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

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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 instars) and adult (male and female) on wheat grains. When larvae in the second or fourth instar were exposed to 300 Gy, no emerging adults were detected. Despite the fact that no emerging adult has been observed in parental adults, *T. granarium* irradiated with 200 Gy. The disinfestation of the Khapra beetle required 200 Gy Furthermore, after being exposed to 200 Gy, the DNA of *T. granarium* larvae, male and female adults, was analysed in all body cells. The image analysis results revealed a complete analysis of DNA migration patterns as well as a sample homogeneity investigation. According to the findings, the comet assay provides a quick, easy, and sensitive visual tool for determining 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* larvae. 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.

As a result, we calculated that a phytosanitary irradiation dose of 200 Gy is suitable 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].

Storage product infestation of grains and cereal products In geographically temperate locations, insect pests that seriously damage stored wheat may vary from 5 to 10%, whereas in tropical regions, they may range from 20 to 30% [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 inflict quantitative damage ranging from 6-33% to more than 73% with a heavy infestation. In addition to gaining insecticidal resistance and the ability to survive for years on end [8, 35].

For many years, fumigation with gases such as phosphine (PH3) and methyl bromide [39] was the primary method of controlling insect infestations in storage. Because it depletes the ozone layer, methyl bromide has been banned worldwide since the Montreal Protocol was signed in 2015[57].

Due to its efficiency, simple application, and low cost, PH3is still widely used to eradicate pest insects that attack stored products

. The high level of insect resistance to PH3 is a key downside of using it. As a result, it is critical to investigate alternate safe strategies for preventing insect pests from attacking stored products [23].

Irradiation as a physical control method is an alternative tool to chemical and pesticides in several countries to control stored product insect pests. Furthermore, the IAEA, WHO, and FAO have declared food irradiation at doses up to 10 kGy to be safe and effective for preventing insect infestation of food [5]. When irradiation energy is delivered to insect bodies, it produces free radicals that destroy their DNA or genetic material. If the insect pest is unable to heal the harm, it will die.

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

The comet assay can identify DNA strand breakage and alkali labile sites by measuring the migration of DNA from circular nuclear DNA [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 on the individual cell level. For examining genetic toxicity and DNA repairing, the comet assay has been widely utilized in radiation biology and clinical research [11

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 objective to analyse the impact of gamma radiation on two stages of *T. granarium* larvae and adults (male and female). In larvae, male, and female adults, assess the DNA damage produced by radiation. Determine the effect of gamma radiation on the antioxidant enzymes SOD, CAT, and GST, as well as the oxidative stress biomarker MDA. Furthermore, the effect of radiation on wheat grain germination.

2. Materials and Methods

2.1. Rearing technique

Stocks of Khapra beetle ,*Trogoderma granarium* E. (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 grains used for the bioassay were 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 techniques

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 and fourth instars, adults (male and female) of *T. granarium*.

All gamma radiation dosages were tested five times, and control replicates of each treatment were left untreated.

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2.3Application of gamma radiations on different stages of *T. granarium.*

2.3.1. Larva

Twenty larvae used for each instar or the two instars, using second and fourth or one of them, please, clarify

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

Following treatment, the tested tubes were moved to maintain the optimal temperature, and the mortality percentage per replicate was calculated. The number of adults that emerged was counted to evaluate the drop in adult emergence relative to the control.

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 doses of gamma radiations. The mortality rate was determined in treated adults.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 till emergence. The reduction of adult emergence percentages in F1 was calculated.

2.4. Application of gamma radiations on DNA

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

The whole body of ten (control and Gamma radiation-exposed *T. granarium* larvae, male and female adults), was minced with 200 l of PBS for each sample. Three sample replicates were used in each group.

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 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.There was three replicates of each sample and each replicate made with ten individuals of insects.

2.4.3. Analysis 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 damaged DNA stage B tail length is 1.1-3.5 times longer than the head diameter. A damaged cell stage C's tail length is greater than 3.5 times its head diameter. Stage D damaged DNA lacks a 'head,' because all of the DNA has gone to the tail [4].

2.5. Application 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, measured as superoxide dismutase (OD) produced per mg protein per minute. The principle of the assay is based on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro-blue tetrazolium salt. After sample lysate (or homogenate) preparation, the chemical reagent quantities were added as recommended, and the absorbance was measured using the spectrophotometer at 560 nm. The percent inhibition was calculated using the following equation:

Percent of inhibition = cont sample cont A A A − × 100 where Acont is the control absorbance, and Asample is the absorbance of the sample.

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. and expressed as OD/Mg protein/min. The total GST activity, caused by the conjugation between CDNB with reduced glutathione, was measured at 340 nm using the spectrophotometer. The rate of increased absorbance due to the conjugation is directly proportional to the GST in the sample according to: 81 2. 2 U Asample g of tissueused

2.5.5. 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 findings show that fourth-instar larvae are more resistant to radiation than second-instar larvae. Irradiation doses of 50 Gy raised mortality to 24.40% in fourth-instar larvae and 300 Gy increased mortality to 94%, equal to 34 and 100% in second-instar larvae, respectively. At 300 Gy (100% decrease), no adult emergence was detected in irradiated larvae of either instar.

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

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

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

3.2. Effects of gamma radiation on adult

The mortality percentages for *T. granarium* adults, male and female, were shown in Table 2. At 50 Gy, the death rate in adult males and females was 26.40% and 10.40%, respectively.

The mortality percentages for *T. granarium* adults, male and female, were shown in Table 2. At 50 Gy, the death rate in adult males and females was 26.40% and 10.40%, 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 supresses 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.

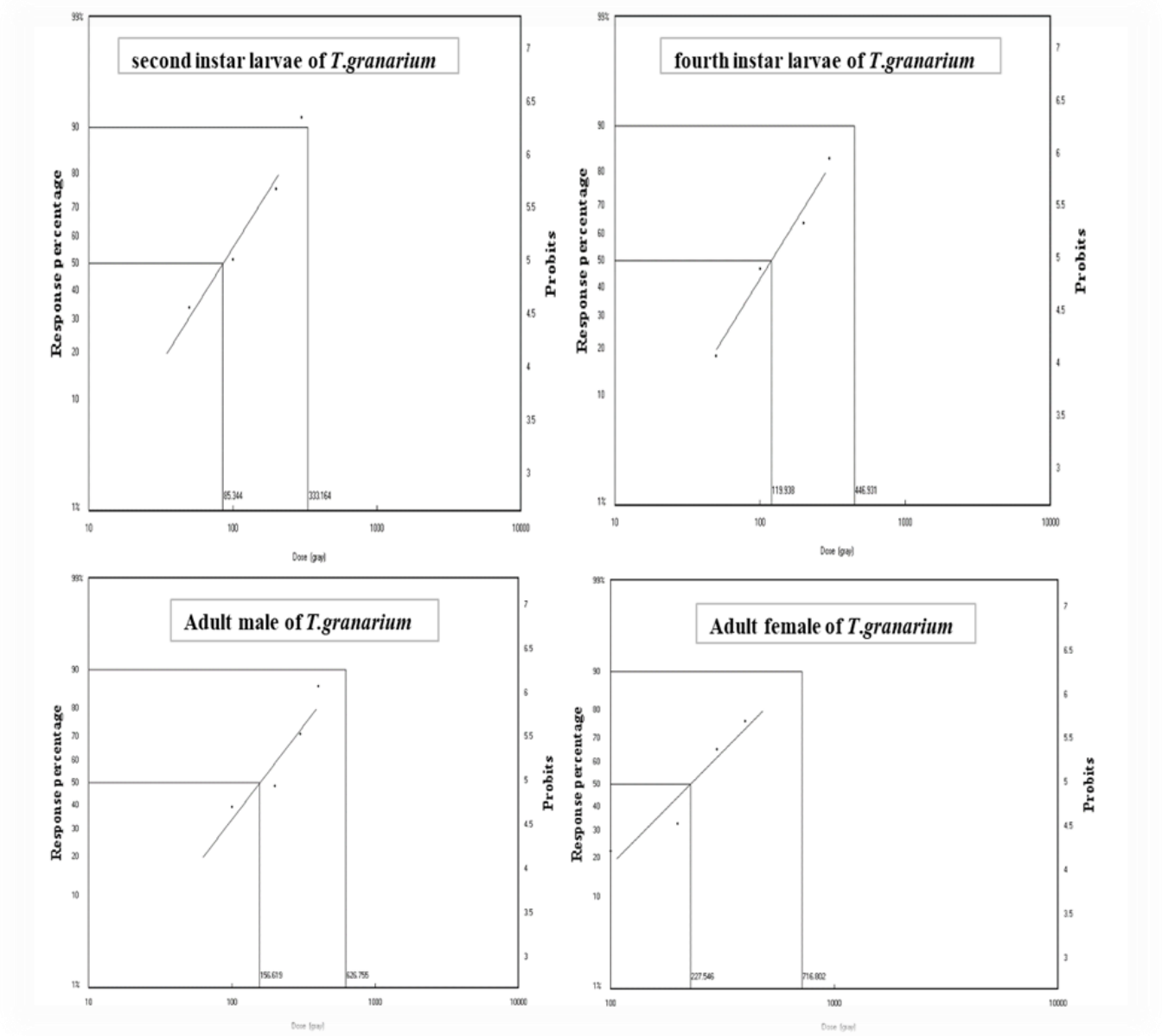

Figure. 1. LD50, LD90 values for two stages of *T. granarium* exposed to different doses of gamma radiation.

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 (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]	-

Table 2. Percentage adult mortality, fecundity, adult emergence and reduction in adult emergence of the 1st generation (F_1) of *T. granarium* irradiated as adults male and female.

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

Table 3. LD_{50}, LD_{90} 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.

3.4. Evaluation of gamma radiation genotoxicity

Gamma irradiation cause DNA damage in cells of larvae and adults. 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]. 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).

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

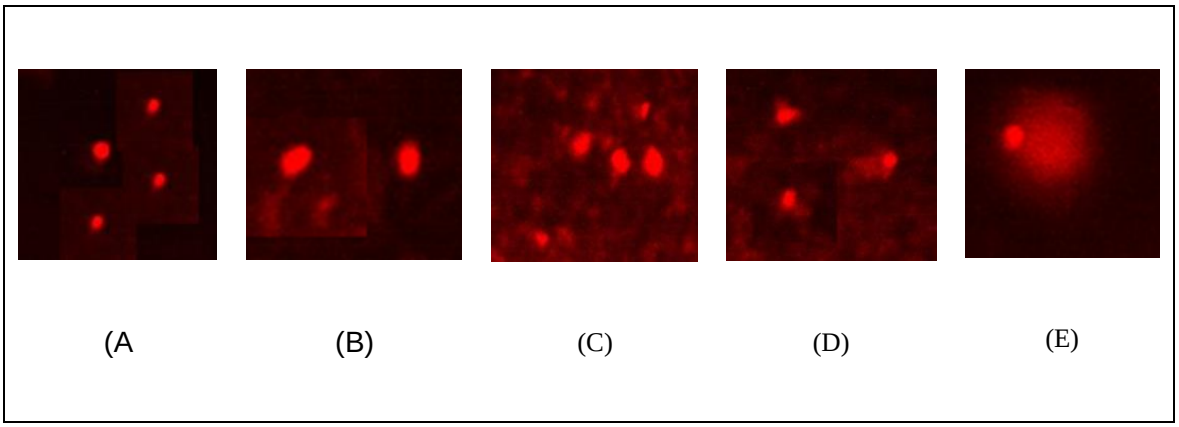

Figure. 2. Different cell damage stages in the comet assay (A) no damage, % tail DNA < 5% (control); (B) slight damage, % tail DNA < 15%; (C) moderate damage, % tail DNA < 50%; (D) higher damage, % tail DNA < 60%; and (E) highest damage, % tail DNA > 60 %.

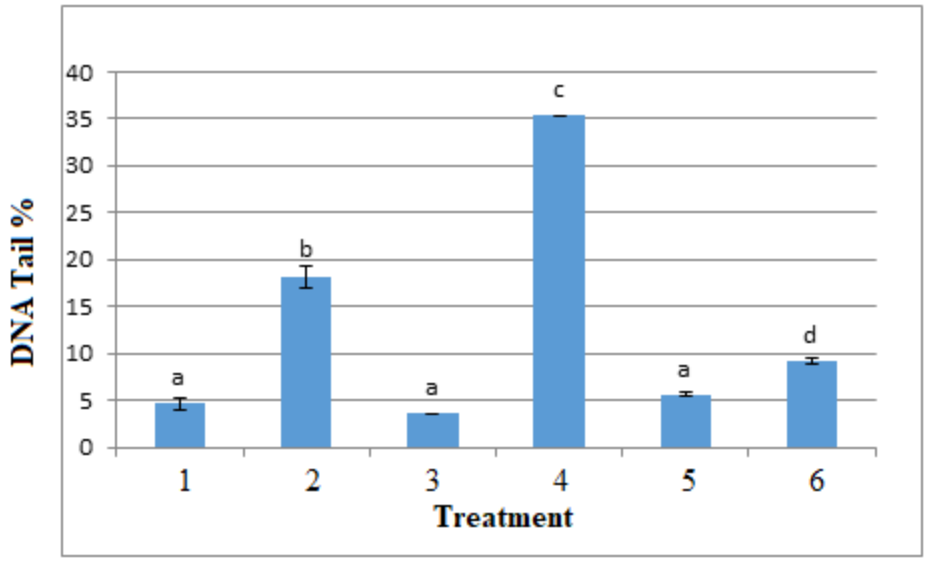

Figure 3. DNA damage analysis, assessed as (DNA tail %) in the body cells of larvae, male and female adults of *T. granarium* under normal and gamma radiation exposure conditions; columns1, 2: control and treated male adults with gamma radiation respectively, columns 3, 4: control and treated female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

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 stages, adult and larvae. 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 demonstrated that different stages of susceptible *T. granarium* play a role in the sensitivity after exposure to gamma radiation.

Table 5.SOD concentration, Catalase concentration, GST concentration and MDA concentration in *T. granarium* larvae (control, irradiated) and adult (control, irradiated) stages.

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

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.

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]

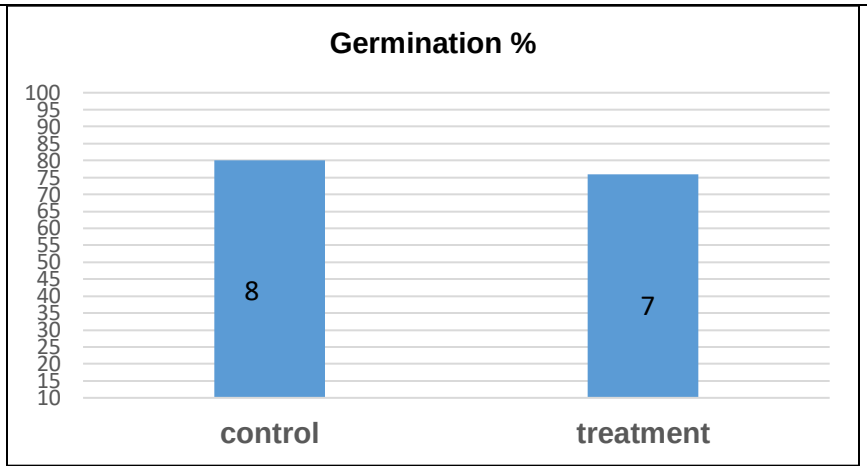

Figure 4. Germination percentage of wheat grains post-irradiation with 300 Gy of gamma radiation compared with controlled grains.

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, and 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%) was observed at 400 Gy in 2nd instar larvae and 500 Gy in 4th instar larvae and adults (male and female). This suggests that 500 Gy is sufficient to manage the Khapra beetle. According to these studies, [56], a 500 Gy treatment efficiently controls all pests of stored products by preventing their reproduction or adult emergence. According to [32], the dose required to stop the reproduction of stored goods pests ranges from 0.05 kGy to 0.45 kGy. Adult emergence was completely suppressed at 300 Gy in second or fourth instar larvae, despite parental adults demonstrating no emerging adult *T. granarium* irradiated at 200 Gy.

Our findings suggested that 200 Gy irradiation could impede adult reproduction. These findings are consistent with the International Standards for [36], which state that sterilising dormant adult reproduction in Coleoptera beetles requires doses ranging from 50 to 400 Gy. According to [29], the effective quarantine irradiation dose for *T. granarium* is 200 Gy. [10]. This discovered that 100 Gy was needed to inhibit reproduction in adult *T. granarium*, while 200 Gy is advised for greater safety. According to our findings, 100 Gy irradiation of both sexes of the adult stages of the Khapra beetle *T. granarium* completely inhibited egg hatching, pupation, and adult emergence.

The effects of gamma radiation on genomic DNA (DNA damage) of *T. granarium* larvaeand adults, as assessed by the comet assayshowing that 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] (Hasan et al. 2008), 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 endeavour 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, and 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

claiming that there was no significant difference in seed viability between wheat seedlings exposed to various amounts of gamma radiation and the control.

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 decrease in radiation resistance in adult *T. granarium* may be due to a diminished capacity of the internal origin of antioxidant enzymes to remove oxygen radicals or repair damage. As a result, we recommended 200 Gy of gamma radiation as a quarantine dose to control *T. granarium*.

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