 1.3 liter jars containing standard diet and reared at the conditions mentioned before. The number of emerging F1 progeny was recorded for each 100 eggs. One day old F1 adults were selected for the following combinations: untreated (U) male x U female; F1 male x U female; U male x F1 female. The total number of F2 progeny from each 100 eggs was counted.

DNA, RNA and total protein content in male parents (P) irradiated as pupae with 150 and 250 GY and in males and females of the first and second generations (F1 and F2) were evaluated. The method described by Ceriotti (1952) was used for the determination of DNA content, RNA were determined according to Ceriotti (1955) and total protein content was determined by the method of Bradford (1976).

T-test at P<0.01 and F-test at P<0.05 were used to evaluate the significance of the results.

# **RESULTS AND DISCUSSION**

The effect of gamma irradiation on the number of the F1 and F2 progenies is presented in Table 1. The data showed that the higher irradiation dose gave the highest reduction in the number of progeny. When the male parents resulting from irradiation of pupae with 150 and 250 Gy mated with unirradiated females, the number of F1 progeny/100 eggs was significantly decreased to 27.5 and 20.4, respectively, while it was 75.0 in the control. The number of F2 progeny produced by F1 progeny from irradiated P males was significantly reduced than that of F1 progeny. Also, the reduction was more pronounced in F1 males than F1 females. For example, the number of F2 progeny from F1 males was 15.1 and 4.9 progeny/100 eggs, while F1 females produced 21.8 and 13.0 progeny/100 eggs at 150 and 250 GY, respectively. The previous studies on lepidopterous insects reported by Shontharam et al. and El-Shall et al. (1997) implicated irradiation as a sterilizing agent, as well as causing causes genetic changes. This might be due to its ability to induce mutation within chromosomes or the so-called chromosomal aberration, or within a gene or cistron of the DNA molecule. These changes may be include changes in the amount of DNA. Table 2 shows that when male parents were irradiated as pupae with 150 GY, the DNA content was insignificantly decreased, while at 250 GY it significantly decreased (P<0.05) than in the control. For example, when male pupae irradiated with 150 and 250 GY, DNA content reached 3.8 and 3.7, respectively and that of the control was 3.87. There was a significant decrease in DNA content in F1 and F2 progenies, but the reduction was higher in F2 progeny than in F1. The F1 females were less affected than the F1 males and the reduction of DNA content was directly proportional to the dose.

Verma and Agarwal (1985) reported that when chromosomes and their DNA are being struck by the ionizing photons or particles or they react with diffusing free radicals produced by radiation, then various abnormalities may occur in them. A single nucleotide in a triplet codon of a gene might be lost or deleted (deletion mutation) or added (insertion mutations). The poly-sugar phosphate backbone of DNA may be broken which might ultimately lead to chromosomal mutation such as breaks, deletion, addi-

Table 1. Average number of adult progeny/100 eggs of *E. cautella* from male parents (P) (treated as full grown pupae), and F1 generation mated with the opposite sex.

Crosses	Mean no. of adult progeny/100 eggs						
	Control	150 GY	250 GY				
	N♂ xN♀						
IPxU♀	75.0 aa	27.5 ab	20.40 ac				
		(63.31)*	(72.8)*				
F1♂ x U ♀	76.4 aa	15.10 bb	4.9 bc				
		(80.2)*	(93.6)*				
U♂x F1♀	75.2 aa	21.8 cb	13.0 cc				
		(71.0)*	(82.7)*				

The first letter for the significance within column

The second letter for the significance within rows.

Table 2. Effect of substerilizing doses of gamma irradiation on DNA content of *E. cautella* male parents (P), F1 and F2 progenies treated as full grown pupae.

Dose (GY)	Crosses		Mean DNA content (μg/insect)					
	-		Male		Female			
			Mean (µg/M)	Relative %	Mean (µg/M)	Relative %		
0	U♂ xU♀		3.87 a	100	4.191 a	100		
	I♂	P1	30.80 aa	98.2	5	=		
150	I♂xU♀	F1	3.20 bb	82.7	4.18 ba	85.1		
	F1 ♂ x U ♀	F2	2.42 cc	62.5	3.22 cb	65.6		
	U∂ x F1 ♀	F2	2.85 dd	75	3.81 dc	77.6		
	18	P1	3.70 ba	95.6	-	-		
250	18 xU ♀	F1	3.00 cb	77.5	4.00 ba	81.5		
	F1♂ xU♀	F2	2.11 dd	54.5	3.04 cb	61.9		
	U∂ x F1 ♀	F2	2.47 ee	63.8	3.61 dd	73.5		

<sup>-</sup> The first letter for significance within column between control and 150 GY (P, F1, F2) or between control and 250 GY (P1, F1, F2) (F-test P>0.05; Duncan's)

<sup>\*</sup> Means followed by the same letter are not significant (T-test; P>0.01).

<sup>-</sup> The second letter for the significance between the two doses 150 and 250 GY (T-test P>0.05). Relative % = Treated/Control %.

tions and translocation.

The heterozygous translocation is of a significant value, since it produces semisterile organisms. Proshold and Bartell (1973) reported that biological differences between the F1 progeny and the control were caused by chromosomal rearrangements. More complex translocation per irradiated sperm would occur in the F1 progeny and thus increasing the level of sterility and reducing the number of F2 progeny.

On the other hand, Chapman (1982) stated that the repeated cell divisions in the testes entail the synthesis of large amounts of DNA and RNA. It is known that DNA molecule acts as a template for the synthesis of different RNA molecules which copydown the coded genetic information of DNA molecule during DNA dependent synthesis (i.e, transcription). The present study was, therefore, extended to show the effect of gamma irradiation on RNA content. The results in Table 3 demonstrated a significant decrease in RNA content in male parents, F1 and F2 progenies. The reduction was more obvious in F2 progeny than F1 than P. Male progeny was more affected than female progeny. Similar results were obtained by Fadel *et al.* (2000). Thus, it could be concluded that gamma irradiation caused inhibition of DNA and RNA synthesis, which was directly proportional to the dose.

The process of synthesis of proteins involves one of the central dogma of molecular biology, which postulates that genetic information flows from nucleic acids to protein. Table 4 shows that there were insignificant differences in total protein content between normal and male parents irradiated as pupae with 150 GY. The dose of 250 GY caused significant increase in male parents. El-Bermawy *et al.* (2000) suggested that irradiation may activate some genes which produce different types of proteins. However, data in Table 4 indicated that total protein content decreased in F1 and F2 males and females. The highest effect was observed in F2 progeny produced from F1 males. Sobeiha *et al.* (2000) found that the amino acid content of gamma irradiated *Spodoptera littoralis* was greatly affected. The relationship between inhibition of nucleic acids, proteins and sterility was revealed by LaChance and Leverich (1968) they concluded that inhibition of DNA synthesis in nurse cells may impede endomitotic replication of chromosomes and thus may affect vitellogenesis. It appears, therefore, that due to deficiencies of protein, the oocyte fails to synthesize enough yolk, the eggs do not

Table 3. Effect of substerilizing doses of gamma irradiation on RNA content of *E. cautella* male parents (P), F1 and F2 progenies treated as full grown pupae

Dose (GY)	Crosses		Mean RNA contents (µg/insect)				
			Male		Female		
			Mean (µg/ ♂)	Relative %	Mean (μg/ ♀)	Relative %	
0	U&xU2		8.49 a	100	16.11 a	100	
	18	P1	7.94 aa	93.5	-	=	
150	ΙđχU♀	F1	6.78 bc	79.9	15.03 ba	93.3	
	F1♂×U♀	F2	4.90 cd	57.7	12.57 eb	78.6	
	U♂xF1♀	F2	5.30 de	58.1	14.00 dc	869	
	18	P1	6.14 bb	70.9	-		
250	I♂xU♀	F1	5.40 cc	66.9	13.90 bb	86	
	F1 ♂ x U ♀	F2	4.00 dd	47.9	10.89 cc	67.6	
	U♂x F1 ♀	F2	4.85 ee	58.9	11.81 dd	73.3	

<sup>-</sup> The first letter for significance within column between control and 150 GY (P, F1, F2) or between control and 250 GY (P1, F1, F2) (F-test P>0.05; Duncan's)

Table 4. Effect of substerilizing doses of gamma irradiation on the total protein content of *E. cautella* male parents (P), F1 and F2 progenies treated as full grown pupae

Dose (GY)	(GY) Crosses		Mean total protein content (µg/insect)					
			Male		Female			
			Mean (µg/ ♂)	Relative %	Mean (μg/ ♀)	Relative %		
0	U♂xU♀		149.1 a	100	318.5 a	100		
	13	P1	147.1 aa	98.6	=	н		
150	1♂xU♀	F1	136.5 bb	91.5	259.6 ba	81.5		
	F1♂×U♀	F2	87.3 cc	58.5	155.9 cb	48.9		
	U♂xF1♀	F2	109.2 dd	73.2	212.4 dc	66.7		
	18	P1	159.2 bb	106.7	-	-		
250	18 x U 2	F1	128.5 cc	86.1	230.0 bb	72.2		
	F1 & x U Q	F2	72.1 dd	48.3	141.4 cb	61.5		
	U♂xF1♀	F2	91.44 ee	61.3	158.5 dd	68.9		

<sup>-</sup> The first letter for significance within column between control and 150 GY (P, F1, F2) or between control and 250 GY (P, F1, F2) (F-test P>0.05; Duncan's)

<sup>-</sup> The second letter for the significance between the two doses 150 and 250 GY (T-test P>0.05). Relative % = Treated/Control %.

<sup>-</sup> The second letter for the significance between the two doses 150 and 250 GY (T-test P>0.05). Relative % = Treated/Control %.

develop and ultimately lead to reduction of number of eggs laid per female.

The present study showed that the male parents which were irradiated as pupae with substerilizing doses of gamma irradiation, can produce more sterile filial generations. The inherited sterility is suggested to be due to mutation at the chormosomal or DNA molecule level which was manifested in this study by reduction of the amount of nucleic acids. This reduction could be reflected upon protein content as well. Whether these proteins are enzymes or not, their reduction ultimately lead to such sterility.

#### REFERENCES

- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
- Brower, J.H. and E.W. Tilton 1975. Potential for control of Cadra cautella (Walk.) by release of fully or partially sterile males. Int. J. Isotop., 26: 720-725.
- Ceriotti, G. 1952. A microchemical determination of deoxyribonucleic acid. J. Biol. Chem., 198: 297-303.
- Ceriotti, G. 1955. Determination of nucleic acids in animal tissues. J. Biol. Chem., 214: 59-70.
- Chapman, R.F. 1982. The insects structure and function. 3rd ed., Hodder and Stoughton, 919 pp.
- El-Bermawy, S.M., Z.A. Ahmed and F.I. Ali 2000. Changes in total proteins in larvae of Corcyra cephalonica due to irradiation and heat stress. Arab. J. Nucl. Sci. & Appl., (in press).
- El-Shall, S.S.A., S.E.M. El-Naggar and S.M. Ibrahim 1997. Radiation induced inherited sterility and mating competitiveness in the maize worm, *Mythimna lareyi* (Dup.).
   Arab. J. Nucl. Sci. of Appl., 24: 150-160.
- Fadel, A.M., T.R. Amin and M.H. Al-Elimi 2000. The effect of gamma irradiation on the nucleic acids content of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.).
   Arab. J. Nucl. Sci. & Appl., 33: 317-722.
- LaChance, L.E. and A.P. Leverich 1968. Cytology of oogenesis in chemosterilized screw worm fly, *Cochliomyia hominivorax* as related to endomitosis in nurse cells. Ann. Ent. Soc. Amer., 61: 1188-1197.
- Proshold, F.I. and J.A. Bartell 1973. Fertility and survival of tobacco budworms, Heliothis virescens from gamma irradiated females. Can. Entomol., 105: 377-382.

- Shonstharam, K., A.J. Tomhankar and M.R. Hanwalker 1995. Comparative mating and reproductive performance of radiation sterilized and radiation induced F1 substerile males of *Earias vittella* (Fabricuius). J. Nucl. Agric. & Biol., 24: 232-236.
- Sobeiha, A.K., H.A. Sallam and S.S.A. El-Shall 2000. Amino acid content of the gamma irradiated cotton leafworm, *Spodoptera littoralis* (Boisd.). 5th Con. Nucl. Sci. & Appl. (6-10 Febr., 2000), Cairo, Egypt, 3: 1258-1267.
- 13. Verma, P.S. and V.K. Agarwal 1985. Genetics. 4th ed. S. Chand & Company Ltd., New Delhi.

# تأثير الجرعات المنخفضة من أشعة جاما على محتوى الأحماض النووية والبروتين الكلى لفراشة ايفيستيا كوتيلا وأجيالها

# طارق رئيس أمين '، سلوى عزمى بشرى '

ا معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقى - الجيزة.
 ٢ مركز الإشعاع النووى - معهد الطاقة النووية.

أدى تشعيع ذكور العذارى البالغة من فراشة ايفيستيا كوتيلا بالجرعات غير المعقمة ١٥٠، م ٢٥٠ جراى لانخفاض محتوى الأحصاض النووية ديأوكسى ريبونيوكليك أسيد (د.ن.ا.) و ريبونيوكليك أسيد (ر.ن.ا.) في ذكور الأباء واناث وذكور الجيل الأول والثاني ، أدى تشعيع ذكور العذارى البالغة بالجرعة ٢٥٠ جراى إلى زيادة محتوى البروتين الكلى في الأباء، بينما نقص في الجيل الأول والثاني ، وقد حدث انخفاض واضح في كل من الأحماض النووية والبروتين الكلى في حشرات الجيل الثاني عن حشرات الجيل الأول عن الأباء ، كما لوحظ أن تشعيع العذاري بالجرعات حشرات جيل الذكور عنها في الإناث.

وقد تمت مناقشة العلاقة بين النقص في محتوى الأحماض النووية والبروتين والانخفاض في عدد الحشرات الناتجة.