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The Effect of Vitamin C and / Or Gamma Irradiation on Some Biological Aspects of the Greater Wax Moth, *Galleria Mellonella* (Pyralidae: Lepidoptera)

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ABSTRACT

This study is conducted to determine the impact of unilateral and joint with vitamin C concentrations (0.25Molar) and gamma rays in artificial diets on some biological aspects of the greater wax moth, *Galleria mellonella* L. Vitamin C showed the positive impact of combined treatment with the dose rate 100 Gy on the average number of eggs per mated female and on percentage of the eggs hatched through the crosses T♂ X N♀ and N♂ X T♀. Larval and Pupal period for both sexes did much affect by either radiation or vitamin C or both together. The average weights of larvae and pupae were significantly increased in the most concentrations when compared with the untreated control group. The highest percentage of survived larvae was 96.30 % from the control at the concentration 0.25 Molar of vitamin C and the lowest percentage was 61.11% from the control at dose rate 300 Gy only. While highest rate of the emergence was found by the treatments of vitamin C only or combined with the dose rate 100 Gy. The percentage of sex ratio was in favor of males at the dose rate 300 Gy only. Vitamin C significantly increased the activity of GOT and GPT enzymes by increasing the dose of gamma radiation in larvae of *G. mellonella* as compared to the control group.

Key Words: Gamma Radiation /Galleria mellonella/Vitamin C / Repruduction /Larval and pupal weight

INTRODUCTION

The greater wax moth, *Galleria mellonella* L., is a major pest of the honey bee, *Apis mellifera* L. It feeds on wax and pollen stored in combs of active honey bee colonies [(1)].

The technique of using ionizing radiation to induce sterility in the F_1 generation by irradiating the parents is now the most promising genetic method for suppression of lepidopteran population [2-5]

Classical radiation biology defines principles of irradiation killing based on DNA strand breaks specifically targeted to the nucleus [6]. Recently, the molecular mechanism of ionizing irradiation cell killing has been shown to follow pathways common to multiple other forms of oxidative stress including that induced by chemotherapeutic alkaline agents, ultraviolet irradiation, hyperthermia, and agents which induce oxidative stress [7- 9].

Radioprotectors are compounds that are designed to reduce the damage in normal tissues caused by radiation. These compounds are often antioxidants and must be present before or at the time of radiation for effectiveness [10]. Among this natural matters are vitamins, which have received particular interest [11]. Vitamin C protects against radiation-induced chromosomal damage in mice even when administered after irradiation [12].

Transaminases are the important components of amino acid catabolism; which is mainly involved in transferring an amino group from one amino acid to another keto acid. The aspartate aminotransferase and alanine-aminotransferase serve as a strategic like between the carbohydrate and protein metabolism and are known to be altered during various physiological and pathological conditions [13, 14].

The objective of this work is to improve the pupae of *G. mellonella* treated with gamma irradiation by using antioxidant vitamin C to produce adults more vital in order to be able to compete with normal adults in the field.

MATERIALS AND METHODS

Insect rearing technique

The laboratory strain larvae of *G. mellonella* were reared on a semi-synthetic diet composed of grounded wheat fortified with Treacle (we excluded bee honey because of its known anti-bacterial effect), glycerin, yeast and powdered milk at 28-30^{o}C and 65±5% R.H. Culture used in this study originated from eggs surface sterilized with formalin (10%) vapour treatment as suggested by [15]. Emerged adults were collected and kept in similar empty glass containers (egg laying cages) provided with paper lids (egg laying substrate) to the glass container. Paper carrying the eggs were removed for egg collection and replaced by new ones.

Source of irradiation

The source of gamma radiation used during the present study was from a Cobalt 60 (^{60}Co) irradiator installed in the cyclotron project ,Nuclear Research Center, Abu Zaabal, Egypt; the dose rate of irradiation source was 1 Gray/ second.

Experimental technique

Biological studies

Vitamin "C" was obtained from El-Nasr Pharmaceutical Chemicals Company, Abu Zaabal, Egypt. Vitamin "C" was dissolved in water already prepared for the larval medium at first with the concentrations 0, water, 0.25 Molar, 0.50 Molar, 1 Molar and 1.50 Molar to evaluate the effect of vitamin C on the average percentage larval and pupal weights of *G. mellonella*. Then share the concentration 0.25 Molar was used to evaluate the effect of vitamin C and / or gamma irradiation on the biological aspects of the insect, three experimental groups were set up. The first group consisted of the progeny of F_1 larvae irradiated as parental full-grown male pupae (24-48 hours before emergence of adults) with 100 and 300 Gray (which gives inherited sterility among F_1 progeny). The second one consisted of the progeny of F_1 larvae fed on artificial diet plus vitamin E with 0.25 Molar concentration of vitamin C (Ascorbic acid) then irradiated the F_1 male pupae with the same previous doses. While the third group consisted of larvae fed on artificial diet plus vitamin C alone. A comparable group of untreated insects is used as control.

The third instar larvae (20 larvae) of F_1 progeny were put in a glass jar containing a known weight of the artificial diet (20 gm). Each experimental group was replicate of three times and reared until pupation. The produced pupae were collected and irradiated with the two dose levels 100 and 300 Gy. The number of eggs per mated female, the percentage of hatched eggs, the percentage of gained weight after 10 days of larvae and pupae, the specific growth rate (SGR).

Growth performance evaluated in terms of weight gain (%) and specific growth rate (SGR) by using the formulae:

Weight gain (%) = (final weight – initial weight) / initial weight x 100 and

$$SGR\ (\%\ per\ day) = (\ln W_f - \ln W_i) / t \times 100,$$

Where W_f is the final wet weight, W_i is the initial wet weight and t is the

number of days. T | C % = Treatment | Control X 100

$$\% \text{ Male} = \frac{\text{No.of Males}}{\text{Total No. of adult emergence}} \text{X} 100$$

Biochemical studies

Some of pupae produced from the selected concentration of vitamin "C" was placed in cages and left to emerge. Both males and females mated with normal opposite sex. The third instar larvae were killed in a freezing box and stored until analysis with the control group.

Determination of the transaminase enzymes (GOT&GPT)

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities were determined colourimetrically according to the method [16].

The GOT activity was determined according to the method [17] using a kit of Bioadwic. The enzyme was measured at wave length 546 nm by spectrophotometer. The GPT activity was determined according to the method [17] using a kit of Bioadwic. The enzyme was measured at wave length 546 nm by spectrophotometer.

RESULTS AND DISCUSSION

Several concentrations of vitamin "C" (VC), 0.25, 0.50, 0.1 and 1.5 Molar (M) were used. Their influence was studied on the average weights of larvae and pupae of the greater wax moth, *G. mellonella* (Figure 1). The average weight of larvae and pupae increased gradually at the concentrations 0.25 and 0.50 M, then decreased gradually at concentrations 1 and 1.5 M. So the best concentration (0.25 Molar) was selected because in case of using the high concentrations, the insect did benefit from it.

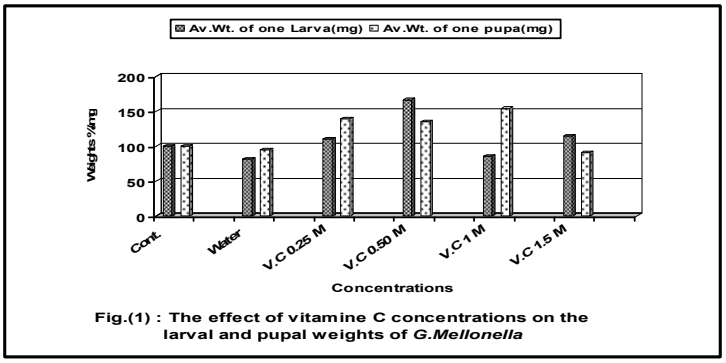

Fig.(1) : The effect of vitamine C concentrations on the larval and pupal weights of *G.Mellonella*

Figure (2) indicated that the effect of Vitamin C at the concentration 0.25 Molar separated or combined with the two dose levels 100 and 300 Gy of gamma irradiation on the average number of eggs per mated female of *G. mellonella* moths when treated males mated with normal females (T♂ X N♀) (male line) and when normal males mated with treated females (N♂ X T♀) (female line). The percentage of the average number of eggs per mated female was very decreased to 34.33 and 33.48 % compared to untreated control through the T♂ X N♀ and N♂ X T♀, crosses, respectively, at the treatment VC (0.25M). While at the dose rate 100 Gy it increased to 51.37 and 67.98 % compared to the control group, respectively. The combined treatment with VC (0.25M) and the dose rate 100 Gy increased to 88.14 % compared to the control at mated T♂ X N♀ Which showed the positive impact of vitamin C. But when the transaction between vitamin C and the dose rate of 300 Gy was decreased these percentages to 41.6 and 42.77 %, respectively, when compared with 65.72 and 68.22 % for the single dose rate of 300 Gy. Thus did not show the positive impact of vitamin C with this dose at the percentage of the average number of eggs per mated female.

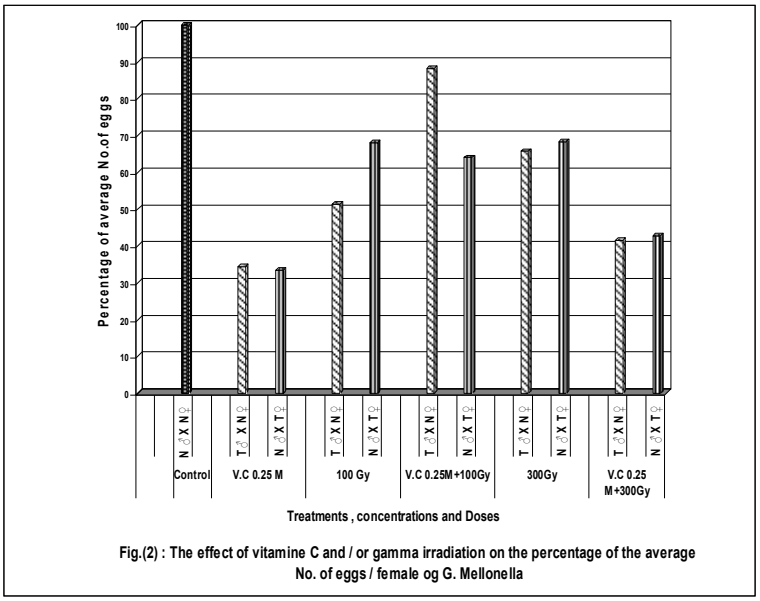

Fig.(2) : The effect of vitamine C and / or gamma irradiation on the percentage of the average No. of eggs / female og G. Mellonella

Many investigators have demonstrated that, vitamin C had an effect on the reproduction of insects, for example, [18] found that ascorbic (Vitamin "C") supplementation did not alter fecundity of *Phyllosoma*. Applying silymarin as antioxidants to the medfly larval medium it decreased the fecundity whatever alone or followed by gamma radiation doses. El-Kholy (2009)[11] applied vitamin

C with the two concentrations (1000 and 2000 mg / 1 kgm) on medfly larval media insignificantly affected the female egg production when compared with the control group. But when exposing the producing pupae with 90 Gy, the mean number of eggs per female per day was significantly decreased comparing with the control.

The percentage of hatched eggs of *G. mellonella* (Fig.3) was 90 and 83.97 % produced from the untreated control through the crosses of T♂ X N♀ and N♂ X T♀ respectively, at the treatment VC (0.25M). While at the treatment with the dose rate 100 Gy was 80.31 and 92.92 % from the control treatment, respectively.

The combined treatment by VC (0.25M) and the dose rate 100 Gy decreased the percentage of hatched eggs (T♂ X N♀ and N♂ X T♀) to 78.05 and 48.04 %, respectively, compared to control.

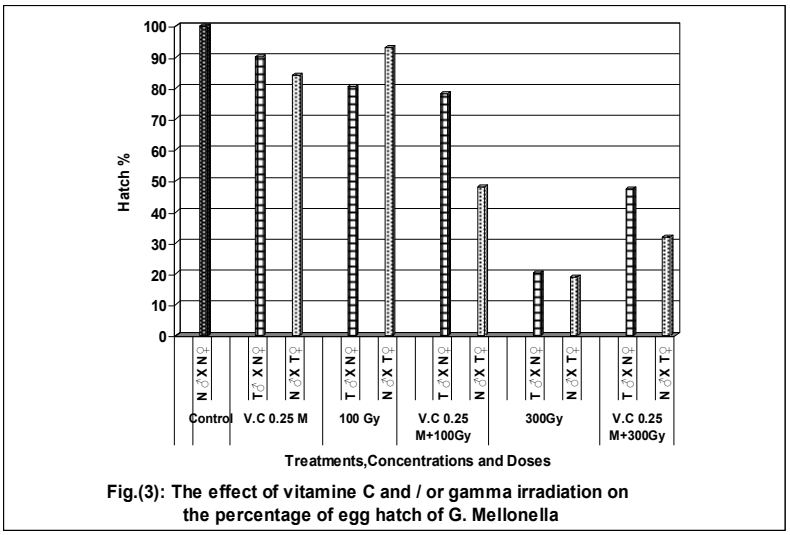

Fig.(3): The effect of vitamine C and / or gamma irradiation on the percentage of egg hatch of G. Mellonella

Also, figure (3) showed that the dose rate 300 Gy drastically decreased the percentage of the hatched eggs through the crosses T♂ X N♀ and N♂ X T♀ to 20.33 and 18.87 %, respectively, from the control treatment. While these percentages were increased to 47.21and 31.8% at the combined treatment VC (0.25M) and the dose rate 300 Gy. It is clear from figure (3) that the dose rate of 100 Gy catalytic role (the role of refresher), making it converge with the positive impact of vitamin C, which is close to the control treatment, and therefore the mutual influence of the combined vitamin and gamma radiation did not show the positive impact of vitamin C with this dose. While showing the

positive impact of vitamin C with the dose rate 300 Gy at these percentages are very low with gamma radiation only, and then increase to almost double when the transaction between the vitamin and gamma radiation, which shows clearly the positive impact of vitamin C. Similar results were obtained by El-Kholy (2009)[11] on medfly, who reported that the combined treatment of VC and gamma irradiation could be successfully used to reduce the adverse effects of gamma irradiation and subsequently can be used in the integrated pest management .

The effect of vitamin C and / or gamma irradiation on the average weight of *G. mellonella* is presented in Table (1) which showed the percentages of the average larval weight gained after ten days. It was almost comparable to the control treatment at the concentration 0.25 Molar of vitamin C, as well as the dose rate 100 Gy was 109.59, 98.86%, respectively, compared to the control treatment, while this percentage increased to 115.05% from the control treatment through the treatment of vitamin C (0.25M) combined with the dose rate 100 Gy. However, this percentage decreased to 80.24% from the control through the dose rate 300 Gy; then increased again to 109.39% from the control during the combined vitamin C (0.25M) and the dose rate 300 Gy.

The specific growth rate (SGR) after ten days (Table 1) take the same previous trend with the weight gain, the combined effect is higher than the radiation dose rate only. The average weight of one pupa per mg (Table 1) was higher at the combined effect than the radiation dose treatment only at all treatments.

Larval duration in days among F_1 generation of *G. mollenella* did not affect by separate or combined of vitamin C and gamma irradiation (Table 2). The percentage of survived larvae was 96.30 % from the control treatment at the VC treatment, then, decreased to 88.89 and 61.11% from the control treatment at the dose rates 100 and 300 Gy treatments respectively, While, it was increased to 96.30 and 85.19 % from the control treatment when the VC combined with 100 and 300 Gy respectively.

Table (1): The effect of vitamin C and / or gamma irradiation on the average weight of *G. Mellonella*

Treatments Conc. Dose	Average larval weight (mg)		Weight gained after 10 days %	SGR (% | day) after 10 days	Average pupal weight (mg)
	Initial weight	After 10 days			
Control (0)	32.00	174.91	446.59	1429.10	290.00
VC 0.25 M	31.67	186.67	489.42	1550.00	347.00
T | C %	98.97	106.72	109.59	108.46	119.66
100 Gy	33.67	182.33	441.52	1486.60	253.33
T | C %	105.22	104.24	98.86	104.02	87.36
VC 0.25 M+100Gy	33.67	206.67	513.81	1730.00	271.33
T | C %	105.22	118.16	115.05	121.06	93.56
300 Gy	36.00	165.00	358.33	1290.00	226.67
T | C %	112.50	94.33	80.24	90.27	78.16
VC 0.25 M+300 Gy	29.00	170.67	488.52	1416.70	260.00
T | C %	90.63	97.58	109.39	99.13	89.66

Also, the percentage of pupation (Table 2) be have the same previous trend, it decreased to 82.76 and 56.89% from the control treatment in 100 and 300 Gy treatments, respectively , then increased to 89.66 and 79.31 % from the control when the VC combined with the two dose levels 100 and 300 Gy, respectively.

The separate or combined vitamin C and gamma irradiation did not clearly affect on the pupal duration, except at the female treatment, it reached to 117.58 % from the control. Also, the percentage of emergence not clearly affected, except, at the dose rate 300 Gy, it decreased to 55.20 % from the control treatment.

The percentage of males (Table 2) was 84.37 % compared to control treatment at vitamin C treatment; it was increased from 82.41 % at the dose rate 100 Gy to 87.75 % from the control treatment at VC combined with the dose 100 Gy treatment. But, it was decreased from 99.26% at the dose rate 300 Gy to 75.94 % from the control treatment when V C combined with 300 Gy treatments.

Table (2): The separate or combined effect of vitamin C and gamma irradiation on some biological aspects of *Galleria mollenella* among F_1 generation

Treatment / Dose	Larval duration (in day) ± SE	% Survived larvae	% Pupation	Pupal duration (in day) ± SE		% Emergence	% Male
				Male	Female		
Control (0)	13.41±0.1164	90.00	96.67	10.22±0.0622	10.35±0.0822	93.33	59.26
VC 0.25 M	13.41±0.0294	86.67	86.67	9.51±0.0451	10.04±0.0157	96.29	50.00
T / C %	100.00	96.30	89.66	93.05	97.00	103.17	84.37
100 Gy	13.22±0.1356	80.00	80.00	9.27±0.05126	9.98±1.9666	89.69	48.84
T / C %	98.58	88.89	82.76	90.70	96.43	96.10	82.41
VC0.25M+100Gy	12.67±0.2222	86.67	86.67	8.99±0.1500	9.35±0.0616	96.30	52.00
T / C %	94.48	96.30	89.66	87.96	90.34	103.18	87.75
300 Gy	14.12±0.2275	55.00	55.00	11.02±0.5172	12.17±0.3889	51.52	58.82
T / C %	105.29	61.11	56.89	107.83	117.58	55.20	99.26
VC 0.25 M+300Gy	13.53±0.3072	76.67	76.67	10.67±7.2419	10.74±7.0324	87.04	45.00
T / C %	100.89	85.19	79.31	104.40	103.77	93.26	75.94

Traditionally, radioprotectors are defined as agents that are administered before radiation exposure, whereas therapeutic agents are administered after exposure. Strict classifications may be irrelevant to the concept that radiation damage involving reactive species represents a continuum of events. In this regard, many naturally occurring antioxidants exhibit a long window of protection, including post-irradiation protection against lethality and mutagenesis(Weiss and Landauer,2000) [19]. Many other authors are reported similar results on different pests by Mitra and Mukhopadhyay, 2003 on rohu carp larvae; Cappellozza *et al*. (2005) [21] stated that vitamin "C" deprivation affected the larval growth in *Bomby mori* Li , *et al.,* (2008) [22] institute that broccoli extracts as an antioxidant agent prolonged the survival time of the fruit fly *Drosphila melanogbaster* for its free radical scavenging activity. El-Kholy, (2008&2009)[23-11] found that percent pupation was significantly increased with increasing sylimarin (antioxidant) and vitamin "C", concentration in the larval diet of the medfly, respectively; sex ratio was not affected by applying the different concentrations of vitamin "C" when compared with the control.

The data presented in Table (3) revealed that the tested vitamin significantly increased the activity of GOT and GPT enzymes by increasing the dose of gamma radiation in larvae of *G. mellonella* as compared to the untreated control. The activity of GOT and GPT enzymes significantly increased to 125.33 and 107.58 % respectively from the untreated control at the concentration 0.25 Molar (M) of VC, while was highly increased to 158.67 and

131.82 %, respectively, from the untreated control at the dose rate 100 Gy. However, the combined effect of VC by the concentration 0.25 M and 100 Gy of gamma radiation decreased than in 100 Gy treatment only; it decreased to 141.33 and 112.88 % respectively when compared with 158.67 and 131.82 %, respectively, at 100 Gy only. AS well as at the dose 300 Gy, the activity of GOT and GPT enzymes significantly increased to 217.33 and 153.03 %, respectively, when compared with the control group. Also, at the treatment (0.25+300Gy) the activity of enzymes was observed to be less than in the case of gamma irradiation only (300 Gy).

Transaminases (GOT and GPT) enzymes help in the production of energy [24]; also, serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during variation physiological and pathological conditions [13]. It is noted from the foregoing results that vitamin C combined with gamma irradiation has significantly reduced the hyperactivity of GOT and GPT enzymes in larvae of *G. mellonella* resulting from the effect of gamma irradiation.

Table (3): The separate or combined effect of vitamin C and gamma irradiation on the Changes in activities of transaminase enzymes (GOT and GPT, µ / L) in *Galleria mollenella* larvae

Treatment / Dose or concentration	GOT µ / L	GPT µ / L
Control (0)	15.0[a]	26.4 [a]
VC 0.25 M T / C %	18.8[b] 125.33	28.4 [a] 107.58
100 Gy T / C %	23.8[d] 158.67	34.8[c] 131.82
VC 0.25 M+100 Gy T / C %	21.2[c] 141.33	29.8[b] 112.88
300 Gy T / C %	32.6 [f] 217.33	40.4[e] 153.03
VC 0.25 M+300 Gy T | C %	29.6[e] 197.33	37.8[d] 143.18
F- Value	103.1166	96.98
L.S.D. 0.05	1.5095	1.82196

Some authors found variably affected according to the compound used. Abd El-Hafez *et al.,* (1985) [25] found that the colorimetric evaluation indicated absence of aminotransferases (GOT and GPT) enzymes from the adult pink

bollworm treated with synthetic pyrethroids and organophosphorous insecticides, Raslan *et al*., (1994) [26] on pink bollworm larvae indicate that all the tested compounds (synthetic pyrethroids, op compounds and carbamates) reduced the GOT activity except Esfenvalerarte exhibited slightly increase in GOT activity, while GPT activity was increased in all insecticides treated larvae. Tawfik (1998) [27] recorded reduction in GOT and GPT activity of pink and spiny bollworm after treatment with NeemAzal, Khedr (2002)[28] reported decrease in the activity of GOT and GPT enzymes of *S. littoralis* (2^{nd} & 4^{th} instars larvae) after treatment with Biorepel. Mead (2000) [29] agree with the present results and recorded an increase in the activities of transaminase enzymes (GOT&GPT). Omar *et al*., (2006) [30] mentioned that Chinmix, Spintor and Biorepel compounds caused increase in the activity of GOT&GPT enzymes in larvae of pink bollworm as compared to control. Kamil *et al.*, (2010) [31] revealed that GOT activity was significantly higher in treated *S. littorals* larvae with Agerin after 48 h compared with the control. But the activity significantly decreased in larvae treated with Dipel 2X and Dipel DF than those treated with Agerin. There were significant increases in GPT and GOT activities at 120 hrs, of larvae treated width Dipel 2X and Agerin.

The present authors hope that these results on the effect of vitamin (C) separate or combined with gamma irradiation on the greater wax moth, *G. mellonella* may serve in two directions, firstly, increased the offspring to make a big mass rearing to use in the insect release in the field (when used the vitamin C only). The secondly, improved the vital activity of the insect, which reduces the effects of physical adverse incident (somatic effect) to the insect as a result of radiation, in the sterile insect release technique (when used the combined vitamin C and the dose rate 300 Gy).

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مجلة البحوث الإشعاعية
والعلوم التطبيقية
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تأثير فيتامين سى وأشعة جاما منفردين أو مجتمعين على بعض المظاهر البيولوجية لدودة الشمع الكبرى

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تستهدف هذه الدراسة التعرف على التأثير المنفرد لفيتامين سى بالتركيز ٠.٢٥ مول والمضاف للبيئة الغذائية أو تأثيره مقترن بأشعة جاما على بعض النواحي البيولوجية لدودة الشمع الكبرى.

ولقد أظهر فيتامين سى تأثيرا ايجابيا عند المعاملة المشتركة مع الجرعة الإشعاعية ١٠٠ جراى على كل من عدد البيض لكل أنثى وكذلك نسبة فقس البيض خلال التزاوجات المختلفة للجنسين.

الفترة العمرية لكل من اليرقات والعذارى بالأيام للإناث والذكور لم تتأثر كثيرا سواء بالإشعاع أو فيتامين سى أو كلاهما معا . معدل وزن اليرقات والعذارى زادت معنويا في معظم التركيزات المستخدمة وذلك بالمقارنة بالمعاملة القياسية .

وكانت أعلى نسبة مئوية لليرقات التي عاشت هي ٩٦.٥٠ ٪ من المعاملة القياسية عند التركيز ٠.٢٥ مول من فيتامين سى. وكانت أقل نسبة مئوية هي ٦١.١١ ٪ من المعاملة القياسية عند معدل الجرعة ٣٠٠ جراى بينما كان أعلى معدل لخروج الفراشات عند المعاملة بفيتامين سى فقط والمعاملة المشتركة من فيتامين سى والجرعة الأشعاعية ١٠٠ جراى ولقد زادت خلال المعاملة المشتركة عنها فى كلا من الجرعتين الإشعاعية ١٠٠ ، ٣٠٠ جراى فقط.

ووجد أن النسبة المئوية للنسبة الجنسية بين الذكور والإناث كانت في اتجاه الذكور عند معدل الجرعة الأشعاعية ٣٠٠ جراى .

وقد وجد أن فيتامين سى أدى إلى زيادة معنوية في نشاط أنزيمات الطاقة جى أو تى ، جى بى تى(GPT), (GOT) وذلك بزيادة الجرعة الإشعاعية وذلك من خلال تحليل يرقات الحشرة بالمقارنة بالمعاملة القياسية.