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CONTROLLING PULSE BEETLE, *Callosobruchus chinensis* L. BY GAMMA IRRADIATION AND BACTERIA *Bacillus thuringiensis*

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ABSTRACT

The mean weight loss of seeds after one month decreased as the result of feeding of treated *C. chinensis.* The reduction in weight is inversely proportion to the radiation dose. The weight of seeds was 95.5 and 98.0 gm at the dose levels 20Gy and 40Gy respectively when compared with 91.16 gm in the control treatment and 93.10 gm when the pest exposed to B.t(Lc_{50}) . The change percent in weight of seeds from control ranged between 1.47 and 6.41 at the dose range 2.5, - 40 Gy. The mean weight loss of seeds after two months was significantly lower at all treatments when compared with both the control and the B.t (Lc_{50}) treatments. The reduction weight is continues during the third period (after three months) at both the control and the B.t (Lc_{50}) treatments. In addition, the decrease in weight of seeds was more appearance in this period of storage. The percentage of change in weight from control increased vertically by increasing the radiation dose and horizontally by increasing the storage time.

The total number of insects after one month decreases by increasing the radiation dose. The propagation after one month was affected by the B.t (Lc_{50}) only or when combined with gamma radiation. The mortality percent of adults was 55.81%, 83.96% and 88% at 2.5Gy, 5Gy and 20Gy combined with B.t. (Lc_{50}) treatments respectively. The propagation after two months was more effected than after one month at all treatments. The mortality percent reached 100% at all treatments except at the 20Gy. The total number of adults decreased significantly by increasing the radiation dose . At the female sterile dose 40Gy recorded 20 adults (10 pairs put per 100 gm seeds to start the experiment) which are dead after one month.

INTRODUCTION

In Egypt the pulse beetles are one of destructive pests of stored leguminous seed such as Pease, cowpeas, beans and lentil. The Cowpea *Vigna anguiculata* is one of the most important high-crops, and the seeds are considered as one of the principal leguminous food for humans in many parts of the world. This crop is likely to be more ixtensively cultivated in Egypt in the near future owing to the new agricultural policy of increasing the areas allocated for vegetable crops, Qurashi and metin (1963).

Irradiation techniques seem to offer solutions that are desirables in many aspects. Pests that accurse in stored products can be control by gamma radiation. Cornwell (1966) and Tilton et al. (1971) suggested that by sterilization a resistance population some measure of protection against re - infestation by the same species could be obtained. Stored products also offer some unique advantages for effective utilization of the radiation technique the

most important is that much lower doses are required for insect disinfestations than for most other food irradiation application.

The needs to replace the commonly used pesticides with less toxic alternatives become more urgent in the last decade. One of the most important alternatives is a biological pesticide, which depends on living microorganisms or their toxins. Using microorganisms as biotic insecticides has some notable successes,Where infestation commonly begins in the field, where eggs are laid on maturing pods, Dobie et al (1991). The pest also can live in stores , so the bioinsecticides used in the field to control the pest and in stores when reinfestation occure.The objective of the present study is to investigate the separate and combined effect of *Bacillus thuringiensis* and gamma radiation on the weight loss in infected seeds and total population of the pest after store for different periods.

MATERIALS AND METHODS

1. Colony foundation

This study have been carried on the strain of the blakeyed cowpea ,*Vigna unguiculata*, the natural host medium for the studied pest *Callosobruchus chinensis* the original strain of the experimented pulse beetle,*C. chinensis* from which the continuous stock were created was firstly obtained from the Faculty of Science laboratories. Ain Shams University. Procedure of obtaining clean peas for the present study according to Haiba(1981).

2.Irradiation Technique

Irradiation of the pupal stage of the presently studied strain of the pulse beetle*, Callosobruchus chinensis* has been irradiated with gamma cell unit (Cobalt -60source) installed at the laboratory of the Middle Eastern Regional Radioisotope Center for the Arab countries, Cairo, Egypt. The radiation dosimetery of the source was about 10 Gy / 7.4 mints, until 7.18 mints during the experimental period.

3.The bioinsecticide treatment

Bacillus thuringiensis. (Berliner) namely bactospein was used in this work.This compound is wettable powder containing 16 million international units (I.U) of *B. thuringiensis* / gram powder. It is non-toxic to humans and animals.

Seeds of *Vigna unguiculata* were used to study, the weight loss in seeds as a result of infestation by the cowpae weevil *C. chinensis.* The amounts of weight losses were determined. All samples were stored under constant conditions of 28±1C and 65±5% R.H. 100 gram of *Vigna anguiculata* seeds offered to one-day –old adults as an oviposition site and a source of food for progenies. Ten pairs of *C. chinensis* added to 100 Grams of seeds in glass jar, left to lay eggs. Insect experiments treated with gamma radiationas as full grown pupae at dosage levels of (0, 2.5, 5, 10, 20, 40, Gy) and adult one day old treated with Lc50 *Bacillus thuringiensis*. The combined treatments between Lc50 B.t. and different dosage levels of gamma radiation were done

(2.5Gy+Lc50, 5Gy+Lc50, 10Gy+Lc50, 20Gy+Lc50and 40Gy+Lc50) five replicates for each treatment and 10pair of adults for each replicate. The tests continued three months. After one month all adults were removed and counted to determine alive and died insects, weight loss of seeds was then determined and the propagation of insect was calculated. The amount of losses was determined by weighing the samples of seeds in the replicates after three periods of storage (after the first month, after second month and after the third month) change in weight loss calculated by the equation.

$$\text{Percent of change} = \frac{\text{Test - control}}{\text{Control}} \times 100$$

All adults counted after each periods (of storage). The adults were separate as alive and died, the later ones removed after counted live adults with the same seeds introduce into the glass jar for the second month. After two and three months, the propagation of the pest counted as previously mentioned.

RESULTS

The mean weight loss of seeds after one month decreased as the radiation dose was increased and when the insect exposed to the combined effect of B.t (Lc_{50}) and gamma radiation as compared with the control. The weight of seeds was 95.5 and 98.0 gm at the dose levels 20Gy and 40Gy respectively when compared with 91.16% in the control treatment and 93.10% when the pest exposed to B.t(Lc_{50}) Table(1).

Table (1) Determine the effect of *C. chinensis* adults treated with gamma irradiation and B.t (Lc_{50}) on weight loss of seeds after different periods of storage under constant temperature 28±1 ^{0}C and R.H. 65±5%

Treatment Gy + B.t($L.c_{50}$)	Mean weight of seeds after one month (gm)	% change	Mean weight of seeds after two month (gm)	% change	Mean weight of seeds after three month (gm)	% change
2.5	93.73±0.32 bc	2.82	81.03±4.02 c	35.21	79.33±4.48 c	48.20
5.0	93.45±0.50 bc	2.52	85.20±3.01 c	42.17	83.40±2.44 d	55.80
10.0	93.70±0.23 bc	2.79	84.00±0.72 c	40.16	81.90±0.78 cd	53.00
20.0	95.50±0.69 d	4.76	93.53±0.57 d	56.00	86.46±1.41 d	61.52
40.0	98.00±0.00 e	7.50	95.16±0.16 d	58.79	5.70±0.10 e	76.84
2.5 + B.t	92.50±0.21 bc	1.47	80.63±0.29 c	34.24	79.00±0.50 c	46.65
5.0 + B.t	93.23±0.53 bc	2.62	83.36±3.09 c	36.91	79.80±2.08 c	48.89
10.0 + B.t	94.33±1.35 cd	3.55	88.53±0.57 cd	47.51	87.06±0.37 d	63.09
20.0 + B.t	95.36±0.14 d	4.76	94.66±0.37 d	58.19	90.90±1.85 de	71.51
40.0 + B.t	98.00±0.00 e	6.41	95.76±0.08 d	59.69	95.96±2.00 e	78.59
B.t ($L.c_{50}$)	93.10±0.37 b	2.13	69.83±0.35 b	16.57	65.10±0.75 b	21.61
Control	91.16±0.52 a	0.00	59.93±5.10 a	0.00	53.53±5.97 a	0.00

Initial weight of seeds/replicate =100gm. 10pairs of insect/replicate = Means followed by the same letter in each column are not significantly different at (p > 0.05)
One month LSD.05 = 1.12, two months LSD.05= 12.30, three months LSD.05= 11.54

$$\text{Percent of change} = \frac{\text{Test- control}}{\text{Control}} \times 100$$

The percent of change in weight of seeds from control increased by increasing the radiation dose in the combined effect it was 1.47, 2.62, 3.55, 4.76 and 6.41 at the dose levels 2.5, 5, 10, 20 and 40 Gy respectively. Table (1) also indicates that the mean weight loss of seeds after two months was significantly lower at all treatments when compared with the control. The mean weights of seeds were 59.93 and 69.83 gm in the control and B.t.(Lc_{50}) treatments respectively while it was 95.76 gm when the pest treated with the dose level 40Gy combined with B.t (Lc_{50}) fig(1). The reduction in weight continues during the third period of storage at both the control and the B.t (Lc_{50}) treatments, it reached to 53.53 and 65.10 gm respectively. Also the decrease in weight of seeds was more appearance at the treatments with low doses, for example it was 79.33, 83.40, 79.00 and 79.8 gm at the 2.5, 5 , 2.5 Gy + B.t (Lc_{50}) and 5 Gy + B.t (Lc_{50}) treatments respectively. Additionally in Table (1), we found that the percentage of change from control increased vertically by increasing the radiation dose and horizontally by increasing the storage time, Fig (2)

Data presented in Table (2) shows that the total number of insects after one month decreases by increasing the radiation dose. It recorded 195.33, 151,176.5 and 98.5 at the dose levels 5Gy, 10Gy, 5Gy + B.t. (Lc_{50}) and 10 Gy + B.t (Lc_{50}) respectively. The propagation after one month was very effected by the B.t (Lc_{50}) only or when combined with gamma radiation. The percent of mortality of adults was 100% at B.t (Lc_{50}) alone, 40Gy, 10Gy + B.t. (Lc_{50}) and 40Gy + B.t. (Lc_{50}) treatments compared with 57.54% mortality in control.

Table (2): Separate and joint effects of gamma irradiation and B.t. on propagation of *C. chinensis* during one, two and three months of storage under 28±1 º C and 65±5% R.H.

Treatment Gy + B.t.	Propagation after one month				Propagation after two months				Propagation after three months				Total No. of adults after 3 months
	Means total No. of insect	Av. No. of dead insect	Av. No. of a live insect	% Mortality	Means total No. of insect	Av. No. of dead insect	Av. No. of a live insect	% Mortality	eans al No. insect	Av. No. of dead insect	v. No.of a live insect	% Mortality	
Control	333.66	192.00	141.66	57.54 a	1171.00	811.66	359.33	69.31 a	361.00	361.00	00.00	100.00 a	1364.67
0 + B.t.	393.33	393.33	00.00	100.00 c	387.00	387.00	00.00	100.00 c	5.66	5.66	00.00	100.00 a	785.99
Gamma irradiation only treatments													
2.5 Gy + 0	215.00	120.00	95.00	55.81 a	168.00	168.00	00.00	100.00 c	70.00	70.00	00.00	100.00 a	358.00
5 Gy + 0	195.33	164.00	31.33	83.96 b	108.33	108.33	00.00	100.00 c	45.00	45.00	00.00	100.00 a	317.00
10 GY + 0	151.00	144.00	7.00	95.36 c	151.00	151.00	00.00	100.00 c	38.00	38.00	00.00	100.00 a	333.00
20 GY + 0	29.00	26.66	2.33	91.93bc	112.33	102.00	10.33	90.80 c	25.33	25.33	00.00	100.00 a	153.64
40 Gy + 0	20.00	20.00	00.00	100.00 c	00.00	00.00	00.00	------	00.00	00.00	00.00	-------	20.00
Gamma irradiation combined with B.t. treatments													
2.5 Gy+B.t.	225.50	219.50	6.00	97.34 c	132.00	132.00	00.00	100.00 c	60.00	60.00	00.00	100.00 a	411.50
5 Gy+B.t.	76.50	171.50	5.00	97.17 c	178.00	178.00	00.00	100.00 c	39.00	39.00	00.00	100.00 a	411.50
10 Gy+ B.t.	98.50	98.50	00.00	100.00 c	52.50	52.50	00.00	100.00 c	30.33	30.33	00.00	100.00 a	388.50
20 Gy+ B.t.	25.00	22.00	3.00	88.00 b	54.00	43.00	11.00	79.63 b	23.00	23.00	00.00	100.00 a	181.33
40 Gy+ B.t.	20.00	20.00	00.00	100.00 c	00.00	00.00	00.00	-------	00.00	00.00	00.00	------	20.00

Gy = Gray B.t. = Bacillus thuringiensis

Fig1

fig2

The propagation after two months was more affected,. The percent of mortality reached 100% at all treatments except at the sub sterile dose 20Gy and 20Gy + B.t. (Lc_{50}) treatments, it was 90.8 and 79.63% when compared with 69.31% mortality in control treatment. The total number of adults decreased significantly by increasing the radiation dose Table (2). The female sterile dose 40Gy recorded 20 adults (10 pairs put per 100 gm seeds to start the experiment) which are dead and after one month recorded. The highest effect to the propagation happened after three months, the percent of mortality was 100% at all treatments and at the control treatment. The separate and combined treatments of B.t. (Lc_{50}) and different doses of gamma radiation have a great effect on the total number of adults Table (2).

After three months of storage the propagation investigate a significant decrease in the total number of adults, It was, 785.99, 317.33 , 153,64 , 411.5 and 181.33 at B.t.(Lc_{50}) , 5Gy , 20Gy, 5Gy + B.t.(Lc_{50}) and 20 Gy + B.t.(Lc_{50}) treatments respectively when compared with 1364.67 to the control treatment.

DISCUSSION

The present data conclude that the mean weight loss of seeds among three months was increased in control group. The reduction of weight in seeds after different periods was affected by the storage periods, and radiation doses alone or when it combined with B.t. Therefore, it affected vertically by increasing the radiation dose which reduced the fecundity and fertility of the pest and horizontally by increasing the storage time. This discussed by Golebiowska (1969) on *Sitophilus oryzae*, which, showed that of, the observed reduction in weight loss of *Vigna anguiculata* infested with irradiated insects are attribute able to more than one factor irradiated adult insects not only consume less food per individual per day than unirradiated adults but also on the average die much sooner at the higher radiation dosages. The number of larvae is also greatly reduced because reproduction is decreased or prevented. Similar results were reported by Rogers and Hilchey (1960) in study of food consumption by irradiated adult flour beetles; Brower *et al.*, (1972) in bulk grain on *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) irradiation at 25 Krad, the damage caused by the lesser grain borer in 5 weeks was reduced to 10% and that caused by the rice weevil to 3%. Even less damage occurred after the insects were treated with 50 Krad. Hussain (1989) who studied the weight loss in order to assess the requirement of radiation doses for preventing the insect multiplication and subsequent damage in storage, mungbean samples with various development stage of insect were exposed to radiation at 50,100 and 150Gy. After three months of storage no grain damage was noticed when grains infested with different insect stages were irradiated with the dose of 100 or 150Gy. Wiendle *et al.* (1975) showed that the loss in weight occurring after irradiation of rise, maize and macaroni samples infested with *Sitophilus oryzae.* It can be noticed in the samples during the first 20-25 weeks when sterilization did not occur. The loss in weight was due to an increase in insect population and subsequent attack on the substrate .The percentage loss in

weight of all three substrates and at the different doses used was recorded lower than the control group. Also the percentage of loss in weight after irradiation of bean samples infested with *Zabrotes subfasciatus* and *Callosobruchus maculatus*, shows the difference in susceptibility of insects to radiation treatment. Thorayia *et al.* (1995) showed that, the total number of progenies of irradiated adults *T. confusum* was decreased with increasing the radiation dose. There was a reduction effect. The mean total number of adults were 392.5, 190.0, 66.0 and 20.0at the doses 10, 20, 30 and 40Gray respectively after one and half month compared with 569.5 in the control group, the complete sterilizing dose was 40Gray which confirm our results. Samira *et al*. (2001) showed a remarkable decrease in fecundity and fertility of *Spodoptera littoralis* with gamma radiation as fullgrown pupae the sterilizing doses for both females and males were 350 and 450 Gray. The highest decrease in fecundity and fertility mated when both irradiated sexes mated.

This study is done to obtain a greater reaction from the insects, and makes possible the determination of the minimum dose necessary to sterilize the insects without changing the natural environment.

It may be pointed out that visual observation alone of the seeds samples tested is sufficient to determine the sterilizing dose for the pest, whereas when the desired sterilization occur infestation by insects was easily observed.

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مكافحة خنفساء اللوبيا(كالوزبركس كينينسس) بواسطة اشعة جاما والبكتريا (باسيلس ثيورينجينسس)

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- التأثير المشترك لكل من أشعة جاما والبكتريا (التركيز الذى يقتل نصف عدد الأفراد) على وزن البذور المصابة لعدة فترات مختلفة من التخزين:

نقص معدل الفقد فى وزن البذور بعد شهر واحد من التخزين بزيادة الجرعة الأشعاعية المستخدمة وكذلك نقص عند أستخدام الأشعاع بالأشتراك مع البكتريا وكان معدل الوزن يتراوح بين ٥ر٩٥ ؛ ر٩٨ جرام عند الجرعتين ٢٠ جراى؛ ٤٠ جراى غلى الترتيب وذلك بالمقارنة ب ١٦ر٩١ جرام للمعاملة القياسية ؛ ١٠ر٩٣ جرام عند تعريض الأفة للبكتريا فقط وزادت النسبة المئوية للتغير فى وزن البذور عن الكنترول بزيادة الجرعة الأشعاعية المستخدمة والتى كانت ٤٧ر١ ؛ ٦٢ر٢ ؛ ٥٥ر٣ ؛ ٧٦ر٤ ؛ ٤١ر٦ % عند الجرعات ٥ر٢ ؛ ٥ ؛ ١٠ ؛ ٢٠ ؛ ٤٠ جراى على الترتيب وأنخفض معدل الفقد فى وزن البذور بعد شهرين من التخزين معنويا عند جميع المعاملات وذلك بالمقارنة بكل من المعاملة القياسية وأستخدام البكتريا منفردة وأ ستمر معدل الفقد فى وزن البذور خلال الفترة الثالثة وهى بعد ثلاثة شهور من التخزين وكان واضحا فى المعاملة القياسية وكذلك فى المعاملة بالبكتريا فقط.أيضا فان الزيادة فى نقص وزن البذور كانت واضحة جدا فى هذة الفترة الأخيرة زادت النسبة المئوية للتغير فى وزن البذور عن المعاملة القياسية (الكنترول) رأسيا بزيادة الجرعة الأشعاعية المستخدمة وزادت عرضيا بزيادة فترة التخزين.

- التأثير المشترك لكل من الأشعاع والبكتريا على زيادة عدد الحشرات خلال ثلاثة اشهر متتالية لحشرة خنفساء اللوبيا:

نقص العدد الكلى للحشرات بعد شهر واحد بزيادة الجرعة الأشعاعية وعند استخدام البكتريا وحدها أو مشتركة مع أشعة جاما زادت النسبة المئوية لموت الحشرات الكاملة ووصلت الى ١٠٠ %عند أستخدام البكتريا وحدها او الجرعات ١٠ ؛ ٤٠ جراى بالأشتراك مع البكتريا بالمقارنة بالكنترول التى كانت ٥٤ ر٥٧ % تأثر التكاثر بعد شهرين تأثرا كبيرا بدرجة أكبر منها بعد شهر فى جميع المعاملات ووصلت نسبة الموت الى ١٠٠ % فى جميع المعاملات فيما عدا الجرعة ٢٠ جراى وكذلك أشتراك الجرعة ٢٠ جراى مع البكتريا والتى كانت ٨ر٩٠ ؛ ٦٣ ر٧٩ % بالمقارنة بالكنترول ٣١ ر٦٩ % نقص العدد الكلى للحشرات الكاملة معنويا بزيادة الجرعة الأشعاعية والجرعة المعقمة للاناث (٤٠ جراى) سجلت ٢٠ حشرة (١٠ أزواج لكل ١٠٠ جرام بذور ببداية التجربة) والتى ماتت وسجلت بعد شهر واحد بعد ثلاثة ا شهر نسبة الموت وصلت الى ١٠٠ % عند جميع المعاملات وكذلك فى حالة المعاملة القياسية . المعاملات المنفردة للاشعاع أو المشتركة مع البكتريا أدت الى تأثير كبيرعلى العدد الكلى للحشرات الكاملة وأظهرت نقصا معنويا