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Biochemical changes produced by gamma irradiation in the alimentary canal of males *Rhynchophorus ferrugineus* (Olivier)

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Abstract
The effect of gamma radiation (10, 15 and 20 Gy) on amylase and invertase activities and some metabolites in alimentary canal of five days old males *Rhynchophorus ferrugineus* was investigated. The optimal pHs for alimentary canal amylase and invertase activities ranged from 6.2-6.5 to 6-6.5, respectively. Amylase and invertase in *R. ferrugineus* were active in acidic pH, but the activity of invertase was higher than amylase activity. There was a significant ($p \leq 0.05$) increase in amylase, invertase activities and total carbohydrate at the dose level; 10 and 15 Gy, but decreased at the dose level; 20 Gy .Total protein and lipid were a significant decrease ($p \leq 0.05$) at all dose levels tested. In conclusion, it is obvious that gamma radiation affected the activities of amylase, invertase and total nutrient content in alimentary canal of males *R. ferrugineus*. But, the greatest marked decrease was at dose level; of 20 Gy.

Keywords: Amylase, Gamma radiation, Invertase *Rhynchophorus ferruginous*

1. Introduction

Rhynchophorus ferrugineus Olivier (Coleoptera: Curculionidae) is considered a serious pest that affects date palm tree (*Phoenix dactylifera*) and causes heavy losses to farmers. Red palm weevil is one of the hidden pests that remain within the Palm during the larval-stage growth and inside the palm tunnel even when turning out to be pupae. The adult insect can either appear on the trunk or remain inside the tunnel causing damage to the palm. The insect destroys the trunk and consequently produces multiple and overlapping generations.

Digestive enzymes are common enzymes that break down polymeric macromolecules into their smaller building blocks, in order to facilitate their absorption by the body. Digestive enzymes are found in the salivary secretions and regions of the digestive tract of various insects. A-amylases and invertase are digestive enzymes that play an essential role in starch and sucrose digestion and are consequently involved in energy production in insects [1]. Many studies have focused on the characterization of the digestive α-amylases of economically important insect pests, including *Rhyzopertha dominica* [2], *Tenebrio molitor* [3], *Eurygaster integriceps* [4], *Zabrotes subfasciatus* [1], *Naranga aenescens* L. [5], *Xanthogaleruca luteola* Mull. [6] and *Plodia interpunctella* [7].

Radiation effects on the molecular chemical species of the living organism body will provide information that is essential to the knowledge of the radiation-induced molecular alteration that will initiate the biological chain of damage and its final sequel. There are two distinct mechanisms by which biochemical changes can be induced by ionizing radiation, namely, direct action in which ionization takes place directly in the target molecule, and indirect action in which the molecule receives the energy by transfer from another molecule. These mechanisms are well defined in biological system where water is the major constituent [8]. Proteins, carbohydrates, lipid and enzyme molecules are the essential nutritive components in the biological system in insects. Therefore, any disturbance in this component leads to the disturbance in the biological system and adult performance. Thus, we focus in this paper on determining the effect of gamma radiation on amylase and invertase activity at optimal pH. In addition, the effect of gamma radiation on total proteins, carbohydrates and lipids in the alimentary canals of males red palm weevil were investigated.

2. Materials and methods

Red palm weevil, *R. ferruginous* adults were obtained from cocoons collected from infested date palm trees in the Menasheet El keram and Tel Bani Tamim- Qalubia - Governorate, Egypt in 2014. Insect rearing technique was performed according to Mahmoud *et al*. [9].

2.1 Irradiation process

Full-grown pupae were irradiated using Gamma cell-40 (cesium-137 irradiation unit), at National Center for Radiation Research and Technology (NCRRT, Cairo). The dose rate was 0.758 rd/ sec.

2.2 Enzyme sample preparation

A five-day-old male adult was immobilized on ice and dissected under a stereomicroscope in ice-cold distilled water. All parts of the alimentary canal were removed from the body and thoroughly rinsed in ice-cold distilled water. The alimentary canal was homogenized in double-distilled water using a hand-held glass homogenizer (20 mg/ml) and centrifuged in cold at 10.000 g for 15 minutes. The supernatant was used as enzyme solution [10].

2.3 Effects of pH on α-amylase and invertase activity

The pH profiles of the amylase and invertase activity were determined at room temperature in 0.2 M phosphate buffer (pH 5.8, 6.4, 7 and 7.6) and adjusted to acidic and basic pH values, respectively. Before determining activity, the reaction mixtures were incubated at different pHs at room temperature for 60 min.

2.4 Determination of amylase and invertase activity

Amylase and invertase activity of *R. ferrugineus* was determined by the methods of Ishaaya and Swirski [11] with modification. Calculations of enzymatic activity were carried out according to the equation denoted by Abou El-Ghar *et al*. [12], enzymatic activity (µg glucose/min./mg tissue).

2.5 Total protein determination

Total protein was calorimetrically determined by the method according to Gornal *et al.* [13], using kits purchased from Biodiagnostic Comp, Dokki, Giza, Egypt. The method is dependent on Biuret reaction.

2.6 Total lipids determination

Total lipids were calorimetrically determined by the method according to Zollner and Kirsch [14], using kits purchased from Biodiagnostic Comp, Dokki, Giza, Egypt.

2.7 Total carbohydrates determination

Total carbohydrate contents of total tissues content and fat body tissues were calorimetrically determined according to Singh and Sinha [15]. The standard curve was prepared by dissolving 20 mg glucose to 100 ml of distilled water, and from further dilutions serial concentrations of glucose solution were prepared. Determination of the standard curve was obtained from a direct reaction of glucose with anthrone reagent, under conditions similar to those for total carbohydrate determination.

2.8 Statistical analysis

All data obtained for biochemical studies were statistically analyzed and the variance ratios were calculated. The method of ANOVA is involved by using (SPSS) computer program, ver.15.0, and the significance among the samples was calculated at $P \leq 0.05$.

3. Results and Discussion

3.1 Optimal pH for amylase and invertase activities in *R. ferrugineus*

The optimal pH for amylase activity ranged from 6.2 to 6.5 (Fig. 1-a), and the optimal pH for invertase activity ranged from 6 to 6.5 (Fig. 1-b). In general, amylase and invertase in *R. ferrugineus* were active in acidic pH, but the activity of invertase was higher than the amylase activity at a temperature of 37 °C for 60 min (Fig. 1). The insects' diets are utilized after having been changed into simpler molecules through the action of digestive enzymes in the alimentary canal of the larvae. Subsequently, carbohydrase enzyme in the red palm weevil plays an essential function in turning the plant diet into useful biomolecules. Metabolism of carbohydrate-hydrolyzing enzymes and utilization of carbohydrate by insect is controlled mainly by amylase, invertase and trehalase enzymes [16]. So it is very important for determination the activities of amylase and invertase enzymes at optimal pH and This finding comes in agreement with the results reached by many researchers; the optimal pHs for amylase and invertase activities was 5.5 and 6.0, respectively, in red scale insect [11]. Optimal pH values for α-amylases in larvae of several coleopterans range from 4 to 5.8 [17]. α-amylase in *Hypera postica* larvae have an acidic optimal pH (pHs 3 - 6) [18]. Also, α-amylase, extracted from *Hypothenemus hampei,* has an optimal activity at pH 5 [19]. However, in some coleopterans, such as *Trogoderma granarium* (Dermestidae), optimum α-amylase activity mostly occurs at pH range (6 - 9) [20]. In the case of larval *Xanthogaleruca luteola,* showed that the optimum pH for gut α-amylase activity was 5 [21]. Also, the maximum amylase activity at pHs 4 – 5, observed in the gut of *R. ferrugineus,* was reported [2].

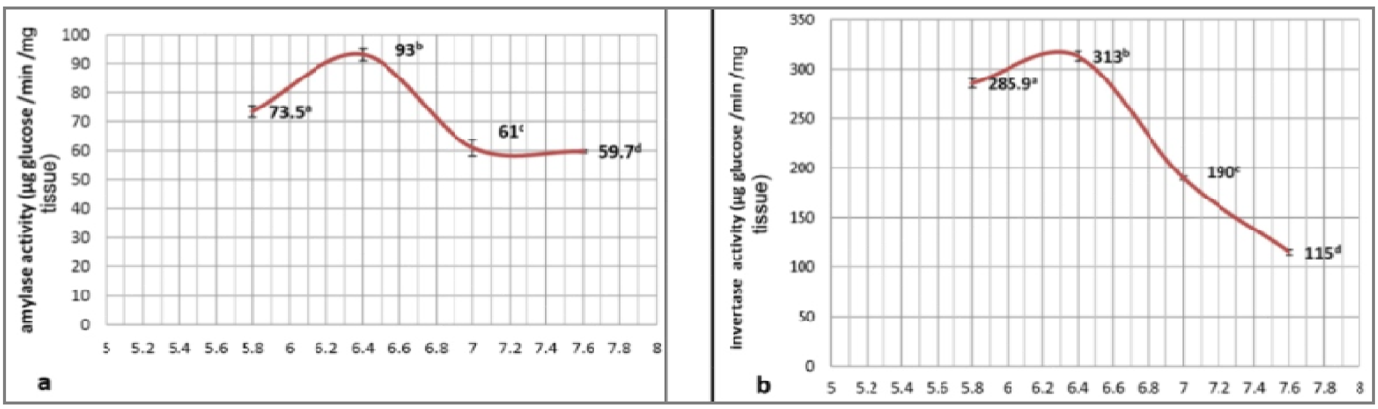

Fig. 1: (a&b): Optimum pH for amylase and invertase activities in the alimentary canal of male *R. ferrugineus*

3.2 Effect of gamma radiation on enzyme activity (amylase and invertase*)*

Amylase and invertase inhibitors represent an important tool in controlling insect pests. In order to achieve this goal, it is necessary to find the effect of gamma radiation (10, 15 and 20 Gy) at optimal pH on enzyme activity and their properties for possible use in *R. ferrugineus* management procedure. As shown from the results, amylase and invertase activities in the alimentary canal of male *R. ferrugineus* were affected by gamma radiation. There was a significant ($p \leq 0.05$) increase in amylase and invertase activity at dose levels of 10 Gy and 15 Gy, but the activity significantly ($p \leq 0.05$) decreased at the dose level of 20 Gy for amylase and invertase as compared with the normal control (Fig. 2 & 3).

Enzyme activities are known to be susceptible to radiation damage. The activity of enzyme may increase or decrease; depending upon the doses of gamma radiation. In general, the physicochemical effects of radiation on enzymes include decrease in molecular weight (due to fragmentation of polypeptide chain), changes in solubility, disruption of secondary and tertiary structure, formation of aggregates, and destruction of amino acids in the chain. Consequently, the biochemical criteria of damage are the loss of the enzyme to perform its function. An increase in the enzyme activity at the dose level; 10 and 15 Gy may be explained as follows: several radiation-induced reactions are reversible at low doses due to Repair Processes that occur.

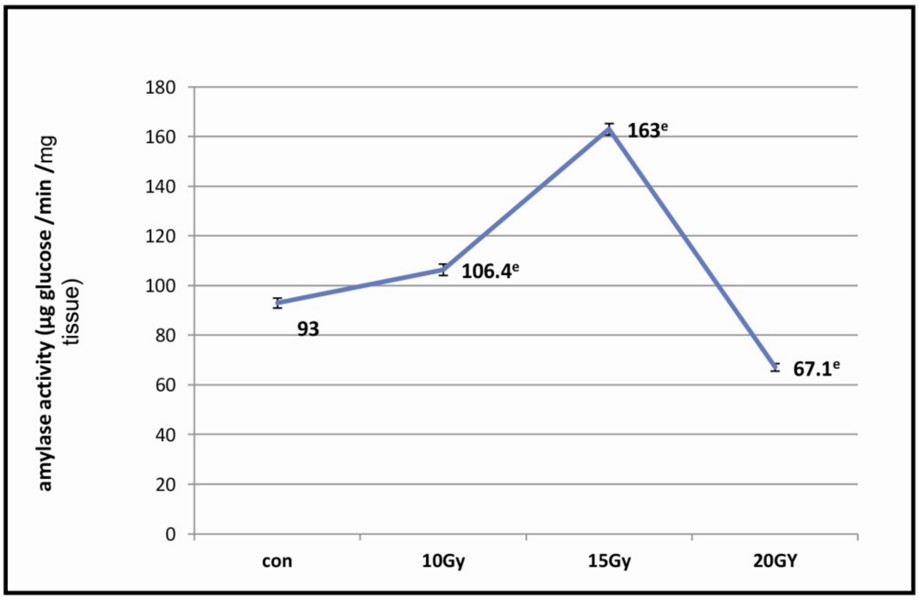

Fig. 2: Effect of gamma radiation (10, 15 & 20 Gy) on amylase activity in male *R. ferrugineus* at optimal pH.

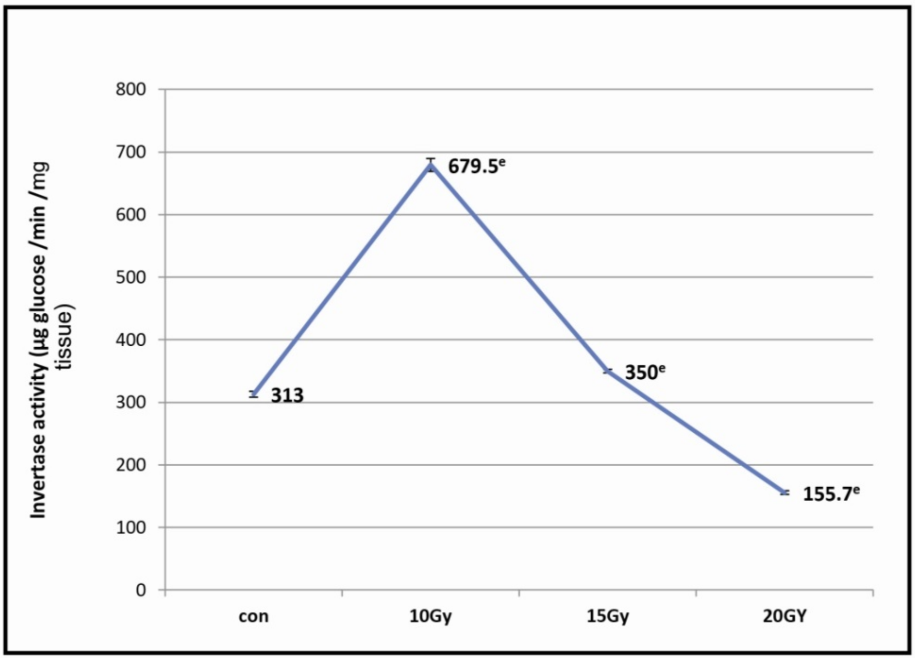

Fig. 3: Effect of gamma radiation (10, 15 & 20 Gy) on invertase activity in male *R. ferrugineus* at optimal pH.

This repair process is called molecular repair. This depends on the degree of molecular injury, the number of affected molecules, and the inherent capacity of the molecule to repair damage. The recovery process that may occur at the cellular level essentially depends on the degree of repair at the molecular level. However, the central issue in radiobiology is depression and inhibition of protein synthesis and enzyme activity, an effect which is related to the radiation dose [8]. Prasad and Sethi [21] reported the reduction in the enzyme activity in all regions of the gut in *Dacus dorsalis* when the radiation dose was increased to 15 and 20 krad. Similar findings were by El-Naggar [22] that the exposure of *Earias insulana* to gamma irradiation lowered the activity of the haemolymph digestive enzymes amylase, invertase and trehalase. On the other hand, reduction in the activity of (amylase and invertase) in the larvae of *Ephestia cautella* as a result the gamma-irradiation (80 Gy) treatment recorded by Boshra, [23]. The exposure of full-grown pupae of *Agrotis ipsilon to* (100 Gy) gamma irradiation lowered the activity of the haemolymph digestive enzymes amylase and invertase in 6th instars larvae [24].

3.3 Effect of gamma radiation on the total nutrient content in the alimentary canal of male *R. ferrugineus*

Studying the effect of gamma radiation on the total content of proteins, lipids and carbohydrates is very important to the recognition of the effect of gamma radiation on the digestion in the red palm weevil. Data summarized and illustrated in Table (1) showed the changes in the total content of male alimentary canal proteins, lipids and carbohydrates at dose levels of 10, 15 and 20 Gy.

Table 1: Effect of gamma radiation on the total nutrient content in the alimentary canal of male *R. ferrugineus*

	Mean ± S.E.					
Treatments	**Total Proteins (mg/g.b.w)**	**Reduction %**	**Total Lipids (mg/ g.b.w)**	**Reduction %**	**Total carbohydrate (mg/ g.b.w)**	**reduction %**
Control	451±5.2	**-**	34.4±1.9	**-**	44.75±0.4	-
10 Gy	412.5±7.2^e	**8.6**	28.1±1.8^e	**18.3**	50.38±0.5^e	+12.6
15 Gy	291.5±4.6^e	**35.4**	22.35±2.9^e	**35**	47.5±1^e	+6.1
20 Gy	172±4.7^e	**61.4**	7.85±1.3^e	**77.2**	25±0.3^e	44.1

^{e}Significantly different from corresponding control value (p≤0.05). Values represent the mean. of 3 replicates for each sample

The results indicated a significant decrease (p≤0.05) in the total proteins and lipids as compared with control. The percentage of reduction in total proteins recorded 8.5, 35.4 and 61.4 at dose levels; 10, 15 and 20 Gy, respectively. The percentage of reduction in total lipids was 18.3, 35 and 77.2 at dose levels; 10, 15 and 20 Gy, respectively. But there was a significant increase (p≤0.05) in the total carbohydrates at the dose levels; 10 and 15 Gy followed by remarkable reduction at dose levels; 20 Gy.

The present results are in accord with the results reached by Amin, *et al.* [25] who reported that total protein and lipid contents were significantly reduced with increasing the gamma dose in both sexes of the newly-emerged adults *Culex pipiens* L. resulted from irradiated pupae by (40 , 60 and 80 Gy). But, the carbohydrates content of both sexes significantly decreased at lower doses of radiation. However, at dose 80 Gy the males showed significant increase, while females showed a significant decrease. Gabarty [24] recorded a significant decrease in the total content of haemolymph proteins, lipids and carbohydrates of irradiated pupae (100 Gy) F1 progeny in the 6th instar larvae of *A. ipsilon*. Gabarty [26] observed a reduction in the total protein tissue content in the 6th instar larvae of *Spodoptera littolaris* due to the effect of gamma irradiation (50, 100 and 150 Gy) on male pupae. The major radiation effects on proteins are aggregate formation, changes in serological characteristics, decrease or arrest of protein synthesis, partial or total loss of function, changes of chemical structure, and disturbances in transamination process. Also, radiation is diminished synthesis of globin and haem, decreased antibody formation, changes in normal Albumin/ Globulin ratio with increase in globulin and decrease of albumin, whereas, also gamma radiation reduced absorption of fats and fat soluble vitamins. In addition, oxidation of unsaturated fatty acids forms (lipid peroxides) hydroperoxides (which exhibit radiomimetic properties) [8]. Carbohydrate serves as a source of energy and may be converted to fats for storage and to amino acids [27]. The main effects of radiation on carbohydrate appear in that decrease of glucose absorption from intestines, degradation, chain breaks, and depolymerization (loss of ability of polymer formation) of carbohydrate molecules especially polysaccharides. Reducing total carbohydrates induces stress in the insects which in turn reduces some of the vital components in the body. Under such stress conditions, the nutrients get catabolized to meet the high energy demand [28]. Ultimately, insects die due to reduced energy metabolism [29].

In conclusion, gamma radiation has significantly affected the total nutrient content, (carbohydrates, proteins and lipids) and the activity of amylase and invertase in the alimentary canal of males *R. ferrugineus*. Nevertheless, there was a great remarkable decrease at dose level; 20 Gy which may induce disturbance in the biology of the red palm weevil.

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