INFLUENCE OF GAMMA IRRADIATION ON LARVAE OF THE OASES DATE MOTH, *EPHESTIA CALIDELLA* (GUEN).

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Abstract

Seven-days old larvae of *Ephestia calidella* were gamma irradiated with doses from 25-300 Gy. Irradiation reduced rate of pupation and adult emergence. Also it increased adult's malformation and shortened their life span. The lethal dose for larvae was 300 Gy. Adult fecundity and fertility were adversely affected by the gamma irradiation especially at 100 Gy. Irradiation of 7-day old larvae (2nd instar) with this dose caused severe biochemical abnormalities which extended to 14 and 18-day old larvae (4th and 5th or full grown larval instar, respectively). Lactate dehydrogenase (LDH), protease, carbohydrases and main metabolites, total proteins and total carbohydrates were significantly inhibited. On the contrary, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were elevated after treatment. The possible explanation was discussed and it was concluded that irradiation of this pest led to severe macromolecular changes which could affect general biology.

INTRODUCTION

Date fruits can be considered as a complete diet since they contain all the necessary ingredients required for human body. Among the difficult problems of packing fruits is the infestation by *Ephestia calidella*, which is widely distributed in Arab countries and Egypt. It is capable of causing significant economic damage. The presence of the Oases date moth larvae inside dates reduced their marketing value. Gamma irradiation is an important possibility for use in insect control. This physical treatment could eliminate the use of insecticides which leave residues and sometimes fail to penetrate, completely, the dates packages when used as fumigants.

Reports that deal with the effect of irradiation on this pest are few. Mikhail (2003) studied the biology of this pest after treating the pupae with gamma irradiation. Several reports on different pests illustrate that irradiation cause variable changes at the biochemical level (Salama *et al.*, 2000, Mohamed *et al.*, 2004, and others) that could led to abnormal metabolism. The object is, therefore, to highlight biological and biochemical abnormalities induced by gamma irradiation of *E. calidella* larvae (7-day old)

MATERIALS AND METHODS

Test moths were obtained from laboratory cultures reared at 26 ± 2°C and 75 ± 5% RH. Insects were reared on artificial diet composed of 1 Kg crushed wheat, 250 gm honey, 250 gm glycerin and 5% brewer's yeast. Newly-emerged moths were allowed to mate and oviposite in inverted glass jars with screen tops. The eggs that fell through wire mesh were collected in open Petri dishes every day. Seven-day old larvae were obtained after 24 hr from egg hatching, and then they were fed with the diet for 6 days. Larvae were irradiated with gamma ray doses 25, 50, 75, 100, 150, 200, 250 and 300 Gy in gamma cell with a dose rate of 20 Gy/min Fifty treated larvae as well as an equal number of control larvae were placed in jars and supplied with the diet until eclosion. Each treatment was replicated three times. Percentage of pupation, adult emergence, adult malformation and adult longevity were calculated. To determine adult fecundity and fertility, moth produced from irradiated 7-day old larvae (sexed in last or fifth larval instar according to Poulton, 1888) were paired in plastic tubes as follows: NM x NF, IM x NF, NM x IF and IM x IF (I= irradiated, N = Normal, M = Male, F = female). Fifteen pairs in 3 replicates were tested in each mating combination.

For biochemical tests, sixty larvae in three replicates for both 100 Gy-treated and control were collected at 7, 14 and 18-day old larvae (2^{nd}, 4^{th}, and 5^{th} instar larvae, respectively). The 100 Gy dose was chosen because it caused the highest depression in adult fecundity and fertility. Then the larvae of each replicate were homogenized in distilled water at 5°C using Teflon tissue grinder for 5 min. centrifugation was done at 4000 r.p.m. for 10 min using a refrigerated centrifuge (5°C). The resultant supernatant was used for analysis. Total proteins were estimated according to the method of Bradford, (1976), and expressed as µg protein/larva. Total carbohydrates were determined in acid extracts by the phenol sulfuric acid reaction of Dubois *et al.* (1956) and expressed as µg glucose/larva. The proteolytic activity was estimated according to Birk et al. (1962) by the casein digestion method. The results calculated as OD units x 10^3/min/larva (1 unit = 1 O.D.). The carbohydrases, invertase, trehalase and amylase were evaluated according to the method described by Ishaaya and Swiriski (1976). The enzymatic activity expressed as µg glucose released/min/larva. Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were determined according to the method of Reitman and Frankle (1957) and the activity resembled by U(unit)/larva. One unit of ALAT corresponds to the amount of enzyme which converts 1 µmole α–ketoglutrate to L-glutamate per minute at pH 7.6 and 37°C in the presence of L-alanine. While 1 unit of ASAT corresponds to the amount of enzyme which convert 1 µmole of 2-oxoglutrate to L-glutamate per minute

at pH 7.5 and 37°C in the presence of L-aspartic acid. The estimated lactate dehydrogenase (LDH) was done as described by Randox kit (Randox Laboratories LTD., United Kingdom, BT29 4QY) using an optimized standard method according to the recommendation of the Deutsche Gesellschaft fur Klinische Chemie (DGKC) (1970). LDH activity was expressed as U (unit)/larva. One unit corresponds to the amount of enzyme which reduces 1 µmole pyruvate per minute at pH 7.0 and 25°C. For all experiments, colour produced was red by a spectrophotometer (Spectronic 1201, Milton Roy Co., USA)

RESULTS AND DISCUSSION

Table (1) shows the effect of gamma irradiation on some biological parameter of *E. calidella* treated as 7-day old larvae. Percentage of pupation and adult emergence were decreased with increasing the dose of irradiation. The lethal dose for 7-day old larvae was 300 Gy. Gamma irradiation particularly at higher doses, increased adult malformation and shortened the life span of the adults. Females were more radiosensitive than males. Table (2) illustrates that irradiation caused significant reduction in fecundity and fertility. The greatest reduction was occurred when both sexes were mated at 100 Gy. The biology of many insect species was affected in the same manner due to treatment with gamma rays, *e.g. E. cautella* (Brower, 1980) and *Corcyra cephalonica* (El-Bermawy *et al.*, 2001).

When 7-day old larvae of the oases date moth, *E. calidella* treated with 100 Gy of gamma irradiation, severe biochemical changes occurred either directly after administration or with time elapsed, i.e. at 14 or 18-day old larvae (Table 3 and fig. 1). All the digestive enzymes tested, protease, amylase, invertase and trehalase were significantly depleted as compared to that of control (84.96, 68.09, 73.21 and 46.84%, respectively). The decline was more pronounced at 14 and 18-day old larvae. Transaminase activities were on the contrary. ASAT and ALAT were significantly elevated in both acute and delayed effects. ASAT activity was 262.6, 249 and 230% as compared to that of control. ALAT sharply increased but the activity began to decline with time. It was 861.74, 473 and293.43% as compared to control for 7, 14 and 18-day old larvae, respectively. The energy enzyme, LDH was greatly reduced in 7-day old larvae as compared to that of control (52.77%), but its activity was increased later. It was 70.76% and 75.12% for 14 and 18-day old larvae, respectively. The larval total proteins and total carbohydrates content was also depleted significantly after irradiation, indicating the great effect, in general, on the larval whole body. Total proteins were only 59.5% as that of control, while total carbohydrates were more

depleted. It was 49.17% as compared to that of control. But the decline was more noted in total protein content than total carbohydrates at 14-day old larvae.

Authors have attempted to clarify the mode of action and the effects of gamma irradiation on several insect species. It is known that irradiation affects ribonucleic acids which ultimately might cause disturbance in protein synthesis including enzymes. Also irradiation could denaturate enzymes. Desroseir (1970) reported that ionizing radiation induced denaturation in protein. The disturbance of enzymes might affect several metabolic processes such as digestion, energy production, metabolic pathway ..etc. which could ultimately affect normal metabolism in the insect. In an attempt to explain the reduction of the biological aspects in *E. calidella*, several enzymatic activities have been examined. As we show, the digestive enzymes, protease, amylase, invertase and trehalase were inhibited. El-Naggar (1999) reported that the gamma irradiation of male moth of *Earias insulana* lowered the activity of the haemolymph enzymes, amylase, invertase and trehalase.

Due to the inhibition of digestive enzymes, it was expected that total proteins and total carbohydrates to be reduced. In the present investigation, these metabolites were significantly reduced. Amin *et al.* (1996) reported that carbohydrates in both sexes of *Culex pipiens*, which gamma irradiated as pupae were decreased with increasing the time elapsed after emergence. Irradiation decreased significantly protein content in the male whole body after 48 hr especially at higher doses.

Maintenance of the balanced "amino acid pool" in insects is the result of various biochemical reactions carried out by a group of enzymes called aminotransferases. Such reactions are mainly responsible for the degradation and biosynthesis of amino acids, linking the glucose and protein metabolism. The present experiments revealed that the activity of ASAT and ALAT was increased sharply after exposure to gamma irradiation especially at 7-day old larvae. This increase began to decline with elapsing of time, especially for ALAT activity, but still higher than control insects. Mohamed *et al.* (2004) recorded elevation in some enzymes activities of *Agrotis ipsilon* after exposure to 100 Gy. The activation of enzymes after irradiation was due to disruption of enzymes, disruption of cellular structure and liberation of proteolytic enzymes due to changes in the permeability of the mitochondrial membrane for enzyme molecules (Hagen, 1975). It seems that in spite of gamma irradiation ability to activate some enzyme systems, its adverse effect on the rest of enzyme systems and general metabolism is greater.

One of the important energy enzymes that related to energy yielding and which was significantly reduced, as shown in the present work, is LDH. Insect tissues other than flight muscles do have lactic dehydrogenase, leading to the formation of two

molecules of lactic acid for every molecule of glucose used with consequent net gain of energy. This system is known to occur in insect or tissue where oxygen is likely to be in short supply (Chapman, 1982). Little is known about the effect of irradiation on this enzyme, but Salama *et al.* (2000) used some dehydrogenases including LDH as a direct measure for *Certitis capitata* vitality after exposure to gamma irradiation.

The present study clarify that irradiation of 7-day old larvae of *E. calidella* with 100 Gy of gamma rays caused severe macromolecular abnormalities exemplified by the effect on several enzymes and important metabolites. Also clarify that these abnormalities lasted for relatively long period during the larval stage, i. e. not regenerated. These might interpret the reduction of the biological parameters that observed in this work.

Table 1. Effect of gamma irradiation on some biological parameters of *E. calidella* treated as 7-day old larvae.

Dose (Gy)	Pupation %	Emergence %	Malformation		Life span (days) ± S.E.	
			Male	Female	Male*	Female*
0.0 (Control)	78.0	90.0	0.0	0.0	5.4± 0.1a	5.7± 0.13a
25	70.2	66.8	0.0	5.1	4.8± 0.24a	5.4±0.30b
50	58.8	60	7.2	12.3	4.5± 0.22b	5.4± 0.17b
75	50.0	45.4	27.9	40.5	4.6± 0.47b	4.8± 0.11b
100	36.6	31.2	45.0	67.7	4.0± 0.16b	3.9± 0.06c
150	17.5	14.4	73.0	86.2	3.2±0.20cd	3.0± 0.15d
200	11.0	8	84.1	92.9	2.8± 0.14cd	2.6± 0.22de
250	6.8	3.6	96.3	100	2.3± 0.42cd	2.2± 0.34e
300	0.0	-	-	-	-	-

*Means followed by the same letter are not significantly different (P> 0.05, Duncan's multiple range test).

Table 2. Effect of gamma irradiation on average number of eggs per female and egg hatch of *E. calidella* treated as 7-day old larvae.

Dose (Gy)	Average no. of eggs/ female ± SE			Egg hatch (%)		
	IM x NF*	NM x IF*	IM x IF*	IM x NF	NM x IF	IM x IF
0.0 (Control)	250.2±5.2a	240.6±3.4a	240.6±3.4a	86.0	84.4	84.4
25	140.8±3.5b	102.5±2.6b	88.5±1.1b	50.5	40.6	33.2
50	99.0±2.6c	70.8±3.7c	47.6±0.78c	36.8	25.5	14.5
75	66.8±3.4d	50.2±2.4d	35.0±1.5d	24.0	13.2	8.0
100	40.6±2.8e	27.0±1.9e	20.0±0.48d	19.6	7.5	2.8

I = irradiated, N = Normal, M = male, F = Female.

*Means followed by the same letter are not significantly different (P> 0.05, Duncan's multiple range test).

Table 3. Effect of gamma irradiation on some biochemical components of *E. calidella* treated as 7-day old larvae with dose of 100 Gy (Mean ± SE).

Parameters§	Larval age (Days)					
	7-day old		14-day old		18-day old	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
Protease	0.82±0.02	0.70±0.01 *	1.95±0.03	1.26±0.01 ***	1.25±0.01	0.83±0.01 **
Amylase	11.44±0.25	7.79±0.12 **	35.5±0.33	15.51±0.88 ***	43.97±0.51	22.0±1.02 ***
Invertase	124±0.79	91.36±0.99 ***	282±2.05	112.2±3.0 ***	290.9±2.4	113.7±1.12 ***
Trehalase	27.75±1.47	13.0±0.85 **	80.26±1.5	19.95±1.21 ***	93.46±0.96	24.16±2.35 ***
ASAT	1.82±0.08	4.78±0.12 ***	1.00±0.06	2.49±0.25 ***	0.36±0.02	0.83±0.11 **
ALAT	2.98±0.30	25.68±0.29 ***	0.96±0.13	4.55±0.23 ***	0.76±0.05	2.23±0.17 ***
LDH	11.56±0.20	6.10±0.01 ***	32.02±0.12	22.66±0.12 **	40.95±0.24	30.76±0.14 **
Total protein	58.80±2.05	35.13±0.57 **	275±5.63	122.41±1.96 ***	355±10.02	211±2.20 ***
Total carbohydrates	76.99±4.8	37.86±0.67 ***	390±9.89	160±10.33 ***	521±14.0	230±12.0 ***

Abbreviation used: ASTA and ALAT= aspartate and alanine aminotransferase, LDH = lactate dehydrogenase

§ Protease units= O.D. unit*10^3/min/larva, Amylase, invertase and trehalase units= µg glucose/min/larvae, ASAT, ALAT and LDH units = U/larva, total protein and total carbohydrate units = µg/larvae

N.S. = nonsignificant, * = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level (Student's t-test)

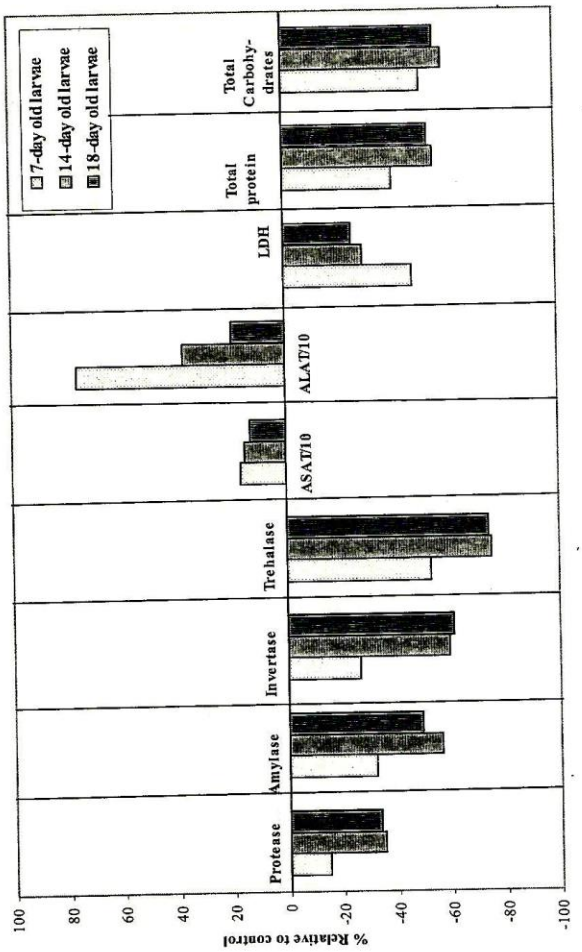

Figure 1. Percentage increase or decrease in the various biochemical parameters of *E. Calidella* treated as 7-day old larvae with 100 Gy of gamma radiation.

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تأثير أشعة جاما على يرقات فراش بلح الواحات

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١. معهد بحوث وقاية النباتات – مركز البحوث الزراعية– الدقى – الجيزة

٢. مركز البحوث النووية–هيئة الطاقة الذرية

نظرا لأن الدراسات التى تتناول تأثير الإشعاع على آفة فراشة بلح الواحات قليلة نسبيا ونظرا لأهمية هذه الآفة من الناحية الاقتصادية فقد تم دراسة تأثير أشعة جاما على مختلف النواحى البيولوجية والكيميائية لهذه الحشرة. فقد تم تعريض اليرقات عمر ٧ ايام (العمر اليرقى الثانى) لجرعات مختلفة من أشعة جاما تبدأ من ٢٥ الى ٣٠٠ جراى. وقد وجد أن التشعيع يقلل من معدل التغذية وخروج الفراشات ويقلل من عمرها كما يزيد من معدل تشوه هذه الفراشات. كما وجد أن التشعيع يقلل من الخصوبة وخاصة عند الجرعة ١٠٠ جراى ولذلك تم اختيار هذه الجرعة لإجراء بعض التقديرات البيوكيميائية فى هذه الآفة. وقد لوحظ أن تشعيع اليرقات عمر ٧ ايام يسبب تغييرات حادة على المستوى البيوكيميائى وهذه التغييرات تستمر لفترة تمتد حتى اليرقات عمر ١٤ و ١٨ يوم (العمر اليرقى الرابع والخامس على التوالى). وقد وجد أن المعاملة بالاشعاع تقلل من مستوى انزيم البروتييز والانزيمات المحللة للكاربوهيدرات ومستوى انزيم الطاقة (اللاكتات دهيدروجينيز). كما تسببت المعاملة أيضا فى نقص مستوى البروتينات الكلية والكاربوهيدرات بجسم الحشرة. كما انه على العكس من ذلك زاد مستوى الانزيمات الناقلة للأحماض الامينية. وقد استخلص من هذه الدراسة أن تشعيع هذه الآفة يسبب تغييرات حادة على المستوى البيوكيميائى الذى ينعكس بدوره على بيولوجية هذه الآفة .