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Gamma irradiation effects on larvae of the rice moth, *Corcyra cephalonica* (Staint) (Lepidoptera-Pyralidae)

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The effect of gamma irradiation on the specific activity of ATP-ase, AChE and amino acid contents in different larval ages of *Corcyra cephalonice* (Staint) were studied. In general, the effect of irradiation on enzymes activity was highly noticed with AChE enzyme than ATP-ase enzyme. The higher the dose of gamma irradiation, the higher the rate of enzyme inhibition and vice versa. Also, the influence of gamma radiation on free and protein hydrolysate amino acid contents was more pronounced by increasing the irradiation dose and time after treatment. Increasing the dose was accompanied by reducing the free and protein amino acid contents. The results revealed that reduction in percent pupation, adult emergence, fecundity and fertility of the resulting adults depended upon the dose and larval age at the time of treatment. The severe reduction in fecundity and fertility was observed in 25 days old larvae treated with 80 Gy.

Key words: Gamma irradiation, larvae, *Corcyra cephalonice*.

INTRODUCTION

The doses of ionizing radiation are required to induce full sexual sterility in Lepidoptera. Such doses have a great effect on behaviour and reproductive physiology of moths. Irradiation induces a disturbance in biochemical metabolism resulting from inhibition of energy transfer due to the inhibition of enzymes. Labrecque and Smith (1968) mentioned that gamma radiation influences egg production by interrupting any of the complicated steps in vitellogenesis by hormonal, biochemical or genetic factor.

ATP-ase and ACHE are known as important catalytic factors for energy transfer system. Thornburg (1972) showed that gamma radiation may interrupt energy supplies and block all key enzymes which may stop normal metabolism. The change in enzyme properties may lead to the conclusion that radiation adversely affects the energy production and its utilization which in turn induces slower rate of metabolism. Also, 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 (Wigglesworth, 1972).

The present work was designed to investigate the effect of gamma irradiation on the activity of ATP-ase and AChE enzyme on the larvae of the rice moth, *Corcyra cephalonica* (Staint) and their effect on the fecundity and hatchability and also, to evaluate the relation between amino acid contents and the degree of sterility.

MATERIALS AND METHODS

Test insect, *C. cephalonica* (Staint) was obtained from laboratory culture reared on a mixture of wheat bran (75%) and whole wheat flour (25%) at 25 ± 2°C and 65 ± 5% R.H.

To study the effect of larval gamma irradiated on the development of 20 and 25 days old larvae, they were irradiated with 40 and 80 Gray (Gy) and were left on a certain amount of food in

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Table 1. Effect of gamma irradiation on the development of *C. cephalonica* larvae.

	Age of irradiated larvae (days)							
	20 days old larvae				25 days old larvae			
	Transfer of larvae to pupae (%)	Reduction in pupation (%)	Transfer of pupae to adults (%)	Reduction in adult emergence (%)	Transfer of larvae to pupae (%)	Reduction in pupation (%)	Transfer of pupae to adults (%)	Reduction in adult emergence (%)
Check	88.7	-	82.0	-	88.7	-	82.0	-
40	68.0	23.3	64.3	21.6	72.0	18.8	68.7	16.2
80	39.3	55.7	30.7	62.3	41.3	53.4	34.0	58.5

Check: Un irradiated insects.

glass jars to estimate the rate pupation and adult emergence. Each treatment was represented by fifty irradiated larvae and replicated three times. In all cases, control insects were investigated concurrently with tested larvae.

Immediately after adult emergence, male and female combinations were paired as follows: (1) irradiated male (I♂) x unirradiated female (U♀); (2) U♂ x I♀. Ten pairs of adults for each treatment were replicated 4 times. Adult fecundity and fertility was recorded.

The present study was conducted to estimate the activity of enzymes ATP-ase and AChE and also amino acid contents in irradiated and unirradiated larvae of *C. cephalonica*. Larvae of 20 and 25 days old were irradiated with 40 and 80 Gy of gamma irradiation. Three replicates of 10 larvae in each dose were investigated at 27 and 30 day.

Extraction and determination of *in vivo* ATP-ase activity was carried out according to Umbreit et al. (1964). This method measures the amount of inorganic phosphate produced from hydrolysis of ATP. The enzyme specific activity was computed as µmole/pi/h /protein.

To determine AChE activity, the colorimetric method of Augstinsen (1961) was utilized to determine the AChE activity at the previously mentioned larval ages.

Also, protein hydrolysate and free amino acids were prepared for chemical analysis according to the method used by Abdel-Hafez et al. (1972).

RESULTS AND DISCUSSION

The rates of pupation and adult emergence of *C. cephalonica* irradiated as 20 and 25 day old larvae are given in Table 1. The obtained data showed that in general, all the investigated dosages affected the pupation of irradiated larvae. The percentage of adult emergence for irradiated larvae with 80 Gy were decreased to 30.7 and 34.0% for 20 and 25 day old larvae respectively. These percentages were positively correlated with the larvae age and negatively correlated with the dose of irradiation. In general, a gradual decrease in percent pupation and adult emergence occurred with increase of the radiation dosage. These finding are in agreement with that of Aguilar and Arthur (1993), Thorayia et al. (1995), Boshra and Ahmed (1996), Ahmed and Hassan (2001), Abdalla (2004), Abdel Baki and Bosly (2010) and Mohamed (2012).

Table 2 shows the results obtained representing the reproductive capacity of adults from irradiated larvae. The results revealed that there was a great reduction in fecundity and fertility. However, the reproduction potential of adults produced from irradiated larvae depended upon both sexes and larval age at the time of treatment.

The average number of eggs deposited by females paired with irradiated males at 40 Gy of 20 and 25 day old larvae was 67.5 and 58.8 egg, respectively, Whereas, the reduction in fecundity was 75.9 and 79.1%, respectively. However, the effect of larval irradiation on the fecundity of the resulted adults was more severs in females than in males, especially in 25 days old larvae with 80 Gy.

Regarding the fertility, there was a complete sterility of adults produced from irradiated 25 days old larvae, when irradiated male were paired with unirradiated female at 80 Gy. From the previous results, it could be concluded that 25 days old larvae were sensitive than 20 days old larvae to genetic effects of irradiation. The treated adults of 25 days old larvae showed the greatest degree of sterility (about 100%). The difference in radiosensitivity between larvae ages was studied by many investigators such as Arthur (1988), Aguilar and Arthur (1995), Arthur and Wiendle (1995) and Carmo and Roseli (1995). Boshra and Ahmed (1996), Samira et al. (2001), Tsan et al. (2002) Sawiress (2005) and El Orabi et al. (2007).

Table 3 represents the ATP-ase and AChE activity of irradiated 20 and 25 days old larvae after different days of exposure. Examination of data indicated that gamma irradiation reduced the activity of the enzymes in treated larvae.

The rate of reduction or inhibition differed according to the applied dosage and larval age. The decrease in enzyme activity was more pronounce at the highest irradiated dosage .The higher the dose of gamma irradiation, the higher the rate of enzyme inhibition and vice versa.

Moreover, the reduction of ATP-ase and AChE activity was clear with 25 days old larvae than 20 days old larvae. Considering the time after administration, the data proved that with time progression after application, the rate of enzyme inhibition increased. This finding was more evident by increasing irradiation dose.

Reviewing these results, it can be noticed that, AChE activity were reduced than ATP-ase activity in irradiated larvae at two ages, especially, 25 days old larvae when

Table 2. Reproductive capacity of *C. cephalonica* adults from gamma irradiated larvae.

Age of irradiated larvae (days)	Dose (Gy)	Mean number of eggs/ female		% Egg hatchability	
		I ♂× U ♀	U♂× I ♀	I ♂×U ♀	U ♂×I♀
20	Check	280.8	280.8	91.0	91.0
	40	67.5 (75.9)	118.0 (59.9)	20.3 (77.7)	40.8 (55.2)
	80	49.3 (82.4)	83.3 (70.3)	16.0 (82.4)	27.5 (69.8)
25	Check	280.8	280.8	91.0	91.0
	40	58.8 (79.1)	29.5 (89.2)	5.3 (94.2)	7.3 (91.9)
	80	28.3 (89.9)	13.8 (95.1)	0.0 (100)	3.5 (96.2)

I: Irradiated; U: unirradiated; The values in brackets means: 1, Percent control of fecundity C-t /C×100; 2, Percent control of viability C-t/C× 100. Where C = Average no. of deposited eggs/female in the check; C = % hatched eggs/females in the check; T = Average no. of deposited eggs/females in the treatment. T = % hatched egg/females in the treatment.

Table 3. Effect of gamma irradiation on ATP-ase and AChE activity of *C. cephalonica* larvae at two different larval ages.

Age of irradiated larvae (days)	Dose (GY)	Enzyme activity after irradiation							
		ATP-ase				AChE			
		27 days old larvae		30 days old larvae		27 days old larvae		30 day old larvae	
		Sp Act.	Rel Act%	Sp Act	Rel Act%	Sp Act	Rel Act%	SP Act	Rel Act%
20	Check	3.87	100.0	3.74	100.0	7.14	100.0	6.32	100.0
	40	3.11	80.4	2.88	77.0	5.22	73.1	4.40	69.6
	80	2.76	71.3	2.41	64.4	4.75	66.5	3.88	61.4
25	check	4.27	100.0	3.91	100.0	7.48	100.0	6.68	100.0
	40	3.27	76.6	2.52	64.5	5.18	69.3	4.18	62.6
	80	2.50	58.5	2.15	54.9	4.64	62.0	3.08	46.1

SP.Act% = Specific activity; 1)ATP-ase (µmole/pi/hr/mg/potein); 2) AChE (µM/g); Rel Act% = relative activity percentage. Relative activity percentage = specific activity of enzyme in irradiated larvae / specific activity of enzyme in unirradiated larvae ×100.

irradiated with 80 Gy. The reduction of ATP-ase by gamma irradiation may be attributed to disturbance in biochemical metabolism which is due to the inhibition of energy transfer, through the inhibition of ATP-ase activity (Labrecque and Smith, 1968). Also, the reduction of ATP-ase activity might provide an explanation for the reduction of the reproductive capacity of *C. cephalonica*. Therefore, the change in enzyme properties caused by radiation may lead to the conclusion that radiation adversely affects the energy production and its utilization by the insect as indicated by lower specific and relative activity which in turn induces slower rate of metabolism. Our conclusion is supported by the finding of Abdel Megeed et al. (1987a), Abdel Baki et al. (1990, 2000).

AChE inhibition by gamma irradiation may be involved in the biochemical mechanism for its sterilizing action. This may be attribute to the fact that irradiation affected the protein structure of the enzyme. In this respect, Desrosier (1970) stated that ionizing radiation induced denaturation in protein. It is interest to note that there are correlation between the decrease of ACHE activity in larval stage due to irradiation and the damage in biological features expressed as reduction in egg production and hatchability. Salama et al. (1971) reported that the active process of spermatogenesis takes place during the larval stage. The effect of irradiation on AChE activity of insects was studied by Castillon et al. (1971), Abdel Baki and Sobeiha (1989) and Abdel Baki and Ibrahim (1997), Emam et al. (2003) and Zamzam et al. (2004).

The effectiveness of gamma irradiation on the amino acid contents of 20 and 25 days old larvae after different days of irradiation is given in Table 4. Examination of the tabulated data clearly shows that protein hydrolysate amino acids highly exceeded the free forms in both the check and irradiated larvae.

Generally, the influence of irradiation on free and protein amino acid contents was more pronounced by increasing the irradiation dose and time after treatment. Increasing the dose was accompanied by an increase in the rate of reduction of free and protein acid contents. The protein amino acid contents progressively decreased

Table 4. Effect of gamma irradiation on the amino acid contents of *C.cephalonica* larvae at two periods after irradiation.

Age of irradiated larvae (days)	Dose (Gy)	Amino acid contents detected at											
		27 days old larvae						30 days old larvae					
		F.A.A			P.A.A			F.A.A			P.A.A		
		A.C (mg/g)	Relative%	E%	A.C mg/g	Relative %	E%	A.C mg/g	Relative %	E%	A.C mg/g	Relative %	E%
20	Check	350.7	100.0	——	683.9	100.0	——	323.0	100.0	——	771.0	100.0	——
	40	317.0	90.4	-9.6	527.6	77.1	-22.9	269.0	83.3	-16.7	554.2	71.9	-28.1
	80	275.3	78.5	-21.5	450.3	65.8	-34.2	233.6	72.3	-27.7	435.5	56.5	-43.5
25	Check	345.7	100.0	——	707.1	100.0	——	309.1	100.0	——	772.3	100.0	——
	40	293.0	84.8	-15.2	511.5	72.3	-27.7	214.2	69.2	-30.8	414.6	53.7	-46.3
	80	242.3	70.1	-29.9	429.2	60.7	-39.3	181.9	58.8	-41.2	303.5	39.3	-60.7

A.C = Amino acid contents (mg/g); F.A.A = Free amino Acid; P.A.A = Protein hydrolysate amino acid. Relative% = Amino acid contents in irradiated larvae/amino acid contents in unirradiated larvae (check) × 100. E% = Effective variation (%) = relative (%) in irradiated larvae ----- relative (%) in unirradiated larvae (check).

with the advancement of larval development. However the rate of free amino acids reached 29.9 and 41.2% at 80 gray after 2 and 5 days of treatment of 25 day old larvae, respectively.

The present results show that the higher the gamma irradiation dose, the lower the amounts of both free and protein amino acid content which agreed with the findings of Abdel Megeed et al. (1987b), Ibrahim and Abdel Baki (1989), Abdel Baky et al. (1991), Abdel et al. (2000) and ElBermawy et al. (2000).

Also, the present data on the reduction of amino acid protein contents could explain somewhat the accompanied reduction in egg production of adult from irradiated larvae, it agreed with the explanation of poor oviposition by Leopold (1976) and Yamaoka and Hiroa (1976) which suggested that ovipositional stimulating factor is neither a lipid nor a large protein.

This data led to the conclusion that the reduction of the enzymes activity and amino acid contents cause several biological damage such as malformation, infecundity and sterility in produced adults.

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