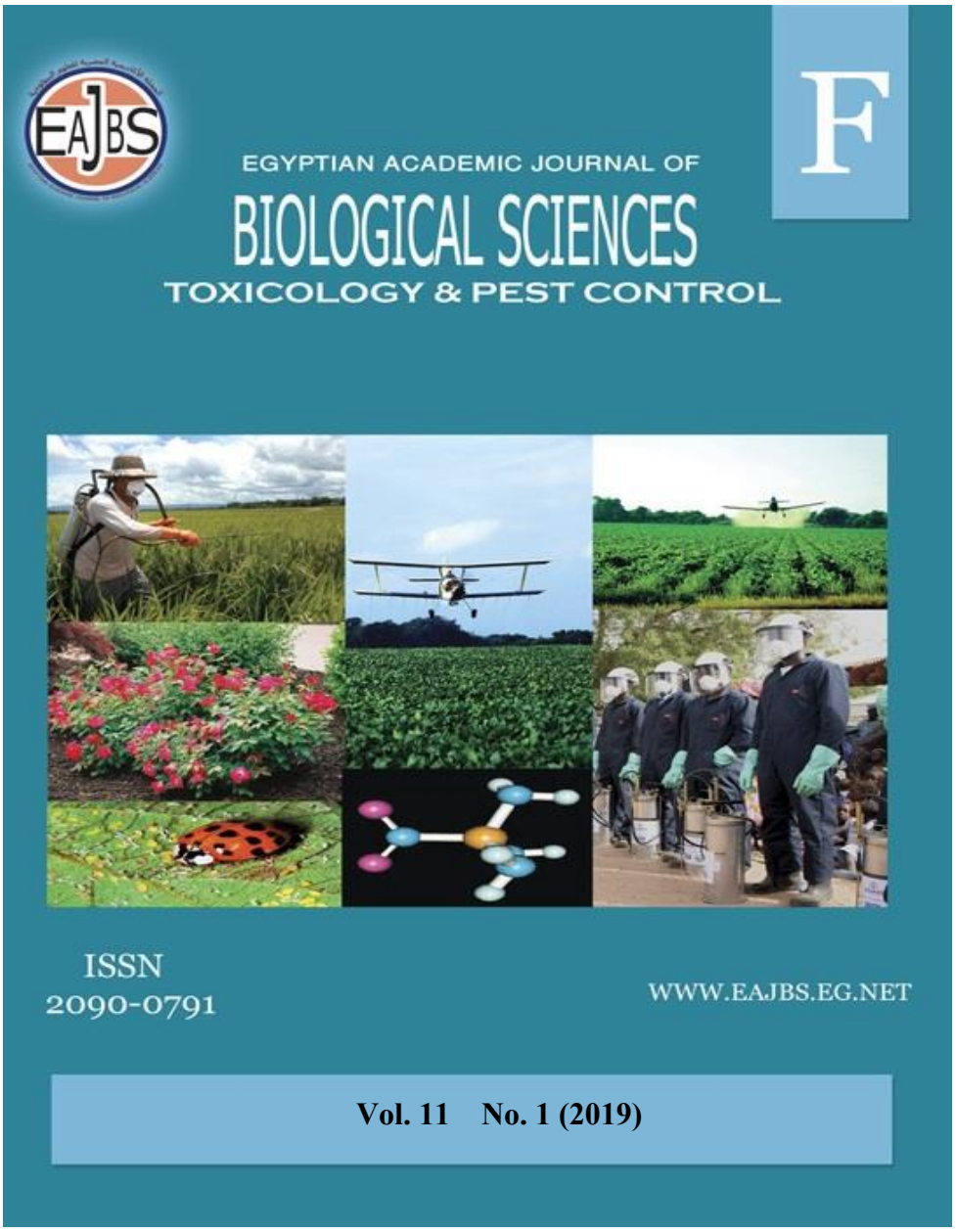


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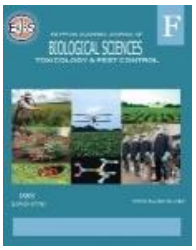

# Efficiency of the Gamma Irradiation in Controlling the Red Flour Beetles, *Tribolium castaneum* Herbst, and Preventing Its Secondary Infestations

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## ABSTRACT

The present study was carried out to investigate the effect of different doses of gamma irradiation (0, 50,100,500 and 1000 Gy) on the mortality percentage of both of larvae and adult stages of the red flour beetle, *Tribolium castaneum* Herbst. (Coleoptera-Tenebrionidae). Also, the effect of gamma irradiation on both of the Quinones secretion and the microflora associated with the pest stages was investigated. The results showed that the incremental of adult and larval mortality percentages were significant ($P < 0.05$) affected by an increment of gamma irradiation dose. The results of Gas chromatography-mass spectrometry patterns exhibited a strong inhibitory effect of irradiation on Quinones secreted by both stages. The used doses succeed in reducing the count of microflora were associated with both larval and adult stages. This reduction reached zero for fungi and bacteria isolated from the surface at the dose 1000 Gy. Obtained data suggested that the irradiation of red flour beetles with 1000 Gy is a promising tool for the control of this destructive pest and prevent its harmful effects associated with its infestation.

## INTRODUCTION

*Tribolium castaneum* Herbst. (Coleoptera- Tenebrionidae) is a major pest in human stored food. It has been found to associate with a wide range of commodities (Abdullahi et al., 2011). It can quickly build up to huge populations at warm and humid facilities. Serious infestations can cause spoilage and decomposition to stored products. The flour beetles don't cause damage by feeding only, but also cause more problems by contaminating the stored products, with large numbers of dead bodies, cast skins, and fecal pellets, as well as liquids secretions (quinones), and pungent odours in grain. The nauseous smell and taste caused by infestations of confused and red flour beetles can result in poor food consumption by livestock and rejection by grain buyers.

For Tenebrionidae including *T. castaneum*, α-benzoquinones such as methyl 1,4benzoquinone (MBQ) and ethyl-1,4-benzoquinone (EBQ) are the major components of the defensive secretions in threat situations (Unruh et al.,1998). Lis et al., (2011) suggested that the benzoquinones secreted by flour beetles may cause carcinogenic changes in cells and tissues of humans and animals. Moreover, the infestation by some

insects encourages the growth of fungi which produce mycotoxins besides the contamination of commodities with insect bodies, (Kumari et al., 2011; Bosly & Kawanna, 2014; Gabarty & Abou El Nour, 2016). Sinha & Sinha, (1992) found that fungal growth was observed on legs, antennae, at the joints and evenly over the whole body surface of insects. Thus, controlling insects during storage may reduce the spread of fungal spores within the storage system. Chemical fumigation and other methods are widely used for disinfestations of stored product with this pest.

Owing to the huge restrictions on the methods available for the control of stored product pests, alternative methods are required. Gamma irradiation is an effective strategy against this pest as it causes mortality beside sterility in insects depending on the dose and the stage, which was exposed (Warchalewski et al., 2000; Tandon et al., 2009; Abbas & Nouraddin, 2011; Kabbashi et al., 2012). The present study aims to shed a light onto using some doses of gamma-irradiation (which already used for extending the shelf life of wheat flour) to avoid the *T. castaneum* infestation and subsequently, its Quinones secretion that naturally occurred in infested wheat flour has been avoided. Moreover, special emphasis on the effect of gamma irradiation in reducing the growth rate of the microflora associated with *T. castaneum* has been done.

## MATERIALS AND METHODS

The experiments were conducted at the insect pests' laboratory of the Biological Application Dept.,Nuclear Research Center-Atomic Energy Authority, Abo-Zaabal, Egypt in August 2017.

**Insects Rearing and Irradiation Samples Process:**

A stock culture of *Tribolium castaneum* Herbst. (Coleoptera- Tenebrionidae) maintained in a rearing medium as described by (Ayvaz et al., 2002) at 28$^{o}$C±1$^{o}$C, 60%±5% RH. Samples of larvae and adult groups of known age were separated from the stock culture and used for each experiment. Irradiation was performed on the Cyclotron Project, Nuclear Research Center, Abu Zaabal, Egypt, using Cobalt 60 (Co 60)gamma cell; the dose rate of irradiation source was 0.37 Gray/ second.

**The Entomocidal Activity of Gamma-Irradiation against Insect Stages:**

Different stages of the insects (larvae 17 day-old and adults less than one-week-old) were espoused to different doses of gamma irradiation (0, 50,100, 500 and 1000 Gy). Each treatment was observed daily for a week and numbers of alive and dead insects were counted. To study the effect of gamma irradiation on larval growth rate, larvae were collected and irradiated at the same tested doses then kept on a sufficient amount of the rearing medium. The growth rate of the untreated and treated larvae was calculated according to (Waldbauer, 1968) as follows:

G.R. = Wg /D×A Where G. R = Growth rate Wg= weight gain D = Duration of feeding period A = Initial weight of insects + final weight of insects' ÷ 2. Three replicates (20 larvae or adults per replicate) were used for each dose level and control.

**Effects of Gamma-Irradiation on Quinones Secreted by Insect Stages:**

To determine the effect of gamma-irradiation on the Quinones secreted by *T. castaneum* adults and larvae, each stage was exposed to the lowest and the highest doses (50 and 1000 Gy). Five irradiated and non-irradiated insect stage were added to 0.33 gm of clean and free infestation wheat flour and maintained for five days to collect the Quinones from the flour. Each sample was replicated six times. These samples were analyzed by Gas chromatography-mass spectrometry (GC-MS). The chemical composition of samples was performed using Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x

0.25 mm x 0.25 µm film thickness). The oven column temperature was initially held at 40°C for 5 min. then increased by 5°C /min to 250°C withhold 5 min. The injector temperature was kept at 230°C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 2 min and diluted samples of 1 µl were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 20–350 in full scan mode. The ion source and transfer line temperatures were set at 200 and 250°C respectively. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

**Effect of Gamma-Irradiation on Microflora Associated with the Insect Stages:**

Ten different ages of adult beetles *T. castaneum* and ten larvae 17days-old were taken from the laboratory culture and subjected to 50 or 1000 Gy. Three replicates were used for each dose and control. After treatment, microflora was isolated from the surface and the internal of testing adults and larvae. For microflora isolation, tested stages put separately in the sterile saline followed by vortexing for 5 min (stoke), and then serial dilutions of this stock were prepared. Inoculate 100 ųl from each dilution in Petri dishes containing potato dextrose agar (PDA) media for fungal isolation another Petri dish containing nutrient agar (NA) media for bacterial isolation. The plates were incubated at 28°C for 72 hr and at 37°C for 48 hr for fungi and bacteria, respectively. After incubation periods the colonies were counted and recorded. These steps were done in each stage. The surfaces of the tested insects were sterilized using 70% alcoholic ethanol and then washed twice using distilled water. The sterilizing insects were crushed using mortar and pestle. The sterile saline solution was added to the crushed insect parts, followed by vortex for 5 min (stock). Serial dilutions were prepared and inoculate 100 ųl from prepared dilution on (PDA) media for fungal isolation and on (NA) media for bacterial isolation. The incubation was done as described before. After incubation periods the colonies were counted and recorded.

**Statistical Analysis:**

Mortality percentage data were initially normalized by arcsine transformation before using the SPSS package, ver.17.0 to an analysis of variance ANOVA. The data obtained from three replicates were tabulated as mean ± SE, and Duncan's test at P = 0:05 was used for ranking means.

## RESULTS

**Effect of Gamma-Irradiation on Different Stages of Insect:**

Data obtained in Figures (1& 2) illustrated the effect of different doses of gamma-radiation on both larvae and adults of *Tribolium castaneum* Herbst. (Coleoptera-Tenebrionidae). The results indicated that the mortality percentage increased by increasing gamma-irradiation doses from 50 to 1000 Gy among the observation days. The irradiation doses have a significant effect on the larval mortality percentage of the 4th day and continue till the end of the examination (7th day), while of the 1st day untill the 3rd day there was no significant difference in the mortality percentage among doses as shown in (Fig.1) in the control treatment there was no mortality in the adult stage within the assessing period (seven days) (Fig. 2). Although *T. castaneum* adults were more tolerant to gamma-irradiation treatment, it significantly differed from its control at the 1st day till the 7th day. Mortality percentage proportionally increased with the increment in irradiation dose e.g. at 1st day, the mortality percentages were 0, 1.5, 1.7 and 1.8 % in 50, 100, 500 and 1000 Gy, respectively, while at 7th day, the mortality percentages were 5.5, 22.5, 30.4 and 96.6 % for the same doses, compared with the control value (0%).

Approximately complete mortality of treated adult was achieved with a dose of 1000Gy. Concerning to the effect of gamma-irradiation doses on the growth of treated larvae, (Fig. 3) exert a dose-dependent effect on irradiated larvae. This effect was manifested as a decrease in weight gain of treated larvae accompanied by a decrease in the growth rate of these larvae. In control treatment, the gain was 0.203 gm and the growth rate was about 0.4, while they were 0.012 gm and 0.04 in the larvae treated with the lowest dose, 50Gy.The remarkable reduction in both of the weight gain and the growth rate in the larvae treated with highest doses 500 and 1000 Gy were (-0.019 gm and -0.118) and (-0.158 gm and -0.216) respectively (Fig. 3). This may be due to the increasing of larval mortality, thus the final weight of treated larvae was pronouncedly decreased. Also, exposure of larvae to low dose (50 and100 Gy) subsequently decrease feeding and gave of *T.castaneum* population control.

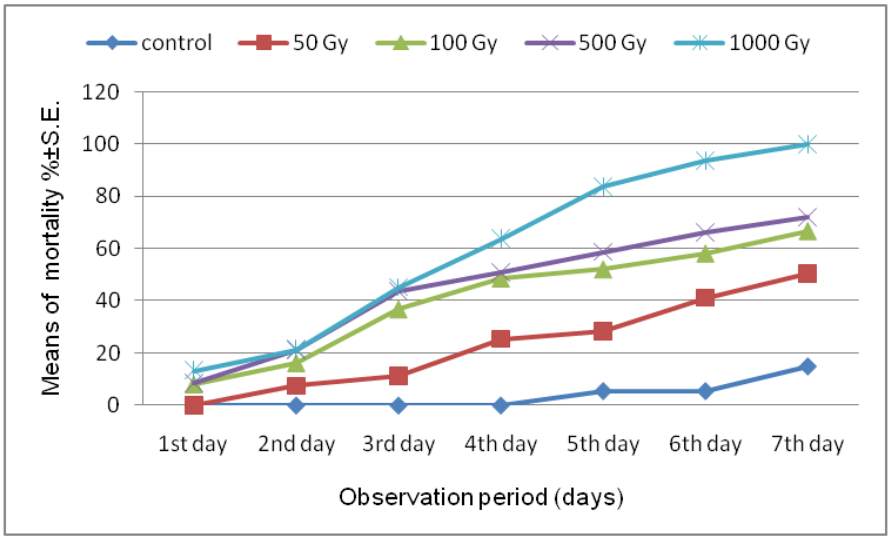


**Fig.1. Effect of gamma irradiation doses on the accumulative mortality percentages of *Tribolium castaneum* larvae within the observation period.**

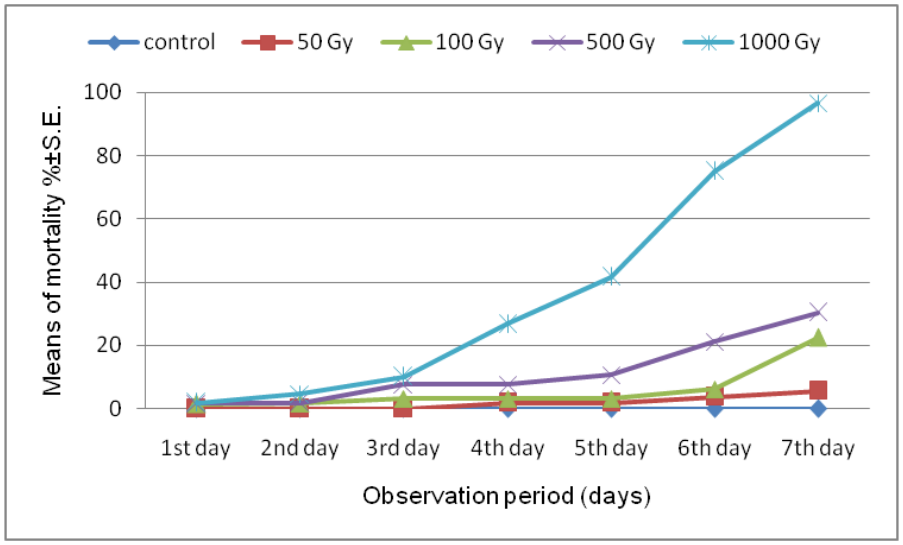


**Fig.2.** Effect of gamma irradiation doses on the accumulativ mortality percentages of *Tribolium castaneum* adult within the observation period.

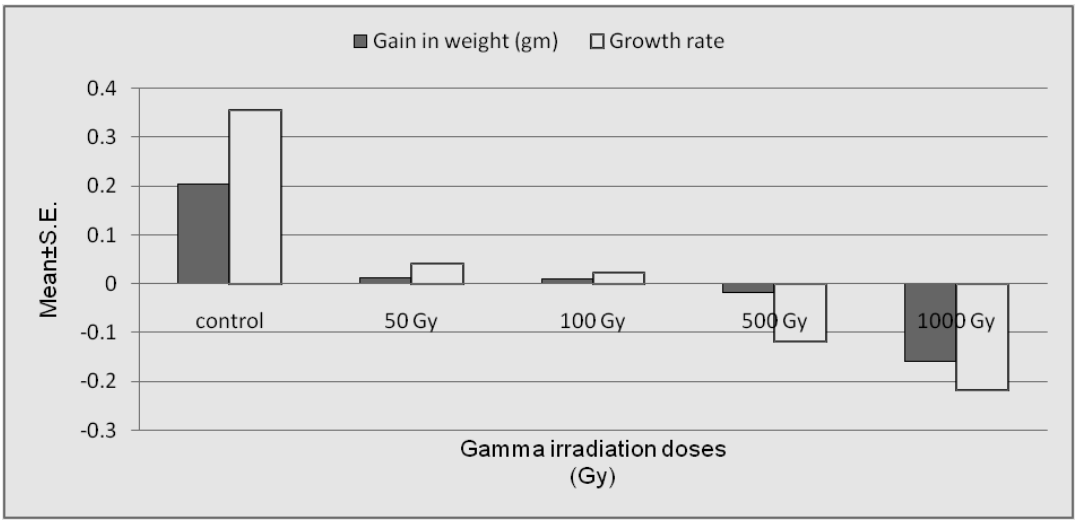


**Fig.3.** Effect of gamma irradiation doses on the gain of weight and growth rate of irradiated larvae

**Effect of Gamma-Irradiation on Quinones Secreted by Insect Stages:**

The relative amounts of quinones detected in the wheat flour which was artificially infested with *T. castaneum* stages are tabulated in Table (1). We compared the amounts of Quinones extracted from non-irradiated insect stages found in the infestation media with those extracted from irradiated insect stages with 50 and 1000 Gy. GC-MS analysis of extracts revealed that the Quinones secretion was reduced relatively in the adult stage than that in the larval stage. Five and eight Quinones substances were existed in un-irradiated adults' and in un-irradiated larvae respectively, (Table 1). The Quinones, 2,4-Decadienal, (E, E) and1-Pentadecene were the most abundant substances in adult control and Quinones, 1,2-Benzenediol,3-Fluoro, and Butanal were the most abundant substances in control larvae. As noticed in Table (1) the contamination flour with Quinones substances was negatively correlated with gamma-irradiation doses. Quinones substances, 2-Methyl-1,4-benzoquinone, 1-Pentadecene and2,4-Decadienal, (E, E) recorded 6.01, 6.30 and 7.01 in untreated control adults, these area percentages showed clear 2.03, respectively (Table 1). The GCMS analysis of the Quinones secreted from irradiated adults with 1000 Gy showed two types of substances which were unique compared to un-irradiated adults; Butanal and Nonanal. In Table (1) it was noticed that most Quinones secretions present in un-irradiated larvae were absent in larvae irradiated with 50 and 1000 Gy. At the same time, the persisted ones were severely affected by irradiation treatment.

**Effect of Irradiation Processing on Microflora Associated with the Insect Stages:**

The effect of gamma irradiation with doses 0,50, and 100 on microflora associated with *T. castaneum* stages are shown in Table (2). Firstly, as easily seen in this table, the internal bacterial and fungal counts are mostly more than that on the surface isolating. In respect to the microflora of the larval stage, data of the same table clarify that both of fungi and bacteria were greater in number than that of the adult stage. Gamma-irradiation decreased surface and internal counts of bacteria and fungi compared with the control treatment (Table 2). The counts of bacteria isolated from the surface were inhibited by about zero% at the irradiation doses of 50 Gy in case of adult and larval stages of *T. castaneum*. While the internal counts of bacteria were inhibited completely at the irradiation dose of 1000Gy.

There was a significant decrease in the count of fungi isolated from the surface

of the adult stage of the half at the dose of 50 Gy compared with the control treatment. It was shown that the dose of 1000 Gy inhibited the counts of fungi isolated from the surface of both stages about zero%. Whereas the counts of fungi isolated from both the adult and larval stages were significantly decreased by about 3.3% and 3%, respectively as recorded in Table (2).

**Table1.** Effect of gamma irradiation on quinones secreted by *Tribolium castaneum* stages

| Compound Name* | Molecular formula | Molecular Weight | Area % (concentration) of quinones secretion of adult stage | | | Area % (concentration) of quinones secretion of larvae stage | | |
|---|---|---|---|---|---|---|---|---|
| | | | In control treatment | In 50 Gy treatment | In 1000 Gy treatment | In control treatment | In 50 Gy treatment | In 1000 Gy treatment |
| 2-Methyl-1,4-benzoquinon | C7H6O2 | 122 | 6.01 | 1.54 | --** | -- | -- | -- |
| 1-Pentadecene | C15H30 | 210 | 6.30 | 2.96 | -- | 2.00 | -- | -- |
| 2-Ethyl-1,4-benzoquinon | C8H8O2 | 136 | 1.57 | -- | -- | -- | -- | -- |
| 2,4-Decadienal, (E,E) | C10H16O | 152 | 7.06 | 2.03 | -- | -- | -- | -- |
| Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate | C16H28O3 | 268 | 2.53 | -- | -- | -- | -- | -- |
| Butanal or Butyraldehyd | C4H8O | 72 | -- | -- | 3.39 | 6.42 | 2.38 | -- |
| Nonanal | C9H18O | 142 | -- | -- | 0.35 | 1.53 | 0.77 | 0.23 |
| 1,2-BenZenediol,3-Fluoro | C6H5FO2 | 128 | -- | 1.22 | -- | 11.49 | -- | -- |
| 2-Aminoethanethiol | C2H7NO3S | 157 | -- | -- | -- | 1.47 | 0.99 | 0.77 |
| Hydroquinone | C10H14O4 | 198 | -- | -- | -- | 5.29 | -- | -- |
| 1-Hexadecene | C16H32 | 224 | -- | -- | -- | 2.87 | -- | -- |

***All quinones substances were detected in irradiated and non-irradiated insects**
**** Noquinones substances were detected**

**Table 2.** Microflora counts associated with irradiated and non-irradiated *Tribolium castaneum* stages.

| Microflora Type | Treatments | Surface count | | Internal count | |
|---|---|---|---|---|---|
| | | Adult stage | Larvae stage | Adult stage | Larvae stage |
| Bact. Count CFU/100µl means ± S.E. | Control | $6.7\times10^3\pm 0.67^{a}$ | $14.7\times10^3\pm1.67^{a}$ | $6.0\times10^3\pm1.7^{a}$ | $22.3\times10^3\pm1.5^{a}$ |
| | 50 Gy | $0.0\pm0.00^{b}$ | $0.0\pm0.00^{b}$ | $3.3\times10^3\pm1.2^{ab}$ | $1.3\times10^3\pm.33^{b}$ |
| | 1000 Gy | $0.0\pm0.0^{b}$ | $0.0\pm0.0^{b}$ | $0.0\pm0.0^{b}$ | $0.0\pm0.0^{b}$ |
| | $F_{2,6}$= | 100.0 | 69.1 | 7.9 | 211.9 |
| | *P* = | .000 | .000 | .02 | .000 |
| Fungi count colony/100 µl means ± S.E. | Control | $6.7\pm1.20^{a}$ | $11.0\pm1.15^{a}$ | $8.7\pm.33^{a}$ | $16.0\pm2.1^{a}$ |
| | 50 Gy | $3.0\pm1.15^{b}$ | $8.3\pm.88^{a}$ | $8.0\pm1.00^{a}$ | $5.7\pm1.2^{b}$ |
| | 1000 Gy | $0.0\pm0.0^{b}$ | $0.0\pm0.00^{b}$ | $3.3\pm.33^{b}$ | $3.0\pm.52^{b}$ |
| | $F_{2,6}$= | 12.0 | 46.8 | 20.2 | 23.1 |
| | *P* = | .008 | .000 | .002 | .002 |

## DISCUSSION

Wheat is the most important grain crop in Egypt, where represents almost 10 percent of the total value of agricultural production and about 20 percent of all agricultural imports. The stored wheat flour is subjected to the invasion with the red flour beetle, *Tribolium castaneum* Herbst. (Coleoptera- Tenebrionidae) (Wakil et al., 2003). These beetles can cause a grey tint to the grain flour, which they are investing and give off a displeasing odor; also their presence encourages mold growth. Gamma irradiation is one of the alternative strategies for food protection, insect preventing infestation, food spoilage and increasing the shelf life with the aid of reducing the microbial load (Kanemaru et al., 2005b). Some recent researches continue to use acute mortality as the criterion for the efficacy of irradiation. Our results demonstrated that irradiation has negatively affects *T. cataneum* and kills both tested stages, but the larvae stage was more sensitive than the adult stage. The highest dose (1000 Gy) was capable of killing all tested stages, approximately introduced (100 % mortality) throughout the period of examination. The present findings corroborate with those of (Tuncbilek et al., 2003; Tandon et al., 2009; Kabbashi et al., 2012) who reported that the mortality of irradiated Tribolium sp. was augmented as the irradiation dose increased. (Ayvaz & Tuncbilek, 2006) explain the possible mechanisms of induction of the insect mortality by gamma-irradiation through cell cycle disruption following damage to DNA. Besides, larval mortality, Larval growth was remarkably inhibited in response to the increase of irradiation dose. A similar response was reported by (Abdel–Kawy, 1997; Ahmed and Hassan, 2001; Abdel Baki and Bosly, 2010) who proved the opposite relationship between the growth rate of the larvae and the dosages of gamma radiation. Al khlafe and Abdel Baki, (2013) mentioned that the possible mechanisms of induction changes by gamma radiation may lead to a conclusion that radiation adversely influences energy production and its utilization through the insect as indicated by the slower rate of metabolism. Consistent with many authors, (Happ, 1968; Unruh et al., 1998; Lis et al., 2011) tenebrionid beetles, mainly Tribolium species infest saved products and contaminate them with their defensive secretions (Quinones).

These secretions act as protection for their individuals from other insect species. Contamination of wheat flour appears to be a greater serious problem, wherein rheological properties of the dough are touchy to electro-active compounds, which include Quinones. Otherwise, many other problems due to Quinones secretion are its actual toxicity, allergenic and even carcinogenicity to people (Ladisch et al., 1967). In mammals, its toxicity may also cause respiratory depression, pores, and skin blanching and cyanosis earlier than death. So using the manipulate method to handiest kill of the pest is not sufficient, but this method ought to have an effect on the reduction of Quinones production. Unfortunately, there is still inadequate information concerning controlling and preventing these compounds by any mean to be compared with the results obtained within this assay. However, in mild of our findings, we can say that gamma irradiation has a severe effect on the Quinones substances production with the aid of *T. castaneum* stages, especially at 1000 Gy. For knowledge, fungi normally accompanied or followed the insect invasion (Miller, 1995). Additionally, insects can act as vectors of fungi, serving as internal and external carriers of spores. A number of bacteria and fungi were isolated from adults of *Tribolium castaneum* Herbst by Kumari et al., 2011). They detected a variety of microorganisms was found which include the pathogenic bacteria, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli, Enterobacter* spp., and pathogenic fungi, *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Penicillium* spp., *Fusarium* spp. *and Rhizopus oryzae*. It also

serves as a mobile source of fungal metabolites and mycotoxins (Barney et al., 1995). Insects and fungal infestation of stored food commodities, including wheat flour are considered a very serious problem in Egypt and worldwide. Our study indicates that a large number of bacteria and fungi were observed associated with *T. castaneum* particularly, larval stage. Concerning the effect of gamma irradiation on the count of isolated microflora, these counts were adversely correlated to the irradiation doses. It was clear that the negative association between the microflora counts and the irradiation dose was more pronounced in the case of insect surface isolation than the internal one. Many other investigators have been studying different methods to control the fungi and inhibit mycotoxins production in the storage food as Mohale et al., (2010) who assessed the losses caused by *Tribolium confusum* and its control by diatomaceous earth and its effect on *Aspergillus flavus* spore dispersal during storage of groundnuts. Also, (Khan et al., 2016) who investigates the insecticidal potential of spinosad, thiamethoxam, imidacloprid, and indoxacarb, against *Rhyzopertha dominica* / *Sitophilus oryzae* artificially infested wheat grain and subsequently its effects on aflatoxin production. Hence, in the present study gamma irradiation doses significantly reduced the numbers of both microflora types either the numbers of both microflora types either in surface or internal isolation of *T. castaneum* stages. It is already established that gamma irradiation has successes to control insect pest infestation. However, this study highlights the success of this treatment in preventing the additional damages associated with insect pest infestation.

**Conclusion**

From aforementioned results, it can be concluded that a dose 1000 Gy gamma irradiation can be used as a safe and effective strategy in the control of *Tribolium castaneum* as well as the additional harmful effects caused by this insect pest

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## ARABIC SUMMARY

### فاعلية التشعيع الجامى فى مكافحة خنفساء الدقيق الصدئية ترايبوليم كاستنيم هيربست ومنع أصاباتها الثانوية

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أجريت هذه الدراسة لمعرفة تأثير الجرعات المختلفة من التشعيع الجامى (0 ، 50 ، 100 ، 500 و 1000 جراي) على حنفساء الدقيق الصدئية ، تريبيولوم كاستنيوم هيربست. (غمدية الأجنحة: الخنافس). كما تم اختبار تأثير التشعيع الجامي على كل من إفراز الكينون والميكروفلورا المرتبطة بمراحل هذه الافة. وقد أظهرت النتائج أن زيادة نسب الموت في الطورين المختبرين كانت معنوية ($P < 0.05$) نتيجة لزيادة جرعة التشعيع. ايضا أظهرت نتائج نماذج الطيف الكتلي للغاز تأثيرًا مثبطًا قويًا للإشعاع على الكينونات التي تفرزها كل من الطورين. كما نجحت الجرعات المستخدمة في الحد من عدد الميكوفلورا المرتبطة بكل من مراحل اليرقات والحشرات الكاملة. حتى وصل هذا الانخفاض إلى صفر بالنسبة للفطريات والبكتيريا المعزولة من السطح بجرعة ١٠٠٠ جراي. وتشير النتائج المتحصل عليها إلى أن تشعيع اطوارخنافس الدقيق الصدئية بالجرعة ١٠٠٠ جراي هو وسيلة واعدة لمكافحة هذه الآفة المدمرة ومنع الاثار الضارة المصاحبة للإصابة بها.