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## **Influence of low doses of gamma irradiation on cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)**

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### **Abstract**

Phytosanitary irradiation for food commodities has been widely accepted in recent years. Gamma irradiation has been used as a phytosanitary treatment against microbial diseases, insect infestation and food spoilage. The goal of the current study was to determine the lowest possible dose of gamma irradiation that will induce long-term sterility of insects through generations. The effect of four gamma irradiation doses examined were; 20,40, 50 and 70 Gy. Irradiated males were crossed with normal females. For the cowpea beetle *Callosobruchus maculatus*(F.), adult fecundity, hatchability, adult emergence, sterility% was investigated. 100% adult mortality was achieved by 70 Gy dose. Fecundity, hatchability, number of adults emerged, sterility% were significantly reduced when males exposed to 20, 40, and 50 Gy compared to the control. The effect of parental irradiated males exposed to 20 Gy on F2 generation was also studied. Fecundity, hatchability, number of adult emerged, sterility% were significantly reduced in F2 compared to F1 and control progeny. Interestingly, for F1 generation, the effect of gamma rays on adult emergence% exhibit a hermetic effect response although it was not significant. These results demonstrat that pulse irradiation relying on low-doses of gamma radiation induce inherited semi-sterility through generations and is a very promising phytosanitary food technology for post-harvest treatments.

**Keywords:** inherited sterility, gamma radiation, low-dose effect, sterile male technique, sterile insect technique, hormesis.

## Introduction

Inherited sterility (IS) has evolved as an alternative to the standard sterile insect technique (SIT) (IAEA, Ibrahim *et al.* 2017). Male insects irradiated with sub-sterilizing doses of gamma or x-ray radiation and mated with virgin females resulted in F1 generation with more sterility than their parents. Previous studies have been shown that IS has a long-term impact on pest control programs against many insects such as moths and mosquitoes (JANG *et al.*, 2012 and Shetty *et al.*, 2016).

On the other hand, food irradiation has been widely used as a phytosanitary treatment and insect disinfestation methods. It is an environmentally friendly safe treatment and it has been used against many pests as an alternative to chemical control (Ibrahim, *et al.*, 2017, Ihsanullah and Rashid, 2017).

The original data for this paper has been previously published (Ibrahim, *et al.*, 2017). Here, the effect of low doses gamma radiation on the cowpea beetle *Callosobruchus maculatus* and the inherited effect on F1 and F2 generations were examined. The first objective was to determine the lowest gamma radiation dose to be used for insect disinfestation. The second one was studying the long-term effect of low doses gamma radiation on IS through generations.

This work will hopefully increase the effectiveness of using SIT and other releasing biological control programs by induced IS across different generations.

This work also expands on the importance of the potential for radiation hormesis effect that could appear as a result of using low-doses in food irradiation.

## Materials and Methods

### Insect rearing

The insect used was cowpea beetle, *C. maculatus*. The insect culture was maintained on cowpea seeds (*Vigna unguiculata* (L.) Walpers) in Entomology Department, Ain Shams University for many years. Insects were maintained at  $27 \pm 2$ °C and relative humidity of  $60 \pm 5$ %.

### Preliminary test

It has been reported that the highest dose of gamma radiation permitting reproduction was 50 Gy and the sterilizing dose was 70 Gy (Hasan and Khan 1998). Therefore, five doses of gamma radiation (0, 20, 40, 50, 70 Gy) were selected to determine the lowest dose that permitting reproduction and induce IS for both F1 and F2 generations. No. of eggs, no. of hatched larvae, no. of adult emerged, adult emergence% were determined for F1 offsprings

From the insect rearing jar and prior to adult emergence, each 1-egg-seed was transferred to an Eppendorf tube to prevent any mating prior the experiment.

### Irradiation technique

Newly emerged adult males (1-day old) were irradiated with one of five doses of (0, 20, 40, 50, 70 Gy) using <sup>60</sup>Co Indian gamma cell (Gy 4000 A), located at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The dose rate was 0.713 Rad/s.

### Biological assay

For biological assay a 20 Gy dose was selected because it was the lowest dose that caused IS (Ibrahim, 2017) and at the same time produce sufficient individuals to study long-term effect in F1 and F2 progeny. After exposure to radiation, the newly emerged adult males were mated with virgin normal females in 9 mm Petri dishes with cowpea seeds. Each replicate has two males and two females. Five replicates were made for each treatment, 20 Gy and the control one. Insects were also maintained at  $27 \pm 2$ °C and relative humidity of  $60 \pm 5$ %. No. of eggs, no. of hatched larvae, no. of adult emerged, adult emergence%, sex ratio, and sterility% were recorded per each treatment for both F1 and F2 resulted from irradiated male parents.

The percentage sterility was calculated according to Chamberlain's formula (1962):

$$\% \text{ sterility} = 100 - \left( \frac{a \times b}{A \times B} \right) \times 100$$

Where:

- a = number of eggs per female in the treatment.
- b = Percentage of hatched eggs in the treatment.
- A = number of eggs per female in control
- B = Percentage of hatched eggs in control.

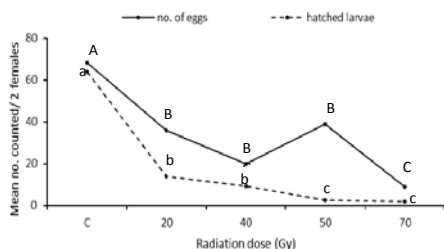
### Statistical analysis

For preliminary test, adult emergence % data were analysed using non-parametric tests. Otherwise, the results were analyzed with ANOVA and followed by posthoc analysis using LSD-test with the help of SPSS program. The level of significance used was  $P < 0.05$ .

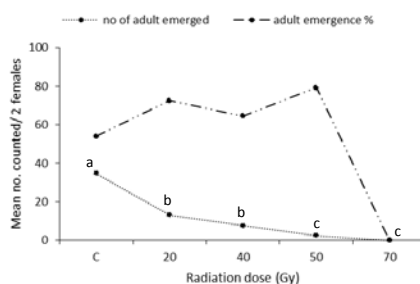
## Results

### Preliminary test

There was an overall significant effect between treatments for both number of eggs and number of hatched larvae ( $P < 0.05$ , Fig. 1). All doses significantly reduced the number of eggs and the number of hatched larvae compared to the control group (Fig. 1). There was also an overall significant effect between different doses for number of adult emerged ( $P < 0.05$ , Fig. 2). On the other hand, results of adult emergence% were not significantly different from each other ( $P = 0.06$ , Fig. 2). Interestingly, adult emergence% for those exposed to 20, 40, and 50 Gy seemed to be better than that of the control group although this effect was not significant ( $P = 0.06$ , Fig. 2).



**Fig. 1** Number of eggs and number of hatched larvae after crossing irradiated males of *C. maculatus* with normal females at 0, 20, 40 and 50 and 70 gamma radiation doses. Different letters indicate significant differences,  $P < 0.05$ . Capital letters for no. of eggs laid and small letters for no. of hatched larvae.



**Fig. 2** Number of adult emerged and Adult emergence % after crossing irradiated males of *C. maculatus* with normal females at 0, 20, 40 and 50 and 70 gamma radiation doses. Different letters indicate significant differences between no. of adult emerged among doses,  $P < 0.05$ .

### Biological assay

There was an overall significant effect between treatments for number of eggs, number of hatched larvae, hatchability %, no. of adult emerged and adult emergence % (For all,  $P < 0.05$ , Table 1).

**Tab. 1** Biological aspects of the F1 and F2 progenies resulted from irradiated parental males *C. maculatus* with 20 Gy dose of gamma radiation and crossed to normal females. Different letters indicate significant differences among different treatments,  $P < 0.05$ .

Conc.	No. of eggs	No. of Hatched larvae	Hatchability %	No. of adult emerged	Adult emergence %
0 GY	68.25±1.89 a	64.00±1.87 a	93.77±0.8 a	34.75±2.1 a	54.14±1.76 a
20 Gy F1	35.80±11.2 b	13.80±4.75 b	40.93±9.2 b	13.25±4.9 b	72.54±9.46 b
20 Gy F2	5.60±2.16 c	5.00±2.35 c	68.91±16.1 c	2.50±0.05 c	22.92±14.0 c

The male sex ratios were  $38 \pm 1.00\%$ ,  $42 \pm 3.00\%$ , and  $17 \pm 17\%$  in the control, F1, and F2 progenies, respectively with no significant difference ( $P = 0.162$ ). The adult percentage sterility was significant between the control ( $0.00 \pm 0.00$ ) and the individuals of F1 ( $70.87 \pm 8.70$ ) and F2 ( $88.31 \pm 7.01$ ), ( $P = 0.000$ ). There was a significant difference in percentage sterility between the F1 and F2 generations ( $P = 0.049$ )

## Discussion

### Preliminary test

The present data shows that doses of 20, 40, and 50 Gy gamma radiation significantly reduce the number of *C. maculatus* progeny compared to the control group. A dose of 70 Gy completely inhibits *C. maculatus* production. These data agree with previous work that showed that complete sterility is achieved by 70 Gy and the highest dose that permits *C. maculatus* reproduction is 50 Gy (Hasan and Khan 1998).

Data illustrating *C. maculatus* adult emergence %, demonstrate that low-doses of 20, 40, and 50 Gy seems to have a hermetic dose effect. In dose-response relationships, the hermitic effect or hormesis is a biphasic effect. Some doses of certain stimuli, whether it chemicals or radiation or even any stressors have a positive (stimulatory) effect on an organism at low doses compared to normal individuals (the control group where high doses have an inhibitory effect and concurs with Baldwin and Grantham, (2015).

### Biological assay

A low-dose of 20 Gy induces IS and has a long-term effect on both F1 and F2 progenies for *C. maculatus*. The same effect of low-doses of gamma or X-rays radiation has been reported for other insects (JANG *et al.*, 2012 and Shetty *et al.*, 2016).

## Conclusion

This paper showed that irradiated male *C. maculatus* with low-doses of gamma radiation induces inherited sterility through F1 and F2 progenies. This is a very promising new technique for insect control and could be an alternative to SIT and other chemical control programs. At the same time, attention should be directed to the potential of the hormesis effect that could exist. Otherwise, low-doses induces IS might have very dramatic results instead.

Finally, hormesis effect should be investigated more in relation to different stimuli such as insecticides, radiation, physical and even environmental stressors.

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## Radio Frequency Heat Treatment for Controlling Cigarette Beetle in Dried Tobacco

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### Abstract

Tobacco (*Nicotiana tabacum* L.) is one of many agricultural commodities produced in Thailand. There are Virginia (flue-cured tobacco) and Burley (air-cured tobacco) types and Cigarette beetle, *Lasioderma serricorne* F. is the most important insect pest that attacks dried tobacco. The efficacy of radio frequency (RF) heat treatment at 27.12 MHz was examined to control cigarette beetle on dried tobacco. Various growth stages of cigarette beetle were prepared within samples of dried tobacco and were exposed to RF at 55, 60, and 65 °C for 1, 2 and 3 minutes. The results showed that pupal and adult stages of cigarette beetle were the most tolerant stages to RF heat treatments. The RF treatment at 65 °C for 3 minutes is able to cause complete mortality of egg, larval, pupal and adult stages of cigarette beetle.

**Keywords:** dried tobacco, *Lasioderma serricorne*, tolerant stage, heat treatment

### Introduction

Virginia and Burley tobacco production in Thailand is found in 10 provinces mostly in the north and northeast of Thailand covering about 21,000 ha and is valued at approximately 2 billion US dollars in 2016-2017 cigarette sales. In Virginia and Burley tobacco storage, cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) is the most damaging insect pests of dried tobacco and grain products such as corn, beans and dried herbs in Thailand. Insecticides have been used as the main control measure for managing stored tobacco pests. To avoid chemical contamination in the commodity other control measures such as storage sanitation and pest exclusion to remove sources of infestation should be considered. Several studies have been performed using radio frequency (RF) energy to control stored product insects. Mitcham et al. (2004) tested RF energy to control Codling moth (*Cydia pomonella*), navel orangeworm (*Amyelois transitella*, and Indianmeal moth (*Plodia interpunctella*), at 55°C for 5 minutes and was able to gain complete control.. Lagunas-Solar et al. (2007) also reported that using RF at, 10 kHz to 1050 MHz resulted in >99% mortality of Anguomois grain moth (*Sitotroga cerealella*).

The use of radio frequency, as a heat treatment, has been investigated to control many kinds of insect pest associated with agricultural products (Nelson, 1996). Experiments have been conducted to control many stored product insects to determine the tolerant stages of insects to the RF heat (Table 1.). For heat treatment with radio frequency, commodities are allowed to increase temperature rapidly due to the vibration of water molecules. Nutapong (2012) tested the efficacy of RF to kill cigarette beetle on packaged dried tobacco which was infested with cigarette beetles in all stages. The results showed that the adult stage was the most tolerance to RF heat treatment at higher temperatures (104 °C) for complete control Radio frequency also has less effect on various kinds of grains compared with conventional heat (Srikam et al., 2014; Wangsapa et al. 2016; Zhou