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Histological Variants Induced by Gamma Irradiation in the Alimentary Tract of *Callosbruchus maculatus* (Coleoptera: Bruchidae)

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This study was conducted to evaluate the histological alternations induced by gamma irradiation in the alimentary tract of the 4th instar larvae of cowpea beetle *Callosbruchus maculates* (cowpea weevil). Using gamma rays at doses of 0.25kGy, 0.50 kGy, 0.75 kGy, 1.0 and 1.5 kGy, and mortality was recorded at 48 and 96 hours after irradiation. The highest mortality 100% had been recorded at 1.5 kGy gamma irradiation, there was no significant difference ($P \geq 0.05$) between irradiation at 1kGy and 1.5kGy, but the significance was observed ($P \leq 0.05$) compared with other treatments. The lethal dose (LD_{50}) of gamma irradiations at 48 and 96 hours were 0.4663 kGy and 0.4275kGy respectively. Results revealed that the mortality augmented by increasing the radiation doses; indicating that the effectiveness of the application was dose-dependent. Histological variations triggered by irradiation showed obvious deformations, cytoplasmic vacuolization and apical degeneration of cells. Also, The tissues exhibited marked signs of lysis and necrosis of cells. Hence, gamma irradiation could be incorporated into the control strategy of *C. maculatus* as a physical control means for the reduction of loss caused by stored product insects.

Keywords: Stored-product insects, Cowpea Weevil, Gamma Irradiation and Histological alternations

Introduction

Stored product pests give rise to serious losses in quantity and quality of post-harvest food grain, the cowpea weevils *Callosbruchus maculatus* is an essential economic insect that disturbs the cowpea germs, causing weight loss during the storage period [29, 28]. It was used as a model insect in many biological laboratories due to its rapid generation time and ease of maintenance. The control of storage pests was depending on the use of chemical techniques; these compounds have been recorded to be operative towards *C. maculatus* and other insects [3]. Because of the problems of numerous insecticides including; unavailability, high persistence, being expensive, resistance and risks to the environment and public health, research is being oriented to alternative control measures such as heat treatments, use of insect hormones, bio-pesticides, irradiation, and integrated pest management [15, 31].

Irradiation was used as phytosanitary management for agricultural products since the International plant protection convention (IPPC) mentioned the recommendations and simplified trade based on these phytosanitary methods [15, 16]. Ionizing irradiations especially gamma rays and X-rays [18, 15] and non-ionizing irradiations including microwave irradiation and Ultraviolet (UV) rays [13] were used to minimize replication and persistence of a diversity of different pests.

Gamma radiations solve the dangerous environmental problems caused by the abuse of

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pesticides. It is safe and reliable when the safety and quality-guarantee guidelines are monitored. Also, low doses of radiation may reflect beneficial effects [21, 20]. Irradiation is useful as a measure of control tactic that reflects a great potential and has been utilized with great achievement towards major agricultural pests. A novel trend is being oriented to biological control methods such as sterile insect techniques (SIT) [6] through exposing the insects to irradiation at doses adequately enough to induce the favorite effects and release in the field to suppress the population. This technique is applied to control insect pests by generating sterility, inducing a disturbance in biochemical metabolism or mortality of the insects [8, 22, 19, 30, 4]. The efficiency of gamma irradiation is wide-ranging between different insect orders and age-related to the same species [26]. Limited studies investigated the influence of radiation on the ultrastructure of insects [27, 17]. Therefore the present study aims to illustrate the influence of gamma radiation on cowpea weevil and to recommend the use of this physical method incorporation with the integrated pest management tactic of *C. maculatus* on stored cowpea.

Material and Methods

Insect culture

Adults of *Callosobruchus maculatus* were collected from grain shops. They were maintained in glass jars covered with a muslin cloth. A stock culture was reared on cowpea seeds at room temperature (28-30˚C and 65-75% relative humidity), the lifecycle took 45-48 days for completion [9].

Radiation facility

Irradiation was conducted at the National Center for Radiation Research and Technology (NCRRT), the Atomic Energy Authority, Cairo, Egypt with a Cobalt-60 gamma cell at a dose rate of 0.46 Gy /min, at doses of (0.0, 0.25, 0.50, 0.75, 1.0 and 1.5) kGy. Irradiation doses were selected based on previous studies. The amount of dose gained by the insect was calculated in terms of the energy absorbed in the body tissue and was defined in Kgray. 1 KGy =1000 Gy. Counting of the insects was done after 48h and 96h to determine the number of those alive or dead and the mortality percentage was related the radiation dose.

Assessment of irradiation effect on insect survival

Five pairs of newly emerged 4thinstar larvae of *C. maculatus* were obtained from ordinary culture , maintained into Petri-dish containing 50 healthy cowpea seeds and subjected to gamma rays (cobalt 60 source) at 0.25 kGy, 0.50 kGy, 0.75 kGy, 1.0 kGy, and 1.5 kGy. Mortality was recorded at 48 and 96 hours after treatments.

Histological technique

For histological examination irradiated and non-irradiated larvae, they were washed in 0.7%NaCl and fixed in 10%neutral formalin for 3-5 days. Specimens then underwent washing by distilled water and then dehydrated in ascending series of ethanol for 2 min (100: 70%). All the specimens cleared in xylene and were impregnated in hot paraffin. Implanting was prepared in hot paraffin wax by typical plastic cups. Transverse sections were cut at 3µ thickness and were kept back on a hot plate at 40°C to spread the wax ribbons. Following water evaporation in the oven (at minimum 24 h), the slides were immersed for 3-5 min in xylol and 2 min in each concentration of the previously prepared series of ethyl alcohol. Then, they were stained in hematoxylin for 30-45 min, the slides were washed with water for 2 min. subsequently, the slides were stained in counterstain (1% eosin) for 5-10 sec and were quickly soaked in an ascending alcohol series (70, 80, 90 and 96) and placed in absolute alcohol for 10 min and passed into two changes of xylol for 10-15 min/each. Lastly, the slides were fixed in Canada balsam and shielded with cover glass and were desiccated at 40°C for a day. The sections were observed by a light microscope (XSZ-107BN, China) [7].

Statistical analysis

For the bioassay test, the LC_{20} value was estimated according to [14] via "LdPLine®" software, [http://embakr.tripod.com/ldpline/ldpline.htm].

Data of all investigations were expressed as mean ±deviation error (SD) and analyzed using the SPSS11.5.0 software (SPSS Inc., 2012).

Results and Discussion

Bioassay of Gamma Irradiation

Invasion of Cowpea Bruchids began in the field and remained until storage. Bruchids are insignificant pests in the field, but they are considered a major pest grade during storage [24]. They cause significant harms expressed by seed damage and a decrease in weight, commercial value and seed germination [25, 2]. Gamma radiation has proved to be a technical alternative to conventional methods for controlling the stored-product insects [1]. Moreover, it is also used in agriculture for higher crop production, improving animal health and food processing [35]. Timbadiya et al.[34]mentioned the advantages of gamma irradiation applications as follows; a) improves the quality and quantity of crop production b) reduces pesticide use c) does not generate undesirable residues in treated foods d) damages insects that having resistance to insecticides e)No recovery of insect pests f) Safe to natural enemies. However, there are some disadvantages, thus it requires controlled dose exposure and proper safety measures. Control of insect pests through radiation is carried out by two methods (a) sterile insect techniques (b) exposing infested products, e.g. stored grain pests [12].

Influence of gamma rays on the mortality percentage of *C. maculatus* is presented in (Table1). Gamma irradiation was very effective at 48 hours after irradiation; there was a significant reduction in the population of *C. maculatus* at all expressed doses in comparison with non-irradiated (control) larvae. The highest mortality of 100%had been recorded at 1.5kGy gamma irradiation, while the control had zero value. There were not any significant differences among irradiation at 1 and 1.5kGy, but there was a significant difference ($P \leq 0.05$) to 0.25kGy, 0.50kGy, 0.75kGy, and the control. The same remark was noted at 96 hours following irradiation; where irradiation at 1.5 kGy had the maximum value of 100%. In conclusion, the irradiation processes at 1kGy and 1.5kGy have no significant differences, while they significantly differed from other doses. The mortality percentage of *C. maculatus* was directly proportional to the doses of gamma rays.

Table (1): Influence of gamma irradiation on the mortality percentage of *C. maculatus*

Treatment kGy)(	Percentage Mortality (%) of*C. maculates* (mean ± SD)	
	48h	**96h**
0.0	$0.00^{e}\pm0.00$	$0.00^{e}\pm0.00$
0.25	$22.41^{d}\pm1.871$	$28.20^{d}\pm1.483$
0.50	$40.50^{c}\pm1.581$	$44.20^{c}\pm0.837$
0.75	$74.13^{b}\pm0.707$	$76.80^{b}\pm0.837$
1.0	$98.91^{a}\pm0.837$	$99.40^{a}\pm0.894$
1.5	100^{a}	100^{a}
Slope	4.0443±0.3138	3.7980±0.3047

N = 5 replicates per test.

The significant difference ($P \geq 0.05$) represented by the same letters

The significant difference ($p < 0.05$) represented by different letters

For the detection of lethal dose (LD_{50}) of gamma irradiations outcomes (Table 2) reveals that 0.4663 kGy is adequate to kill 50% of *C. maculatus* after 48 hours of exposure. On the other hand, 0.4275 kGy is required to eradicate 50% of *C. maculatus* infestation after 96 hours of gamma irradiation. A cessation of feeding was noted gradually by time until death.

Table (2): Results obtained by statistical analysis

	48h	**96h**
LD_{50}	0.4663	0.4275
χ^2	24.9645	27.9114
χ^2 tabulated	7.8	7.8
R	0.9516	0.9352

These outcomes have proven that the efficacy of the treatment was dose-dependent where the mortality of *C. maculatus* increased through augmented doses of irradiation. This is maybe due to higher diffusion of gamma rays into the insect body which caused a greater destruction and thus produced more mortality. This explanation is in agreement with a previous [5] which investigated that irradiation effect on the cell cycle disruption resulting damage to DNA through which the mortality of insects occurs. Similar observations were made by Zhao et al. [36] who worked on the rice weevils exposed to microwave irradiation and stated that the efficacy of the treatment was dosage-dependent.

Histological investigation

The histological alternations of the normal 4th instar larva of *C. maculatus* and others subjected to the detected (LD_{50}) of gamma irradiation were examined. Fig. (1A) demonstrates the first section of the alimentary tract(Foregut) recognized as a single layer of epithelia resting upon a basement membrane, their free borders are covered by a thick intima. However the tissues of irradiated ones (Fig 1B) underwent partial lysis through local detachment between small groups of cells resting on the basement membrane. Spaces between epithelial cells appeared, the disintegration of the intima layer and ruptured of the salivary gland were observed. The histological observations of fat body cells revealed marked a rupture following treatment.

The second section of the alimentary tract (Midgut) was demonstrated in Fig. (2) .The normal larval tissues (Fig. 2A) appeared as short cubical cells, some of them exhibited large nuclei; some of the midgut epithelia exhibited a brush border. The midgut does not present a well-defined peritrophic membrane. The midgut included flatter cells with clear cytoplasm (clear cells). The cells exhibited diverse degrees of apical enlargement into the gut lumen, decreasing intercellular connections with adjacent cells and deterioration of nuclei and brush borders. Neighboring epithelial cells seem to be strictly appressed and only a thin white line of the extracellular matrix is visible the cells. The ventral nerve cord was situated peripherally, it is comprised of a mass of nerve fibers enclosed by nerve cells in the normal tissues. Gastric caeca cells were lined by a single layer of cuboidal epithelia in the normal larval tissues. However, tissues of the irradiated insects(Fig.2B)the midgut cells exhibited minor apical lysis through native detachment among cells resting upon a distended basement membrane, cytoplasmic vacuolization, nuclear degeneration and disruption of the lateral junctional complexes. The ventral nerve cord showed an obvious vacuolization and damaged fibers in the irradiated tissues. Cells of gastric caeca were greatly affected by irradiation through signs of necrosis and rupture.

The third section of the alimentary canal (Hindgut) were illustrated in Fig. (3); the normal hindgut epithelial cells(Fig. 3A&B) were lined by two types of cells. One of them was tall columnar epithelia with characteristic round structure expanded from the free borders and the other type of the cells seemed as cuboidal or small columnar cells with a rounded and straight free surface. Malpighian tubules lay between the midgut and the hindgut. They are extremely fine and blind tubes open into the anterior protein of the proctodaeum each tubule has appeared as a lumen surrounded by a single layer of cells. The cells of the hindgut were affected greatly in their nature after irradiation (Fig. 3C); they submitted to fractional lysis through groups of cells, appeared with disruption of junctional complexes and cytoplasmic vacuolization and apical degeneration of cells could be noted. Also, The tissues of Malpighian tubules showed distinct deformations; the lining epithelia of these tubules exhibited marked signs of vacuolization and necrosis.

The degenerative changes in the alimentary tract cells may be a result of a) the creation of free destructive peroxy radicals, which modify irreversibly the organic molecules through the chain of oxidative reactions along the radiation path on the living cells [34] b) breakage of chemical bonds when irradiation used as an isolation treatment ; once this happens to DNA, normal growth of the insect is perhaps prohibited [1]. Niyazi et al. [23] observed extreme radiation damage to the intestinal epithelial tissue along with apoptosis and nuclear damage in sterile males of *Ceratitis capitate*. In other species of insects, the changes in midgut ultrastructure following ionizing irradiation have been associated with reductions of the protease activity [11, 33] and with negative effects on other digestive enzymes [10].

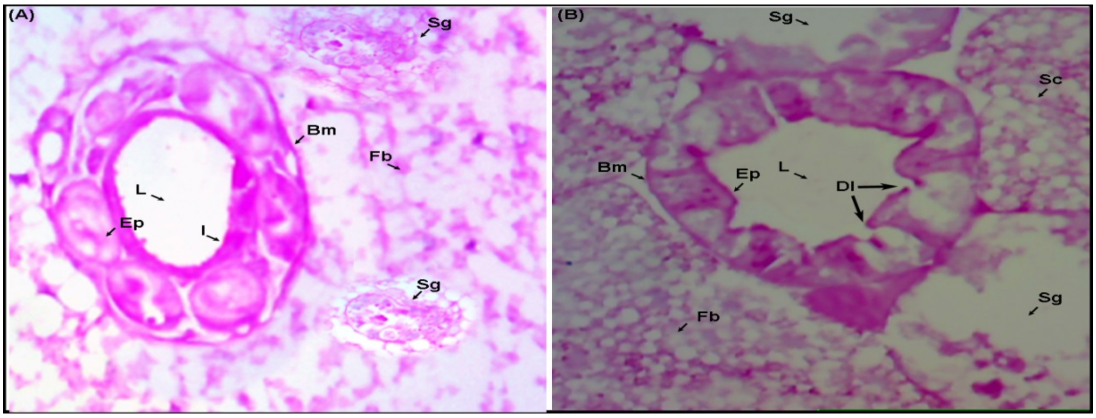

Fig (1): (A) Transverse section in the Foregut of normal 4th instar larva of *C. maculatus*; (Ep) Epithelial cells, (I) thick intima, (Bm) Basement membrane, (L) Lumen, (Fb) Fat body cells, (SG) Salivary gland

(B) Transverse section in the Foregut of irradiated 4th instar larva of *C. maculatus*; (Ep) Epithelial cells ruptured, (Bm) detached Basement membrane, (L) Lumen, (Fb) Fatbody cells, (Sg) ruptured Salivary gland, (DI)disintegrated intima (X400)

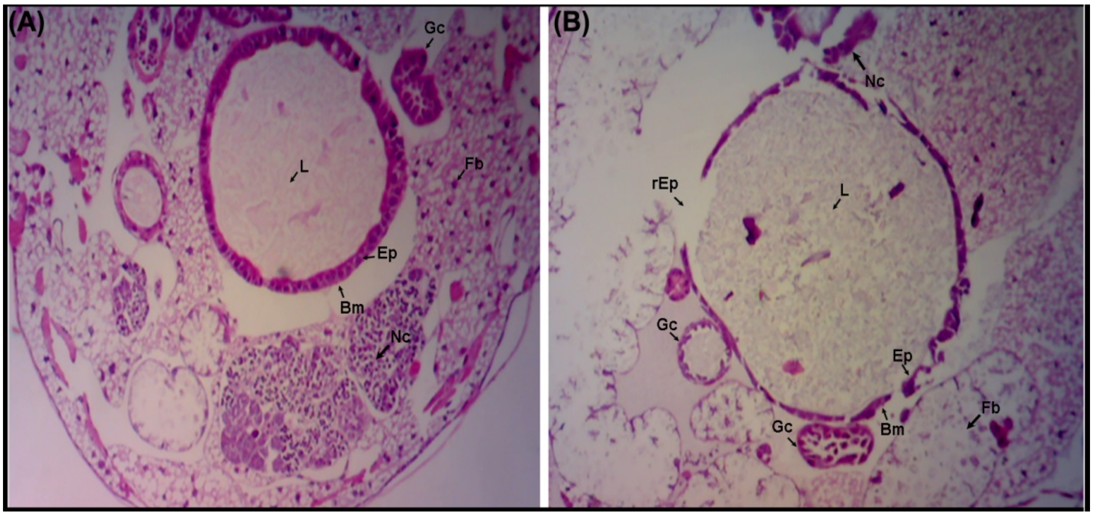

Fig (2):(A)Transverse section in Midgut of normal 4th instar larva of *C. maculatus*;(Ep) Epithelial cells, (Bm) Basement membrane,(N) nuclei, (L) lumen, (Fb) Fat body cells, (GC) Gastric caecum, (NC) Nerve cord

(B) Transverse section in Midgut of irradiated 4th instar larva of *C. maculatus*; (rEp) ruptured Epithelial cells, (Bm) detached Basement membrane, (L) lumen, (Fb) ruptured Fat body cells, (GC) ruptured Gastric caecum, (NC) damaged Nerve cord fibers. (x400)

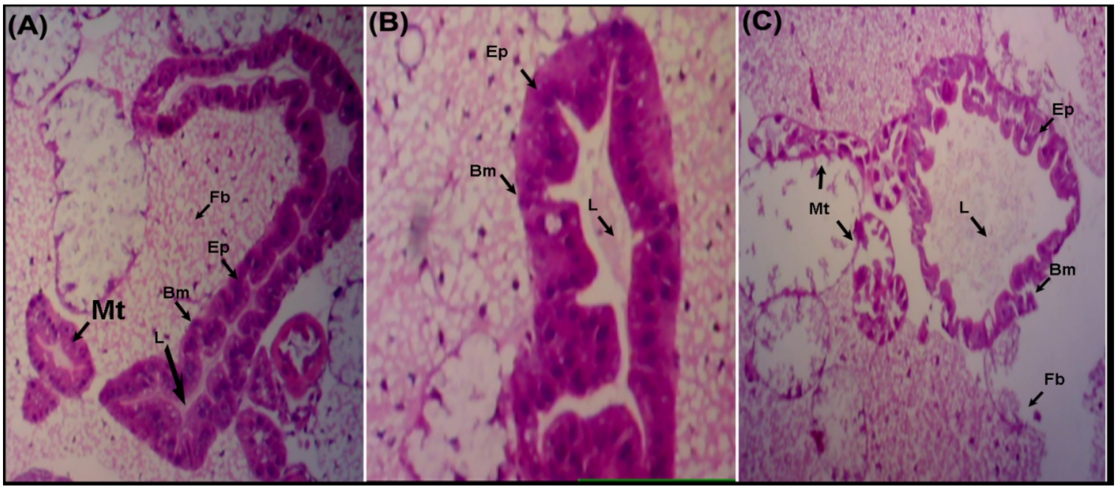

Fig.(3): (A, B): Transverse sections in the Hindgut of normal 4th instar larva of *C. maculatus*; (Ep) Epithelial cells, (Bm) Basement membrane, (L) Lumen, (Fb) Fat body cells, (Mt) Malpighian tubules. (x400)

(C) Transverse section in the Hindgut of irradiated 4th instar larva of *C. maculatus*; (Ep) lyses Epithelial cells, (Bm) detached Basement membrane, (L) Lumen, (Fb) ruptured Fat body cells, (Mt) deformed Malpighian tubules

The degenerative changes in the alimentary tract cells may be a result of a) the creation of free destructive peroxy radicals, which modify irreversibly the organic molecules through the chain of oxidative reactions along the radiation path on the living cells [34] b) breakage of chemical bonds when irradiation used as an isolation treatment; once this happens to DNA, normal growth of the insect is perhaps prohibited [1]. Niyazi et al. [23] observed extreme radiation damage to the intestinal epithelial tissue along with apoptosis and nuclear damage in sterile males of *Ceratitis capitate*. In other species of insects, the changes in midgut ultrastructure following ionizing irradiation have been associated with reductions of the protease activity [11, 33] and with negative effects on other digestive enzymes [10].

Conclusions

The present investigation established that the mortality of *C. maculatus* augmented by increasing doses of gamma rays demonstrating that the effectiveness of the application is dose-dependent. Histological alternations induced by irradiation revealed an remarkable effect. This implies that gamma irradiation can be applied to target insect pests as an eco-friendly technology for insect pest management, without causing any residual effect.

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