Egypt. J. Chem. **Vol. 67,** No. 6 pp. 193 - 201 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Efficacy of Gamma Radiation on Mortality, Reproduction, DNA Damage and Antioxidant Enzymes on *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

Rasha Ahmed Zinhoum [1]*, Mona Mohammed Ali[2], Amira Afify[2]

[1] Agricultural Research Center , Plant Protection Research Institute ,Stored Product Pest Department, Dokki, Egypt.
[2]Cairo University, Faculty of Science, biotechnology Department, Giza, Egypt.

Abstract

Irradiation is a well-known and safe technique for controlling stored product insect pests and food protection as an alternative to pesticides. This study was designed to estimate the effects of gamma radiation doses in the range of 50–500 Gy on two stages of *Trogoderma granarium* larvae (second and fourth instar) and adult (male and female) in wheat grain. When larvae in the second or fourth instar were exposed to 300 Gy, no emerging adults were detected. Although parental adults have demonstrated no emerging adult, *T. granarium* irradiated with 200 Gy. 200 Gy was required for the disinfestation of the Khapra beetle. In addition, the DNA of *T. granarium* larvae, male and female adults, was studied in all body cells after exposing them to 200 Gy. The image analysis results found a detailed analysis of the migration patterns of DNA as well as a homogeneity study of the samples. The study implies that the comet assay is a rapid, simple, and sensitive visual method to evaluate the genotoxic effect of gamma radiation. It also investigated the effect of gamma radiation in the Capacity of three antioxidant enzymes SOD, CAT, and GST, the indicator marker of oxidative stress MDA on two stages of *T. granarium*. There was a change but no significant difference in SOD and Catalase levels between treated and control samples in both stages. The 200 Gy dose level had no effect on the growth of wheat. Moreover, in the adult stage, MDA contents were highly significant between the control and treated sample. Therefore, we estimated that a phytosanitary irradiation dose of 200 Gy is appropriate for *T. granarium*.

*Keywords***:** Gamma radiation; *Trogoderma granarium*; DNA damage; Biochemical studies; germination.

1. Introduction

In most regions of the world, cereals are considered staple foods. The fact that cereals' annual production totals around 2764 million tonnes, the largest single contributor to global food security, further proves the importance of cereals and their products [25]. In particular, wheat, rice, and maize offer excellent sources of vitamins, minerals, carbs, lipids, and proteins. In Egypt, cereals are the most important source of calories and protein for humans [28, 51].

Infestation of cereals and cereal products by stored product insect pests, which severely damage stored cereals, may range from 5 to 10% in geographically temperate regions and 20 to 30% in tropical regions [14]. The Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is considered a quarantine insect in many countries around the world. Different products, including cereals, buckwheat, cereal, pulses, alfalfa, vegetable seeds, herbs, spices, dried fruits, powdered milk, and nuts, are infested by *T. granarium.* Larvae cause quantitative damage that ranges from 6-33%, rising to even more than 73% with a high infestation. In addition to developing insecticidal resistance and the ability to withstand starvation for years [8, 35].For many years, the main strategy for controlling insect infestations in storage was fumigation using gases like phosphine (PH3) and methyl bromide [39]. Because it destroys the ozone layer, methyl bromide has been prohibited globally since the Montreal Protocol in 2015[57]. Due to its efficiency, simple application, and low cost, PH3is still widely used to eradicate pest insects that attack stored products. A significant disadvantage to applying it is the high level of insect resistance to PH3. Therefore, it is vital to look for alternative safe methods to prevent insect pests from attacking stored products [23].

Irradiation as a physical control method is an alternative tool to chemical and insecticide pesticides in several countries to control stored product insect pests. Furthermore, food irradiation with doses up to 10 kGy has been deemed to be safe and effective for preventing insect infestation of food by IAEA, WHO, and FAO [5].

Irradiation energy transported to insect bodies forms free radicals that destroy their DNA or genetic material. The insect pest will die if they are unable to repair this damage. Physical agents, such as gamma radiation and some chemical substances, have been shown to damage DNA in living cells [42]. By measuring the mobility of DNA strand breaks and alkali labile sites, the single-cell gel electrophoresis (SCGE) or comet assay can detect them. By detecting the movement of DNA from circular

*Corresponding author e-mail: ameraafify@yahoo.com.; (Amira Afify).
Receive Date: 16 October 2023, Revise Date: 25 November 2023, Accept Date: 27 November 2023
DOI: 10.21608/EJCHEM.2023.242012.8734

nuclear DNA, the comet assay can detect DNA strand breakage and alkali labile sites [54, 53].

The comet assay for assessing DNA damage is a rapid, simple, sensitive, reliable, and very cheap approach [30]. This technique could analyze DNA damage at the individual cell level. For examining genetic toxicity and DNA repair, the comet assay has been widely utilized in radiation biology and clinical research [11]. The comet assay can be briefed in the following steps: Cells are first mixed with agarose gel on a microscope slide. The membranes of the cells and nuclei are then exposed to a lysis buffer to lyse the cellular and nuclear membranes. Then, the DNA is exposed to an electrical field, followed by the alkaline electrophoresis stage. Cells are finally stained with the proper dye, and the DNA is observed using a fluorescence microscope. Ethidium bromide is used to visualize comets. The software program (Komet software) is used to image analysis of comet-shaped DNA. Measuring some parameters like %Tail DNA, tail length, and Tail moment [50].

Gamma irradiation is one of the biotic stress factors that significantly influence insect life since gathering reactive oxygen species (ROS), which cause oxidative stress and changes in the enzymes in insects that scavenge radicals. ROS are produced naturally as a byproduct of oxygen's usual metabolism. They are crucial for the stimulation of host and defense genes as well as cell signaling. [16, 38]. Oxidative stress can develop in insects and human erythrocytes as a result of environmental stress, UV radiation, bacterial infections, antibiotic use, and pesticide exposure. [43]. Different important components of the antioxidant system exist in insects and other animals. Superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) are considered enzymatic antioxidants, and phenolic substances such as vitamin E, vitamin C, and molecular thiols are considered non-enzymatic antioxidants [17].

SOD catalysis converts the predominant response to dietary pro-oxidant exposure appears to be the superoxide radicals to H2 O2 and oxygen [6]. CAT accelerates the decomposition of H2 O2 to water and oxygen [7]. Lipid peroxidation products or hydroperoxides are removed from cells by glutathione S-transferase (GST) [7, 18]. Malondialdehyde (MDA) is an end–product generated by the decomposition of arachidonic acid and Large PUFAs [22] through enzymatic and non-enzymatic processes. The content of (MDA) is an oxidative stress indicator.

Therefore, this research purpose to assess the impact of gamma radiation on two stages of *T. granarium* larvae and adults (male and female). Measure the DNA damage caused by radiation in larvae, males, and female adults. Determine the impact of gamma radiation on the amount of the antioxidant enzymes SOD, CAT, and GST, as well as the indicator marker of oxidative stress MDA. Furthermore, the influence of radiation on the germination of wheat grains.

2. Materials and methodes

2.1. Rearing technique

Stocks of Khapra beetle ,Trogoderma granarium Everts (Coleoptera: Dermestidae) were maintained at the stored grain and product pests department, Plant Protection Research Institute, Dokki, Giza, Egypt. They were reared on whole wheat in glass jars covered with a fine mesh. Cultures were maintained at 30 ± 1 °C, 60 ± 5% RH. The wheat used for the bioassay was kept in the freezer for ten days to eliminate infestation from the field. The colony was maintained in the same conditions for six generations before treatments.

2.2. Irradiation process

All irradiation processes were conducted at Indian Co-60 gamma Chamber, 4000 A, located at National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The average dose rate of this source was 1.277 kGy /h at the time of irradiation. Irradiation was carried out at room temperature. Alanine dosimeters (Traceable to National Physical Laboratory, UK) were used to calibrate the irradiator and measure the minimum, maximum, and average absorbed dose. Six dose levels of gamma irradiation (50, 100, 200, 300, 400, and 500 Gy) were tested against (larvae second or fourth instar, adults male and female) stages of T. granarium. All tested gamma radiation doses were repeated five times, and similar replicates of every treatment were left untreated for control.

2.3. Effect of gamma radiations on different stages of *T. granarium*:

2.3.1. Larva

Twenty second or fourth instar larvae were put into glass tubes with 10 g of wheat seeds, covered with muslin, secured tightly by rubber bands, and exposed to tested doses of gamma radiation. After treatment, the tested tubes were transferred to keep them at the optimum temperature, and the mortality percentage per replicate was calculated. To determine the decline in adult emergence compared to the control, the number of adults that emerged was counted.

2.3.2. Adult

Newly emerged adults (0-24 h old) were separated into males and females and transferred carefully to glass tubes by sieving (20 adults/ tube), then covered with muslin, secured tightly by rubber bands, and exposed to different tested gamma radiations. The treated adults were taken out and assessed after treatment to determine their mortality rate. Alive adults were separated to males and females, transferred into new glass vials, and examined daily to record the number of laid eggs per female. After that, these eggs were transferred into new glass jars with 10 g wheat seeds and incubated until emerging adults (progeny production). The reduction of adult emergence percentages in F1 was calculated.

2.4. Effect of gamma radiations on DNA

2.4.1. Preparation of samples for the alkaline single-cell gel (SCG) assay

For each sample, the whole body of ten control and Gamma radiation-exposed *T. granarium* larvae, male and female adults, was minced with 200 µl of PBS. In each group, three replicates of the sample were used.

2.4.2. Alkaline SCG assay

The biochemical technique of the comet assay (pH 13) was used to detect DNA single-strand breaks, alkali-labile sites,

and crosslinking [53]. To assess the genotoxic consequences of Gamma radiation, DNA damage was examined in the body cells of *T.granarium*. A volume of 200 µl of the sample was decanted for 10 minutes. On a microscope slide that had been previously treated with 1% normal melting point agarose (NMA), isolated cells (20 µl) were combined with 80 µl of 0.5% low melting point agarose (LMPA). The slides were given a cover slip, which was affixed, and they were put on ice right away. The slides were immersed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 0.25 M NaOH and1% Triton X-100, pH 10) for 24 hours at 4 °C after the coverslips had been removed and the agarose had solidified. The slides were then put in a horizontal gel electrophoresis tank after being lysed. 20 minutes were spent for DNA to unwind in an electrophoresis solution (300 mM NaOH and 1mM EDTA, pH 13). At 4 °C, 24 V, and 270 mA were used for 20 minutes of electrophoresis. The slides were next fixed in methanol, neutralised in 0.4 M Tris-HCl (pH 7.4), and let to dry at room temperature for an overnight period before being stained with ethidium bromide (2 g/ml). A Carl Zeiss Axio Fluorescence Microscope equipped with a 524 nm excitation filter and a 605 nm barrier filter was used to study comets. Ten people made up each sample in the three replicates that were created.

2.4.3. Assessment of DNA damage

The length of DNA movement (tail length) (TL) and the percentage of moved DNA (DNA %) were measured using a comet analysis system 4.0 created by Kinetic Imaging, Ltd (Liverpool, U.K.) coupled to a CCD camera. The nuclear diameter was measured to discriminate between populations of cells of different sizes. Finally, yet importantly, the computer calculated tail moments (TM); figure 1 depicts most of these parameters [50]. Each cell was visually categorised as belonging to one of five damage stages (from undamaged DNA stage 0 to maximally damaged DNA, stage D) based on the relative intensity of the head and tail fluorescence. Per sample, 50 to 100 randomly selected cells were examined (Three slides per treatment and at least 25 cells per slide were examined). There is no tail on stage 0 DNA that has not been damaged. The tail length of damaged DNA stage A is equal to or less than the length of the head diameter. The tail length of damaged DNA stage B is 1.1–3.5 times longer than the head diameter. The tail length of a damaged cell stage C is more significant than 3.5 times the head diameter. Stage D damaged DNA has no 'head,' as all of the DNA has moved to the tail [4].

2.5. Effect of gamma radiation on antioxidant enzyme

2.5.1. Preparation of tissue samples

The analysis was carried out at Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University. About 5gm tissue samples were homogenized in 5ml—ice-cold phosphate buffer, and the homogenized mixtures were centrifuged at 20.000 rpm for 10 min. at 4°C. The part from each supernatant was applied to estimate enzyme activities.

2.5.2. Superoxide dismutase (SOD) assay

According to [45], SOD activity was evaluated and indicated as OD/Mg protein/min.

2.5.3. Catalase assay:

The catalase activity was detected following [2] and expressed as OD/Mg protein /min.

2.5.4. Glutathione S–transferase (GST) assay:

The GST activity was estimated according to [31] and expressed as OD/Mg protein /min.

5.5. Measurement of Malondialdehyde (MDA) Contents: The measuring and determination of MDA levels in extracted tissue samples were quantified following [37]. MDA undergoes a chemical reaction with thiobarbituric acid (TBA), forming a colored mixture. MDA levels concern as an indicator of lipid peroxidation. In addition, MDA can be indicated as OD/Mg protein /min.

2.6. Effect of gamma radiation on Wheat grain germination

To determine the impact of gamma radiation at 200 Gy on wheat grains' germinability, germination experiments were conducted in accordance with the International Seed Testing Association's criteria for seed testing [59].

2.7. Statistics

All measurements were analyzed by using a one-way ANOVA. All statistical analyses were performed at a 5% significance level with the least significant difference using (SPSS) computer program compared to Duncan multiple range tests [19]. Mortality percentages of adult and larvae stages were used to identify the lethal dose values (LD50 and LD90). Bioassay data were statically analyzed by [26] and analyzed by the computer program Ldp Line as described by [47].

3. Results

3.1. Effects of gamma radiation on larvae

Gamma radiation significantly affected the larval stage of *T. granarium* (Table 1). The mortality percentages rise with the increase in radiation dose. At 50 Gy, the larval second-instar mortality was 34%, increasing gradually to reach 100% at 400 Gy. The emergence of adults from irradiated larvae (second instar) decreased as the irradiation dose increased. No emergence adults have been seen in irradiated larvae at 300 Gy (100% reduction), indicating that this irradiation dose prevents larvae from developing. The results indicate that fourth-instar larvae were more tolerant to radiation than second-instar larvae. The irradiation dose of 50 Gy increased the mortality of fourth-instar larvae to 24.40%, and 300 Gy increased mortality to 94%, corresponding to 34 and 100% in the case of second-instar larvae, respectively. No adult emergency was observed in irradiated larvae in either instar at 300 Gy (100% reduction).

Table 1

3.2. Effects of gamma radiation on adult

In Table 2, the results indicated that mortality percentages for *T. granarium* adults, male and female,

dose. At 50 Gy, the mortality percentage recorded was 26.40% and 10.40% in adult males and females, respectively. The results indicate that Females were more susceptible to irradiation than males. This may be due to Khapra beetle females being much larger than males. At the same time, 100% adult (male and female) mortality was recorded at 500 Gy. The fecundity of the adults was significantly reduced with increasing doses of gamma radiation. At 50, 100, 200, 300, 400, and 500 Gy, respectively, the number of eggs per female was 17.60, 15, 11.20, 0.00, 0.0, and 0.0 eggs, whereas the control had 23.60 eggs. No emerging adults have been seen from the parental generation at the dose level of 200 Gy. This

indicates that radiation treatment of *T. granarium* adults at 200 Gy achieves sterility and eliminates reproduction.

3.3. Lethal doses of two stages of T. granarium exposed to gamma radiation

The LD50 and LD90 values, together with their confidence limits, were presented in Table 3 and Fig. 1. According to the results, the LD50 and LD90 values for second-stage larvae were 85.34 and 333.16; for fourth-stage larvae, 119.93 and 446.93; for adult males, 156.61 and 626.75; for adult females, 227.54 and 716.80. According to estimates, the adult stage is the most tolerant.

3.4. Evaluation of gamma radiation genotoxicity

Gamma irradiation of *T. granarium* larvae and adults caused DNA damage in this study. The effects of gamma radiation on genomic DNA (DNA damage) of *T. granarium* larvae, male and female adults, as assessed by the comet assay, are shown in Table 4 and Fig. 2. Due to DNA strand breaks, the fragments of DNA travel from the nucleoid core to the anode during electrophoresis, generating a comet shape [54, 3]. The DNA in the body cells of the control samples was intact and circular (Fig. 2A). Insect body cells' nuclei, which are visible as a tail-like extension indicating DNA damage and strand breaks, display various degrees of DNA damage (Fig. 2A–E): (A) No damage, % tail DNA 5% (control); (B) modest damage, % tail DNA 15%; (C) moderate damage, % tail DNA 50%; (D) greater damage, % tail DNA 60%; and (E) maximum damage, % tail DNA > 60% [46,9] (Møller et al. 2020; Ail et al. 2022). Individual cell strand breaks appear like huge comet tails in this assay. Almost all control cells displayed comet pictures of a circular form with extremely short tails, indicating little or no DNA damage. Most comets had huge tails at 200 Gy in adults. The comet assay was used to quantify the DNA damage of *T. granarium* exposed to gamma radiation, which was quantified as tail length (TL) (μm), DNA tail%, and the tail moment [9]. The treated larvae's male and female adults' TL (m), DNA tail%, and tail moment values are higher than those of the control insects. ($P > 0.05$) This increase is significant in DNA tail% and the tail moment data but not significant in TL (μm) data (Table 4). The comet assay results in this study show that larvae have a lower DNA tail % than adults (Fig.3).

3.5. Effects of gamma radiation on oxidative enzymes

It was detected from the results that biochemical and cellular differences such as oxidative stress enzymes, for instance, SOD, Catalase, Glutathione – S – Transferase GST, and MDA levels in both adult and larval stages in *T. granarium* after irradiation with gamma rays (Table 5). There were marked changes but no significant difference between levels of SOD and Catalase between treated and control samples in both stages, adult and larva. Moreover, it showed a significant increase in levels of GST between the two stages, but there is no significant between the two samples treated and the control in the same stage. On the other hand, highly significant MDA contents were noticed between the control and treated samples respectively in the adult stage, even though there is no significant difference between control and treated samples respectively in the larval stage.

The data and observations taken by these investigations manifested and demonstrated that different stages of susceptible *T. granarium* play a role in the sensitivity after exposure to gamma radiation.

3.6. Effect of gamma radiation on wheat grain germination

Fig.4. depicts the results of the influence of 300 Gy irradiation dose on wheat germination. The results showed that this gamma irradiation dose did not affect wheat germination. Compared to the control, there was no significant difference in seed viability. Control and 300 Gy irradiated wheat seed germination percentages were 80 and 76, respectively.

Table 1. larvae mortality, Adult emergence, and reduction in adult emergence of *T.granarium* irradiated as larvae 2nd instar and 4th instar.

Dose(Gy)	Larva 2nd instar			Larva 4th instar		
	Mortality %	Adult Emergency%	Reduction %	Mortality %	Adult Emergency%	Reduction %
50	34±2.09^{b}	32.80±3.61^{c}	60.09±6.55^{a}	24.40±2.03^{b}	36±3.16^{d}	56.52±3.81^{a}
100	51.60±1.72^{c}	17.60±5.38^{b}	78.59±1.41^{b}	47.20±3.20^{c}	19.20±1.49^{c}	76.81±1.80^{b}
200	76±2.28^{d}	7.20±1.85^{a}	91.24±2.25^{c}	64.00±5.25^{d}	11.20±2.05^{b}	86.47±2.48^{c}
300	91±2.99^{e}	0.00±0.00^{a}	100±0.00^{d}	83.60±1.93^{e}	0.00±0.00^{a}	100±0.00^{d}
400	100±0.00^{f}	0.00±0.00^{a}	100±0.00^{d}	94.00±2.61^{f}	0.00±0.00^{a}	100±0.00^{d}
500	100±0.00^{f}	0.00±0.00^{a}	100±0.00^{d}	100±0.00^{f}	0.00±0.00^{a}	100±0.00^{d}
Control	1.2±0.8^{a}	82.80±3.61^{d}	-	4.0±0.4^{a}	87.20±2.33^{e}	-

Means followed by different letters are significantly different from each other at $P<0.05$.

Table 2. Adult mortality, fecundity, adult emergence and reduction in adult emergence of the 1st generation (F1) of *T. granarium* irradiated as adults male and female.

Dose(Gy)	Adult mortality % (means ± SE)		Fecundity (number of eggs/female) (means ± SE)	Adult emergence % of the 1st generation(F_1)	Reduction% in adult emergence % of the 1st generation(F_1)
	Male	Female			
50	26.40±4.83^{b}	10.40±2.03^{b}	17.60±2.32^{b}	35.01±2.34^{b}	36.76±4.22^{a}
100	39.20±1.49^{c}	22.40±1.60^{c}	15±2.02^{b}	31.33±5.53^{b}	43.39±5.99^{b}
200	48.80±2.65^{d}	32.80±3.20^{d}	11.20±1.15^{b}	0.00±0.00^{a}	100±6.52^{c}
300	71.20±3.44^{e}	65.60±2.99^{e}	0.00±0.00^{a}	0.00±0.00^{a}	100±0.00^{c}
400	80±1.26^{f}	76±2.40^{f}	0.00±0.00^{a}	0.00±0.00^{a}	100±0.00^{c}
500	100±0.00^{g}	100±0.00^{g}	0.00±0.00^{a}	0.00±0.00^{a}	100±0.00^{c}
Control	2.40±1.60^{a}	1.78±0.80^{a}	23.20±1.28^{c}	55.36±4.13^{c}	-

Means followed by different letters are significantly different from each other at P<0.05

Table 3. LD50, LD90 values, and their confidence limits for two stages of *T. granarium* exposed to different doses of gamma radiation.

Stage	LD_{50} (Gary)			LD_{90} (Gary)			Slope ± SE	p
	Value	Confidence limits		Value	Confidence limits			
		Lower	Upper		Lower	Upper		
2nd larvae	85.34	64.71	105.09	333.16	246.00	560.46	2.16±0.34	0.4869
4th larvae	119.93	96.96	145.86	446.93	321.80	785.19	2.24±0.33	0.5541
Adult male	156.61	90.68	207.72	626.75	401.20	2459.35	2.12±0.59	0.3827
Adult female	227.54	174.18	296.03	716.80	473.03	2107.37	1.90±0.62	0.3854

Means followed by different letters are significantly different from each other at P<0.05

Table 4.Quantitative evaluation using comet assay of the DNA damage, expressed as Tail length (TL) (μm)DNA tail % and Tail moment (TM) in whole body cells of larvae, adults (male and female) of *T. granarium* control and irradiated.

Sample	Tail length (TL) (μm)	DNA tail %	Tail moment (TM)
Control male	3.12+0.07 ^{a}	1.1+1.6 ^{a} a	0.5+0.02 ^{a}
Treated male	4+0.4 ^{a}	18.2+1.2 ^{b}	1.56+0.17 ^{b}
Control female	2.9+0.29 ^{a}	3.6+0.01 ^{a}	0.38+0.01 ^{a}
Treated female	3.3+0.3 ^{a}	35.3+0.05 ^{c}	1.3+0.05 ^{c}
Control larvae	2.7+0.27 ^{a}	4.3+0.2 ^{a}	0.17+0.01 ^{a}
Treated larvae	3.5+0.35 ^{a}	9.8+0.9 ^{d}	1.6+0.03 ^{b}

Means followed by different letters are significantly different from each other at P<0.05

Table 5. SOD concentration, Catalase concentration, GST concentration and MDA concentration in *T. granarium* larvae

Stages	Treatment	SOD(OD 480/μg protein/min)	CAT(OD 480/μg protein/min)	GST(OD 480/μg protein/min)	MDA(OD 480/mg protein/min)
Larvae	Control	0.082±0.008^{a}	0.390±0.02 ^{a}	1.01±0.003^{a}	0.085±0.001^{a}
	Irradiated	0.075±0.007^{a}	0.337±0.05 ^{a}	1.04±0.040^{a}	0.080±0.002^{a}
Adult	Control	0.078±0.004^{a}	0.354±0.022 ^{a}	1.20±0.005^{b}	0.125±0.001^{b}
	Irradiated	0.084±0.003^{a}	0.440±0.04 ^{a}	1.25±0.040^{b}	0.304±0.000^{c}

(control, irradiated) and adult (control, irradiated) stages.
Means followed by different letters are significantly different from each other at P<0.05.

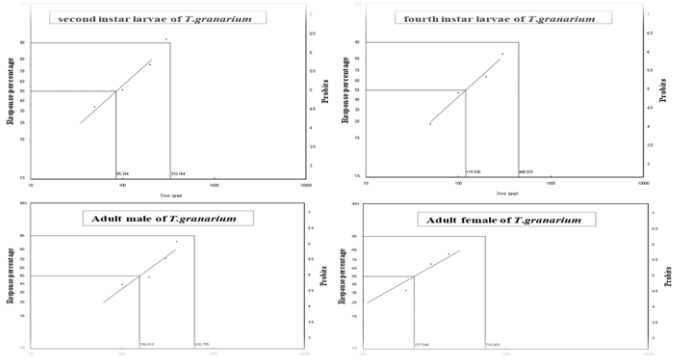

Figure. 1. LD50, LD90 values for two stages of T. granarium exposed to different doses of gamma radiation

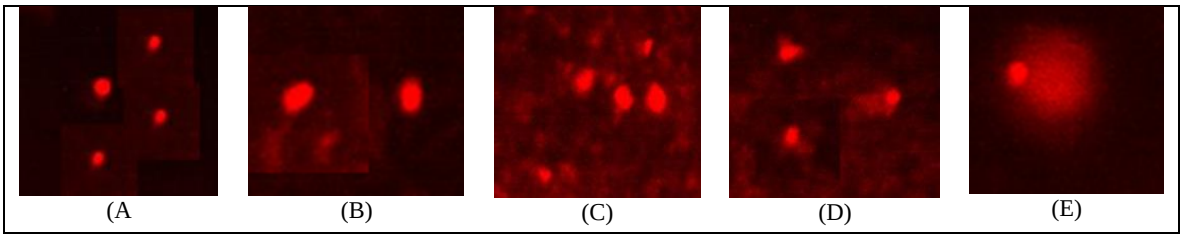

Figure. 2. Different cell damage stages in the comet assay (A) no damage, % tail DNA < 5% (control); (B) slight damage, % tail DNA < 15%; (C) moderate damage, % tail DNA < 50%; (D) higher damage, % tail DNA < 60%; and (E) highest damage, % tail DNA > 60 %.

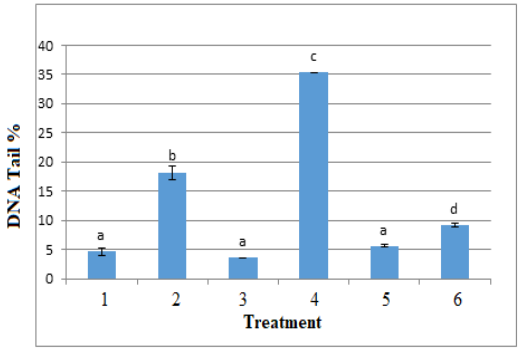

Figure 3. DNA damage analysis, assessed as (DNA tail %) in the body cells of larvae, male and female adults of T. granarium under normal and gamma radiation exposure conditions; columns1, 2: control and treated male adults with gamma radiation respectively, columns 3, 4: control and treated female adults with gamma radiation respectively and columns5, 6: control and treated larvae with gamma radiation respectively.

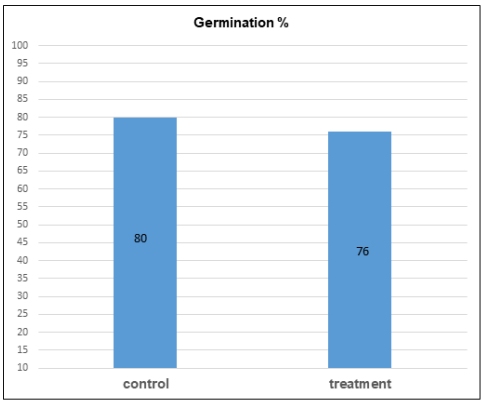

Figure 4. Germination percentage of wheat grains post-irradiation with 300 Gy of gamma radiation compared with unirradiated grain (control).

4. Discussion

Irradiation, such as gamma rays, X-rays, and electron beams, is used as a Phytosanitary treatment to control insect pests in stored and field crops. It is an eco-friendly technology for controlling insect pests in agricultural commodities, with no induced radioactivity or residual effect [12, 21, 27]. In that experiment, gamma radiation doses of 50, 100, 200, 300, 400, and 500 Gy significantly increased the mortality of larvae and adult *T. granarium* with an increased in dose. Total mortality (100%) at 400 Gy in 2nd instar larvae and at 500 Gy 4th instar larvae and adult (male and female). This indicates that 500 Gy is enough to control the Khapra beetle. In line with these findings, [56], the dose of 500 Gy effectively controls all pests of stored products by stopping their reproduction or adult emergence. [32] revealed that the dose to inhibit the reproduction of stored product pests ranges from 0.05 kGy to 0.45 kGy. At 300 Gy, adult emergence was inhibited entirely in larvae in the second or fourth instar, although parental adults have demonstrated no emerging adult *T. granarium* irradiated at 200 Gy. Our results suggested that irradiation at 200 Gy could prevent the reproduction of adults. These results agree with the International Standards for [36], which affirms that for beetles of stored products of Coleoptera, sterilizing inactive adult reproduction requires doses of 50–400 Gy. [29] reported that the effective quarantine irradiation dose for *T. granarium* was 200 Gy. [10] revealed that 100 Gy was required to prevent reproduction in adult *T. granarium*, but a dose of 200 Gy is recommended for more safety. In accordance with our results, [44], irradiating both sexes of the adult stages of the Khapra beetle *T. granarium* with 100 Gy fully stopped egg hatching, pupation, and adult emergence.

The effects of gamma radiation on genomic DNA (DNA damage) of *T. granarium* larvae, male and female adults, as assessed by the comet assay, the number of comets lacking tails was extremely low, implying that gamma radiation had an intrinsic effect on larval and adult cells. Most comets had huge tails at 200 Gy in adults, indicating that the cells throughout the body had been injured and DNA strand breakage had occurred. The most presentable parameter of DNA damage was thought to be the percentage of DNA in the tail region (DNA tail %) [61]. This indicates a high genotoxic potential capable of causing DNA damage in this gamma radiation. Previous research found that ionizing energy induced DNA damage in insect pests. [40, 15]. The most plausible explanation for this is that gamma radiation damaged DNA, either directly or indirectly, as a result of the production of reactive oxygen species (ROS), leading to a variety of oxidative lesions [58,55].

The comet assay results in this study show that larvae have a lower DNA tail % than adults, implying that radiation-generated radicals are quenched. These findings also align with those of [33], who found that the severity of DNA damage caused by gamma radiation differed between *S. zeamais* stages. Moreover, agreeing with earlier studies [52,

34] implies a relationship between insect age and ionizing radiation tolerance [60]. [1] In comparison to the control, the results showed increases in the DNA tail (%), tail moment, tail length, and olive tail moment. The adult *R. dominica* cells across their entire body have DNA damage, according to the results. The proportion of tailed cells compared to intact cells post-irradiation varied significantly. Greater movement of DNA fragments occurring as tail compared to control suggested a considerable increase in DNA damage.

In this experiment, we endeavor to research what category of physiological responses to gamma radiation, especially the estimate and evaluation of complete antioxidants. The capacity of three antioxidant enzymes SOD, CAT, and GST, is also the indicator marker of oxidative stress MDA. The obtaining oxidative injury of the cell macromolecules by exposure to gamma radiation can be reduced by antioxidant security comprised of enzymatic and non-enzymatic reactions. In this instance, oxidative stress occurs when ROS Creation and Oxidant Capacity are unequal to rebuilding the regular and typical *case* [49, 24, 41, 13].

The exposure to gamma radiation was measured by oxidative damage. In our result, we obtained two suggestion points from the data: the relationship between sensitivity to oxidative damage and stage. It showed that the adult of *T. granarium* is more sensitive to gamma radiation than the larval stage by comparing SOD, CAT, GST, and MDA content levels. The data explain that oxidative damage rises with age [48]. However, this study found that the grade of resistance in the *T. granarium* to gamma radiation more than the other species, especially during the larval stages, and older stages showed greater sensitivity to radiation.

According to the obtained results, gamma radiation had proven to be effective and did not harm the quality of wheat. [1], gamma radiation at a dose level of 280 Gy had no influence on the percentage of germination of wheat grains. [20] also agreed with the findings, stating that there was no significant difference in seed viability between wheat seedlings exposed to different levels of gamma radiation and the control.

5. Conclusions

Irradiation is a safe alternative quarantine treatment for the control of the Khapra beetle, *T. granarium*. This study established that the most effective gamma radiation dose for *T. granarium* is 200 Gy, eliminating 100% of adult reproductive. In addition, gamma radiation had a genotoxic damaging effect on the genetic levels of *T. granarium* larvae, male and female adults. The study highlighted the comet assay's remarkable sensitivity in identifying DNA damage in cells exposed to gamma radiation. As in earlier studies, the tail DNA, tail Length, and tail Moment parameters continue to be the more important factors in comet test. Exposure to 200 Gy can be affected directly on a cellular level by causing oxidative damage from generating oxygen radicals from water molecules. The oxidative conception of old age assumes an irregular correlation with oxidative damage. The decline in radiation resistance in adult *T. granarium* might be because of the reduced capacity of the internal origin of antioxidant enzymes to eliminate oxygen radicals or recover the damage. Therefore, we recommended 200 Gy as a quarantine dose of gamma radiation to control *T. granarium*.

Funding: this study was supported by Agricultural Research Center, Plant Protection Research Institute and Cairo University, Faculty of science.

Conflicts of Interest: "The authors declare no conflict of interest."

References:

1. Abotaleb AO, Zinhoum RA, Gabarty A. The comet assay evaluates irradiation dose-induced DNA damage in *Rhyzopertha dominica* and its effect on wheat grains. International Journal of Tropical Insect Science. 2021; 41(1):693-701.
2. Aebi HE. Catalase. In: Bergmeyes, H.U. (Ed.). Methods of Enzymatic analysis. Veriag, Weinheim, 1983; PP: 273-286.
3. Afify A, Ghezawy NA, Ali MM, El-Tayeb TA. Effects of copper chlorophyllin and magnesium chlorophyllin on the humeral immune response, midgut ultrastructural changes, and DNA damage to Culex pipiens larvae. African Entomology.2019; 27(1): 146-158.
4. Afify A. Potential Role of Mosquito Larvae *Culex Pipiens* as a Biological Indicator of Environmental Water Pollution in Egypt", Journal of Mosquito Research .2017; 7 (22): 184-193.
5. Agundez-Arvizu Z, Fernandez-Ramirez MV, Arce-Corrales ME, CruzZaragoza E, Melendrez R, Chernov V , Barboza-Flores M. Gamma-radiation effects on commercial Mexican bread making wheat flour. Nuclear Instruments and Methods in Physics Research.2006; 245(2):455-458.
6. Ahmad S, Pardini RS. Mechanisms for regulating oxygen toxicity in phytophagous insects. Free Radical Biology and Medicine, 1990; 8: 401-413.
7. Ahmad S, Duval DL, Weinhold LC, Pardini RS. Cabbage Looper antioxidant enzymes: tissue specificity, insect Biochemistry, 1991; 21: 563-572.
8. Ahmedani MS, Haque MI, Afzal SN, Aslam M,Naz S. Varietal changes in the nutritional composition of the wheat kernel (*Triticum aestivum* L.) caused by khapra beetle infestation. Pakistan Journal of Botany.2009; 41(3): 1511-1519.
9. Ali M, Ramadan MA, Ghazawy NA, Afify A , Mousa SA. Photochemical effect of silver nanoparticles on a flesh fly larval biological system. Acta Histochemica, 2022; 124(3).
10. Arthur V, Silva LCAS. Control of *Trogoderma granarium* through gamma radiation of Cobalt-60—International Nuclear Atlantic Conference Santos, SP, Brazil, 2007; September 29 to October 5, -ABEN.
11. Augustyniak M, Gladysz M, Dziewie M. The Comet assay in insects — Status, prospects and benefits for science. Mutation Research / Reviews in Mutation Research. 2016 Jan-Mar; 767:67-76.
12. Bell-Gam SP, Nwosu LC, Ileke KD, Aguwa UO. Effect of X-ray irradiation on the F1 generation of *Sitophilus zeamais* Motschulsky and the germination rate of maize grain. The Journal of Basic and Applied Zoology. 2021; 82:25.doi.org/10.1186/s41936-021-00225-y.
13. Birben E, Sahiner UM, Sackesen C, Erzurums S , Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organization. 2012Jan; 5(1):9-19. doi: 10.1097/WOX.0b013e3182439613.

14. Chomchalow N. Protection of stored products with special reference to Thailand. Assumption University Journal of Technology.2003; 7(1):31-47.
15. Cho S-R, Kim M, Shin E, Kim HK, Koo H-N, Kim G-H. X-ray Irradiation-Induced Abnormal Development and DNA Damage in *Phthorimaea operculella* (Lepidoptera: Gelechiidae) Applied Sciences. 2021; 11: 5068.
16. Dalton TP, Shertzer HG, Puga A. Regulation of gene expression by reactive oxygen. Annual Review of pharmacology and toxicology. 1999; 39: 67 - 101.
17. Dubovskii IM, Olifrenko OA , Glupov VV. Level and activities of antioxidants in the intestine of Lavrae *Galleria mellonella* L.(Lepidoptera, Pyralidae) at peroral infestation by Bacteria Bacillus Thuringiensis SSP, Journal of Evolutionary Biochemistry and Physiology.2005; 41 (1): 20-25.
18. Dubuiskiy IM, Martemyanow VV, Voront Sova S L, Rantala M J, Gryzanova, E V, Glupov V V. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of *Galleria mellonella* L. Larvae (Lepidoptera, Pyralidae). Comparative Biochemistry and physiology. 2008; Part C. 148: 1-5.
19. Duncan DB. Multiple ranges and multiple F tests. Biometrics. 1955; 11:1- 42.
20. El-Naggar SM, Mikhaiel AA. Disinfestation of stored wheat grain and flour using gamma rays and microwave heating. Journal of Stored Products Research.2011; 47:191–196
21. Espo E, Eyidozehi K ,Ravan S. Influence of gamma and ultraviolet irradiation on pest control. MAGNT Research Report. 2015; 3(2): 319-326.
22. Esterbauer H, Schaur R J, Zollner H. Chemistry and Biochemistry of 4- hydroxynonal, malonaldehyde and related aldehydes. Free Radical Biology and Medicine. 1991; 11(1):81 - 128.
23. European Mediterranean Plant Protection Organization (EPPO). Phosphine fumigation of stored products to control *Trogoderma granarium*. OEPP/Bulletin, 2012; 42(3): 501-503.
24. Fang Y Z, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition.2002; 18: 872 - 879.
25. FAO Cereal Supply and Demand Brief. World Food Situation. Cereal production and inventories to decline, but overall supplies remain adequate. Food and Agriculture Organization of the United Nations. Available online: http://www.fao.org/worldfoodsituation/csdb/en/ (accessed on November 14, 2022).
26. Finney DJ. Probit Analysis, third ed. Cambridge Univ. 1971. 333p.
27. Follett PA, Snook K, Janson A, Antonio B, Haruki A, Okamura M , Bisel J. Irradiation quarantine treatment for control of *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice. Journal of Stored Products Research. 2013; 52:63-67. https://doi.org/10.1016/j.jspr.2012.09.004
28. Food and Agricultural Organization (FAO). Food balance sheet. 2011; Accessed December 28, 2014
29. Gao M, Chuanyao W, Li S. , Zhang S. In: Irradiation as a phytosanitary treatment of food and agricultural commodities. Irradiation as a phytosanitary treatment for *Trogoderma granarium* Everts and *Callosobruchus chinensis* L. in food and agricultural products. IAEA-TEC-DOC-1427, 2004; 75-85.
30. Garcia O, Romero I, Gonzalez JE, Moreno DL, Cuétara E, Rivero Y, Gutiérrez A, Pérez CL, Alvarez A, Carnesolta D, Guevara I. Visual estimation of the DNA percentage in the tail in the comet assay: evaluation of different approaches in an intercomparison exercise, Mutation Research. 2011; 720(1-2):14-21.
31. Habig WH, Pabst MJ, Jakoby WB. Glutathione – S – transferases the first enzymatic step in mercapturic acid formation. The Journal of Biological Chemistry. 1974; 249(22): 7130 -7139.
32. Hallman GJ. Control of stored product pests by ionizing radiation. Journal of Stored Product Research. 2013; 52: 36-41.
33. Hasan MD, Todoriki S, Miyanoshita A. Detection of gamma radiation-induced DNA damage in maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) assessed using the comet assay. International Journal of Radiation Biology.2008; 84(10): 815-820.
34. Hasan MM, Khan AR. Control of stored product pests by irradiation. Integrated Pest Management Reviews. 1998; 3: 15-29.
35. Honey SF, Bajwa B, Mazhar MS , Wakil W. *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) is an alarming threat to the rice supply chain in Pakistan. International Journal of Entomology Research.2017; 5(1): 23-31.
36. ISPM 18 (International Standards for Phytosanitary Measures) Guidelines for using irradiation as a phytosanitary measure. 2003; Rome, IPPC, FAO.
37. Jain SK , Levine SN. Elevated Lipid peroxidation and vitamin E-quinone levels in heart verticals of streptozotocin–treated diabetic rats. Free Radical Biology and Medicine .1995 Feb; 18(2): 337-341.
38. Kamata H, Hirata H. Redox regulation of cellular signaling. Cell signaling.1999; 11: 1- 4.
39. Kim B, Song JE, Park JS, Park Y, Shin EM ,Yang J. Insecticidal effects of fumigants (EF, MB, and PH3) towards phosphine-susceptible and -resistant *Sitophilus oryzae* (Coleoptera: Curculionidae). Insects, 2019; 10(10): 327.
40. Koo HN, Yun SH, Kim HK, Kim GH. Elucidation of molecular expression associated with abnormal development and sterility caused by electron beam irradiation in *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). International Journal of Radiation Biology. 2019; 95(3): 360-367.
41. Kors Loot A, Gestel CAV, Straalen NMV. Environmental stress and cellular response in arthropods. CRC press .2004.
42. Lee RF, Steinert S. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater)

animals. Mutation Research. 2003Sep; 544(1): 43-64.
43. Lopez Martinez G, Elnitsky MA, Benoit JB, Lee RE Jr, Denlinger DL. High resistance to oxidative damage in the Antarctic midge Belgic Antarctica and developmentally linked expression of genes encoding superoxide dismutase, catalase, and heat shock proteins. Insect Biochemistry and Molecular Biology. 2008; 38(8): 796 - 804.
44. Mansour M. Irradiation as a phytosanitary treatment against *Trogoderma granarium* (Coleoptera: Dermestidae).Florida Entomologist. 2016; 99(2): 138-142.
45. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry. 1972 May; 247(10):3170-3175.
46. Møller P, Azqueta A, Boutet-Robinet E, Koppen G, Bonassi S, Milić M. Minimum Information for Reporting on the Comet Assay (MIRCA). recommendations for describing comet assay procedures and results. Nature Protocols. 2020; 15: 3817–3826. doi:10.1038/s41596-020-0398-1.
47. Noack S, Reichmuth CH. Ein rechnerischesVerfahren zur Bestimmung von beliebigen Dosiswerten eines Wirkstoffes aus empirischen Dosis Wirkungs Daten. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Fortswirtschaft, Berlin-Dahlem. Heft, 1978; 185: 1-49.
48. Parashar V, Fran Kel S, Lurie A G, Rogina B. The effects of age on radiation resistance and oxidative stress in adult Drosophila melanogaster. Radiation Research. 2008; 169(6): 707-711.
49. Pardini RS. Toxicity of oxygen from naturally occurring redox-active pro-oxidants. Archives of Insect Biochemistry and Physiology.1995; 29(2): 101-118.
50. Rady AS, Korraa SS, Elshemey WM , Dakrory A. Evaluation of DNA Damage in Peripheral Blood Lymphocytes Exposed to Gamma Radiation.Journal of Scientific Research in Science. 2020; 37 part 1(1):31-43.
51. Sarwar MH, Sarwar MF, Sarwar M, Niaz AQ, Moghal S. The importance of cereals (Poaceae: Gramineae) nutrition in human health. A review Journal of Cereals and Oilseeds. 2013; 4(3): 32-35.
52. Selman BJ, Hasan MM. The dose-mortality response of *Tribolium* species to gamma irradiation throughout ontogeny. International Pest Control. 1995; 37(4): 114-116.
53. Sharma A, Shukla AK, Mishra M, Chowdhuri DK. Validation and application of Drosophila melanogaster as an in vivo model for detecting double-strand breaks by neutral Comet assay. Mutation Research. 2011; 721:142-146.
54. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research. 1988; 175(1):184-191.
55. Sudpraserta W, Navasumrita P, Ruchirawata M. Effects of low-dose gamma radiation on DNA damage, chromosomal aberration, and expression of repair genes in human blood cells. International Journal of Hygiene Environmental Health.2006; 209(6): 503-511.
56. Tilton EW. Achievements and limitations of ionizing radiation for stored products insect control. Proceedings of the 1st Integration of Working Conference on Stored Product Entomology. Savannah, Georgia. 1974; 354-361.
57. United Nations Environment Programme (UNEP). Scientific assessment of ozone depletion. 2018; Report No. 58: 588 pp, UNEP, Geneva, Switzerland.
58. Unscear (United Nations Scientific Committee on the Effects of Atomic Radiation). Sources and effects of ionizing radiation. Report to the General Assembly with scientific annexes. United Nations sales publication.2000a; E.00.IX.3, United Nations, New York.
59. Wang J, Yu Y, Tian X. Effect of γ-ray irradiation on the germinating characteristics of wheat seed. Radiation Physics and Chemistry.2012; 81(4):463- 465.
60. Willard WK, Cherry DS. Comparative radiosensitivity in the class insect. Journal of Theoretical Biology. 1975; 52(1): 149-158.
61. Zinhoum RA, Negm AAKH, Abd-El Hameed SM, Afify A. The efficiency of some safe methods in controlling *Stegobium paniceum* on *Capsicum minimum* plant. International Journal of Entomology Research. 2019; 4(5):7-14.