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Samah Sayed Ibrahim
Cotton Leafworm Department, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt

Rehab Mahmoud Sayed
Natural Products Research Department, National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt

Corresponding Author:
Rehab Mahmoud Sayed
Natural Products Research Department, National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt

Gamma-ray irradiation's effects on several biological and biochemical processes of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Samah Sayed Ibrahim and Rehab Mahmoud Sayed

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Abstract
The fall armyworm *Spodoptera frugiperda* (J.E. Smith) is invasive pests in Egypt, and is considered the most important Lepidopteran pest to global agriculture. This study targeted studying the effect of different gamma radiation doses on *S. frugiperda*, and to evaluate its potential as a control measure. Five-day-old pupae received radiation treatments at dosages of 100, 200, 300 and 400 Gy. The results showed that irradiation with 100, 200, 300 and 400 Gy had no significant effect on male adult emergency (30%, 33%, 23% and 34%), however adult females were further susceptible than males that recorded 37.74%, 28.30%, 29% and 29.33%, respectively. Afterward irradiation with the sub-sterilizing doses of 200 and 300 Gy, there was no egg hatchability. The results showed that, no significant difference among radiation doses of 100, 200 and 300 Gy in total protein and protease enzymes, while the high dose induced highly significant reduction in the activity of alkaline phosphatase and lactate dehydrogenase (LDH). Gamma radiation is hygienic, an efficient and secure physical restraint tool for controlling *S. frugiperda*, and it can reduce the pest population. It is also highly effective against enzymatic activity of target pests.

Keywords: *Spodoptera frugiperda*, enzymatic activity, gamma-ray, radiation, biology

1. Introduction
Spodoptera frugiperda (J.E. Smith); fall armyworm, a new invasive noxious pest that poses a significant threat to global food security. Fall armyworm infects and seriously harms more than 350 vital commercial crops, so it is also recognized as quarantine pest [1&2]. The financial importance of fall armyworm is regarded to many reasons, one of them is that the adult (male and female) is a powerful flyer, capable of travelling great distances each summer, and has a high rate of reproduction [3]. Besides, more than 350 commercially important crops, including maize, rice, sorghum, sugarcane, wheat, cotton, peanut, soybean, cabbage, beet, onion, alfalfa, tomato, potato, pasture grasses, and millet are damaged by the larvae [4&5]. Farmers were obliged to battle in vain by using chemical insecticides to control it [6]. They eventually used a lot of chemicals, which causes remain of such chemicals in the environment and the insects developed resistance to them [7], as well to *Bacillus thuringiensis* [8]. Due to these problems, it is critical to create sustainable environmentally friendly management technique for *S. frugiperda*. Radiation technology give alternatives that less expensive, riskier, and more dependable than chemical control. As well as, insect life spans are shortened by radiation [9]. Furthermore, Kinipling [10] argues that insect exposure to ionizing radiation sterilizes them by inducing dominant fatal mutations in their genetic makeup that can effectively suppress and eliminate numerous lepidopteran insect pests. Other researches have reported that gamma irradiation induced insect sterility and disturbed the life span of the produced progeny that were in relation to the irradiation doses and the irradiated stage [11-14]. Therefore, the current research was designed to study the influence of gamma radiation on some biological features of *S. frugiperda* as alternative clean control tool. As well as, the accompanying alteration in some enzymatic activities (Protease, Chitinase, alkaline phosphatase, and lactate dehydrogenase)

2. Materials and Methods
2.1 Insect sample
At Department of cotton leafworm, Plant Protection Institute, Agriculture Research Center,

under laboratory conditions 26±1 °C, 65±5% RH, colony of *Spodoptera frugiperda* have been reared for several sensitive generations, the newly hatched larvae were fed a castor oil leaves to six-instar, to prevent larval cannibalism, larvae were individually reared in small plastic jars. After 5 days after pupation, 5pupae/ replicate were put on plastic container for each radiation treatment and control, 5replicates were prepared for the each experiment.

2.2 Gamma radiation technique

The maintained vials of pupae of fall army worm were irradiated with different doses "T1:100, T2: 200, T3: 300 and T4: 400 Gy" at the National Center for Radiation Research and Technology – Egyptian Atomic Energy Authority - Cairo – Egypt. using cobalt-60 gamma-irradiation unit (^{60}Co irradiator), with dose rate 1.083Kg/h at the time of the study. T5 was kept unirradiated (control zero dose). Each tube containing 25 pupae were exposed to radiation doses. Five replicates were prepared for each irradiation treatments and the control.

2.3 Monitoring irradiated stages

After irradiations, the irradiated pupae of *S. frugiperda* were stored under the aforementioned controlled circumstances to report the percentages of pupal mortality, female and male adult emergence, and female and male malformation from treated pupae.

2.4 Experiment method

First laboratory experimental we used 3-5 replications of untreated normal female (NF) and treated irradiated male (IM) were paired for mating and laid eggs. In the second experimental, irradiated female (IF) and irradiated male (IM) were put in glass jar for mating of 3-5 replication, after every 24 h, the moths were fed on cotton wool soaked in a 10% sugar solution., the data were recorded of all biological parameters of life cycle of the target pest on our studies.

2.5 Biochemical analysis

2.5.1. Preparation of insects for analysis

The method of Amin [15] was used to prepare the unirradiated (control) and irradiated males' homogenates. In a chilled centrifuge, 50mg of the tested males were homogenized in 1ml of distilled water and centrifuged for 15min at 8000r.p.m. at 2 °C. The supernatants were stored at least one week to be used in the experiments. 3 replicates were prepared for each irradiated and control samples.

2.5.2. Total protein

Total protein was assessed according to Bradford [16] method. Where 50µl of the sample (adults' homogenates), then phosphate buffer (0.1 M, pH 6.6) was added to complete the volume to 1ml. Five millimeters of protein reagent "100 mg of Coomassie Brilliant blue G-250 in 50ml 95% ethanol was added to 100 ml 85% (W/V) phosphoric acid and diluted to a final volume of 1L" were poured into the test tube, and the liquid was then stirred by either inversion or vortexing. After two minutes and before one hour, the absorbance was measured at 595nm. The blank was made up of 5ml of protein reagent and 1ml of phosphate buffer.

2.5.3. Protease activity estimation

The Proteolytic activity was determined by some modifications in the method of Tatchell *et al.* [17], by measuring the elevation in free amino acids through one-hour incubation at 30 °C. Where reaction mixture contained of 100 µl adults' homogenate, 1ml of 0.1 M phosphate buffer (PH 8) and 100 µl of 0.5% bovine serum albumin, 1.2 ml 20% TCA (trichloro- acetic acid) was added to stop the reaction. Then keep the mixture for 15 min and centrifuged it at 3000 r.p.m. for 20 min. Amino acids in the supernatant were colorimetrically measured as described by Lee and Takabashi [18] by ninhydrin reagent. Where, the reaction mixture was made of supernatant (100 µl), ninhydrin-citrate at PH 5.5 (1.9 ml), 0.5 M citrate buffer at PH 5.5 (0.2 ml) and glycerol (1.2 ml). The mixture was brought to a boil for 12 minutes before being cooled with running water. At 570 nm, the developing colour was read. Zero adjustment was made to the reagent blank, which was made by substituting 100 l of distilled water for the supernatant.

2.5.4. Lactate dehydrogenase (LDH) measuring

To analyze LDH activity, 100 microliters sample was added to 2.5 ml of the reaction mixture (phosphate buffer 68 mmol/L at pH 7.5, pyruvate 0.73 mmol/L and NADH 1.1 mmol/L). Then the initial absorbance was read and repeated after 1, 2 and 3 min. Air was used for zero adjustment. The method described here is derived from the formulation recommended by the German Society for clinical chemistry [19].

2.5.5. Assessment of chitinase activity

Colloidal chitin was prepared as a substrate by solving purified chitin powder (4 gm) in water (100 ml) at 4 ºC and stirred in cold. 30 ml of concentrated H_2SO_4 was poured dropwise to the chitin suspension at 4 ºC, then filtered into ice-cold 50% ethonal (1800 ml) with rapid stirring. The precipitation (colloidal chitin) was rinsed in distilled water till being pH 5 and phosphate buffer (pH 6.5, 0.2 M) was added before use [20]. The reaction mixture due to Ishaaya and Casida [21] method, with some modifications involved 0.2 M phosphate buffer at pH 6.5 (1ml), 0.5% colloidal chitin (200 ml) and enzyme solution (sample 200 ml). After incubation for 1.5 hour at 37 ºC, the enzyme activity was stopped by boiling the tubes. Undigested chitin was precipitated by centrifugation at 8.000 r.p.m. for 15 min. The supernatant was collected in order to measure the N-acetylglucoseamine that chitinase produces as it breaks down chitin. Which was estimated according to Waterhouse *et al.* [22] method. Using phosphate acetate buffer, the volume of the appropriate aliquot from the supernatant was adjusted to 1 ml (0.2 M, pH 6). For each measurement, a 1ml buffer blank and a set of N-acetylglucoseamine standards (10, 20, 40, 60, and 80 g) in 1 ml buffer were used. Each tube was shaken and cooked in a boiling water bath for 10 minutes following the addition of 0.3 ml of saturated sodium borate solution. The tubes were quickly placed in cold water and each received 8 cc of glacial acetic acid. The addition of 1 ml of freshly made, modified Ehrlich reagent came next (1gm p-dimethylaminobenzoate dissolved in 50 ml glacial acetic acid and 2.5 ml concentrated HCl). After shaking, the tubes were left to stand at room temperature for 30 minutes. At 540 nm, the optical density was measured against the buffer blank.

2.6 Statistical analysis

ANOVA was used to examine the data, and Duncan's multiple range test was used to separate the means (P = 0.05). Also, a two-way ANOVA was applied to test the

significant among the tested samples. All statistical tests were conducted using SAS Institute Inc. SAS/STAT Software 1996.

3. Results

Data in Table1 observed that, the Gamma ray with dose100 and 300 irradiations significantly affected the percentage of pupal mortality of *S. frugiperda*, it were recorded 40% and 56%. Compared with the control. As a result, the female's pupae deformity rate was increased to 37% and 58% when the irradiation doses were 100 and 200, Gy, respectively. The present study suggested that the efficient dose for irradiating *S. frugiperda* male pupae was 200 Gy, which produce 58% malformation of female adult than male as shown in (Table 1).

The most stimulatory effect after treatment of 100 and 200 Gray, the pupal duration was 6.93 days, also data in (Table 2) displayed that there was no clear remarkable difference in pupal duration it was 7.32 days after treated with 300 and 400 Gy. Also, certain pre ovipostion biological parameters of *S. frugiperda* could be influenced by 200 Gy irradiation which increased to 4.2 days. Furthermore, after pupae treated with 200 gray the oviposition period are affected and it decreased to 1-day while the post oviposition were increased to 4.6 days, respectively in comparison with control it recorded 3days for two these period (Table 2).

The results in Table (3) and Fig. (1) showed that different scores of adults malformed of *S. frugiperda* were recorded in males and females the wings, the antenna, also the end cuticle was incomplete molting and adhesion a part of old cuticle to the new moths which effect on flight activity of target pest. Highly pupal mortality also increased with the doses increased, the dose of irritated with 300 Gy recorded, it was 56%, as shown in Tables (2 and 3).

Our experimental data investigated that, the influence gamma ray doses on the male longevity were highly significant of 200 Gy increased to 7.43 days and 9.8 days on control, while no significant deference affected on female longevity between all treatment as showed in (Table 4), also the number of eggs /female was insignificantly decreased to 113.7after pupae treated with the same dose 200 Gy , subsequently no egg hatching as the gamma dose increased than in comparison with un-irradiated treatment "control ", that explain in Table 4.

The results obtained represent in Fig. (2), there's no significant ($p \leq 0.05$) differences between the total protein after pupae irradiation with 100, 200 and 300 Gy. While at the high dose 400 Gy, reduction of the concentration of total protein to (35.93±1.14 mg/dl) was recorded in comparison with control 61.77±2.23 mg/dl.

The Proteases enzyme activity (Fig. 3) was significantly decreased reached to 16.10±1.05, 15.00±0.76, 13.43±0.22 and 30.97±0.84 (µg alanine/min/g.b.wt) after irradiation with 100, 200, 300, 400 Gy and un irradiated control, respectively.

Data in Fig. (4) explained reduction in chitinase enzyme activity of irradiated male *S. frugiperda* at the lower doses of gamma radiation (100 Gy) to 38.20±1.30 (µg NAGA/min/g.b.wt), however, the highly doses of radiation 200, 300 and 400 Gy increased the same enzyme to 43.40±0.67, 46.87±1.18 and 59.70±1.54 (µg NAGA/min/g.b.wt), respectively, compared to control 42.50±1.47 (µg NAGA/min/g.b.wt).

Otherwise, the alkaline phosphatase of irradiated males that irradiation with different doses of radiation show severely significantly decreased to 2914.33±48.26, 2457.66±109.74, 3376.66±121.70 and untreated mal of *S. frugiperda* "control", respectively (Fig.5).

In addition of our results discussed at Fig. (6), a significant reduction of lactate dehydrogenase (LDH) were estimated in all treatment of radiation doses, that recorded 3980.33±81.71 (100 Gy), 1274.00±38.30 (200 Gy), 3341.00±83.81 (300 Gy), 1903.33±43.33 (mU/g.b.wt) (400 Gy), in comparison with un irritated male (control) 4990.00±132.04 (mU/g.b.wt).

Results from laboratory experiments explain in table 4, showed the correlation of enzymes that responsible of all metabolism and development of males of *S. frugiperda*. Subsequently the males pupal was irradiated by different doses of radiations.

The results investigated that, significant difference of all enzymes and also total proteins thus impacts of gamma radiation on reproduction and fertility after pupal irradiated and 300 Gy were highly significant 75.97 (Table 5).

Table 1: Influences of gamma radiation on pupae and adults of *Spodoptera frugiperda*.

FAW biological Parameters	Gamma Radiation doses (Gy)				
	0 (Control)	100	200	300	400
No pupal Mortality	0	10	8	14	9
% Pupal Mortality	0	40	32	56	36
% Adults Emergency	100	60	68	44	64
% Normal females ♀	50	62	41	72	43
% Abnormal ♀	0	37	58	0	14
%Normal males ♂	50	85	90	27	56
%Abnormal males ♂	0	14	10	0	0
n (number of pupae = 25 / treatment T, R = 5 pupae / replicate)					

Table 2: Effect of deferent doses of gamma radiations on biological aspects of *Spodoptera frugiperda*.

Gamma radiation Doses (Gy)	Immature and mature stage of *S. frugiperda*				
	Cross	Pupal duration (days)	Pre-Ovi (days)	Ovi (days)	Post – Ovi (days)
100	Im x Nf	7.48±0.13^{b}	2.75 ±0.49^{ab}	5.2±0.49^{a}	2±0.32^{b}
200	Im x Nf	6.93±0.62^{c}	4.2±1.43^{a}	1±0.0^{c}	4.6±1.6^{a}
300	Im x Nf	7.32±0.11^{bc}	3.25±0.75^{ab}	3.3±1.43^{b}	1±0.45^{b}
400	Im x Nf	7.33±0.14^{bc}	2.6±1.15^{ab}	3.2±0.73^{b}	1.75±0.57^{b}
0 (Control)	Nm x Nf	10.65±0.16^{a}	1±0.29^{b}	3±0.0^{b}	3±0.5^{ab}
F		109.5	1.17	6.18	4.45
LSD		0.44	2.79	1.78	2.27
Means followed by different letters in each column are significantly different (P, 0.05)					

Table 3: Score of adult malformation of *Spodoptera frugiperda*

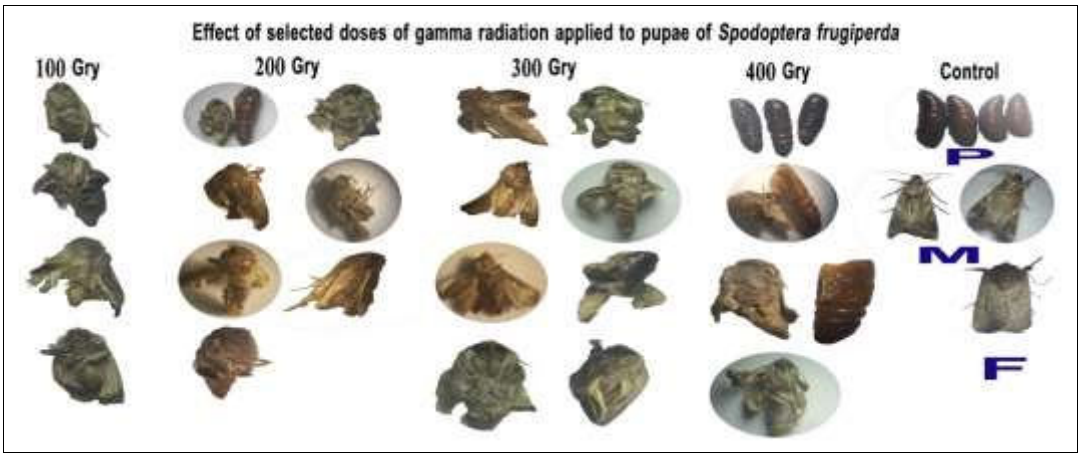
Female and Male *S. frugiperda*		Doses of gamma radiation (Gray)				
Scores	**Characteristics**	**100**	**200**	**300**	**400**	**control**
	Adults seemed to be normal	+				**-**
1	Adults with wings slightly curled	+		++		-
2	Adults wingless					-
2*	Adults wing shortness and deformed	+	++++	++	+	-
3	Adults severely curled	+	++	+	+	-
3*	Sharp curvature of the wings	+	++++			-
4	Adults attached with puprium					-
4*	Adhesion to the abdominal area					-
5	Partial emergency (head and thorax)					-
5*	The antenna is deformed			+	+	-
6	Partial emergency with head only					-
6*[a]	Head sticking to the moulting seam		+	+	+	-
6*[b]	Abdominal adhesion			+		-
7	Posteriorly partial emergency			+		-
7*	End abdominal exit only					-
8	Dead pupa	++++ ++++	++++ +++	++++ ++++ ++++ ++	++++ ++++ +	-
* New record by Dr. Samah S.Ibrahim + present						

Table 4: Effect of deferent doses of gamma radiations on biological aspects of *Spodoptera frugiperda*.

Doses of Gamma radiation (Gray)	Immature stage of *S. frugiperda*				
	Cross	% Adults Longevity		Egg/ female	Hatchability %
		Females	Males		
T1: 100	Im x Nf	12.2±3.2 [a]	9.6±2.27 [a]	712±241.48 [a]	85
T2: 200	Im x Nf	10±1.23 [a]	7.43±1.68 [ab]	113.7±53.85 [a]	0
T3: 300	Im x Nf	8.2±1.16 [a]	5.13±0.63 [b]	533.7±437.65 [a]	0
T4: 400	Nm x Nf	9.2±1.24 [a]	5.80±1.22 [b]	514±143.13 [a]	0
T5:Control	Nm x Nf	9.5±0.5 [a]	9.8±1.82 [a]	788.5±528.5 [a]	91
F		1.66	4.44	1.06	
LSD		3.78	3.05	790.6	
Means followed by different letters in each column are significantly different (P, 0.05)					

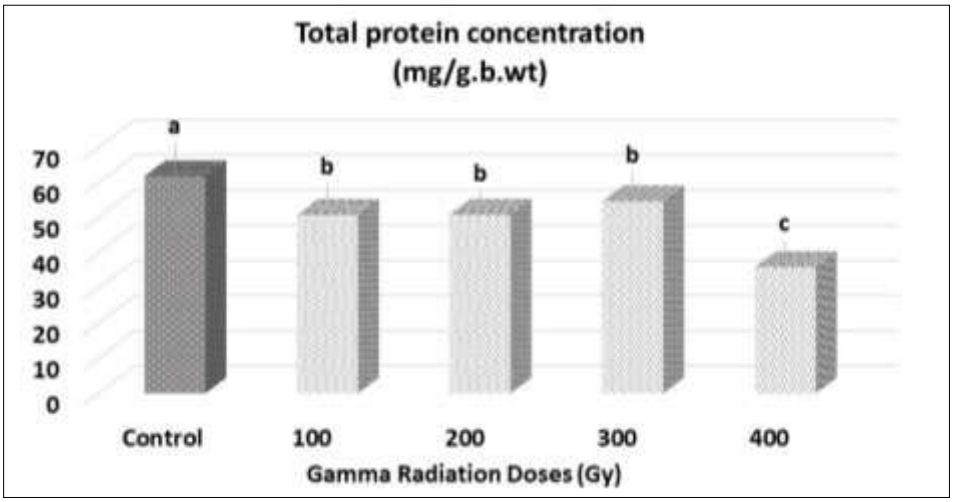
Table 5: Relationship between enzymes and different doses of gamma radiation of *Spodoptera frugiperda*

Total protein and Enzymes in males of *S. frugiperda*						
Total protein concentration (mg/dL)	**Proteases (µg alanine/min/g.b.wt)**	**Chitinase (µg NAGA / min / g.b.wt)**	**Alkaline phosphatase (mU / g.b. wt)**	**lactate dehydrogenase (LDH) (mU/g.b.wt)**	**F**	**LSD**
77.78 [b]	43.82 [d]	110.86 [a]	65.79[cb]	52.59 [cd]	27.60	14.08
Gamma Radiation Doses (Gray)						
100	200	300	400			
72.17 [ab]	61.03 [ab]	75.97 [a]	71.33 [ab]		2.07	2.006
Means followed by different letters in each column are significantly different (p<0.001)						



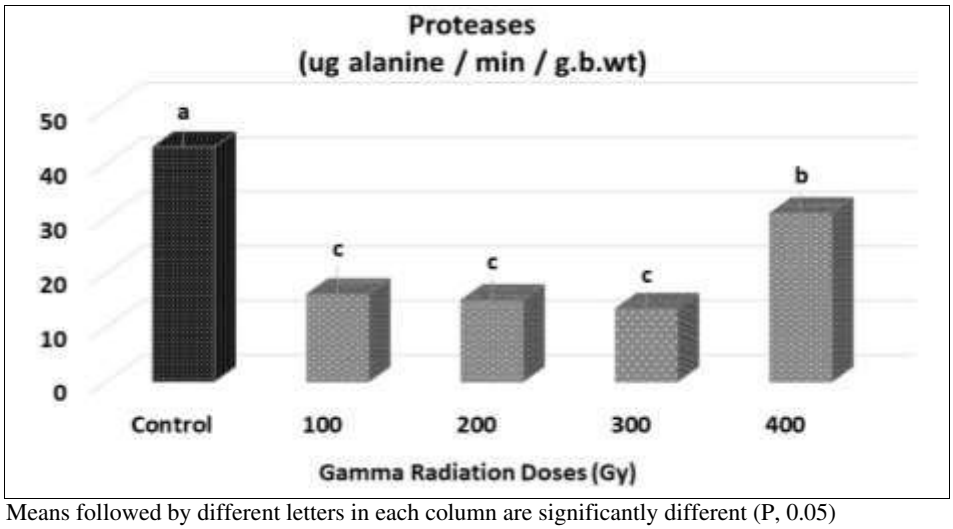
All photos by Dr. Samah Sayed Ibrahim

Fig 1: Adults malformation resulted from gamma irradiated to pupae of *S. frugiperda*.



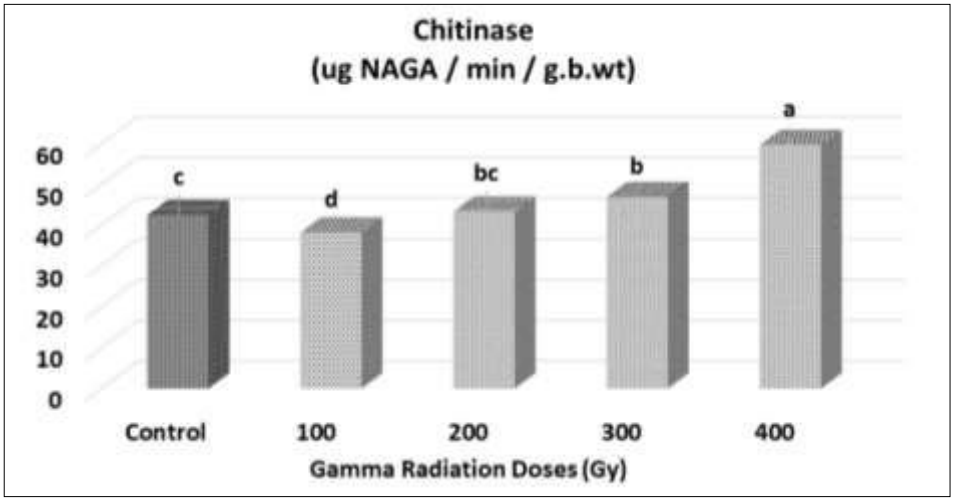
Means followed by different letters in each column are significantly different (P, 0.05)

Fig 2: Impact of gamma radiation doses on total protein of *S. frugiperda* males



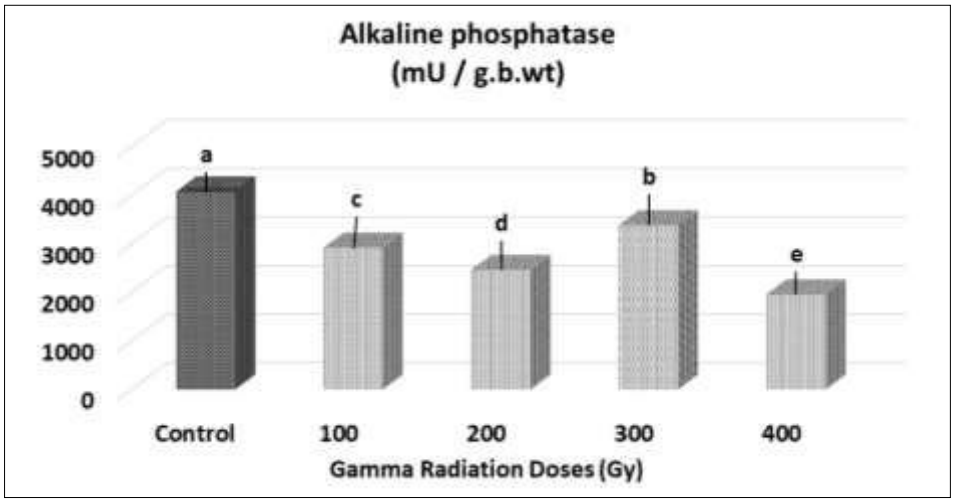
Means followed by different letters in each column are significantly different (P, 0.05)

Fig 3: Impact of gamma radiation doses on proteinase of *S. frugiperda* males



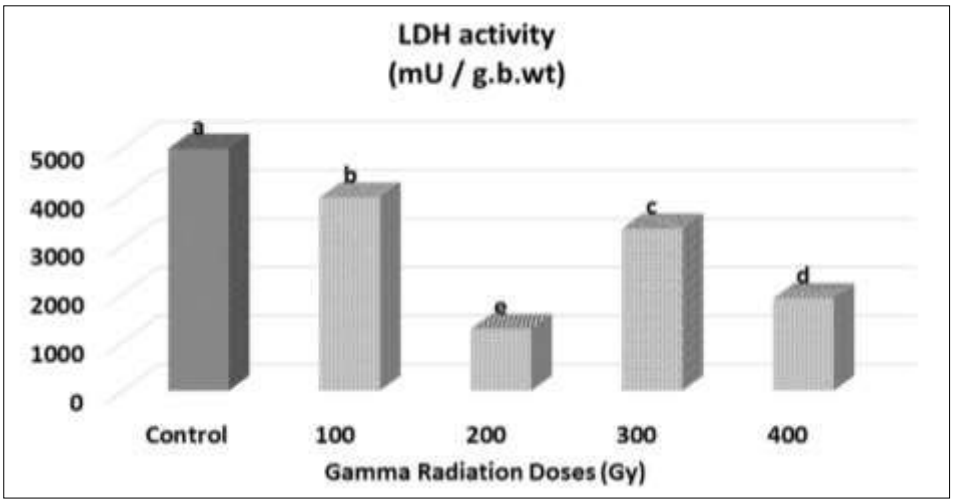
Means followed by different letters in each column are significantly different (P, 0.05)

Fig 4: Impact of gamma radiation doses on chitinase of *S. frugiperda* males



Means followed by different letters in each column are significantly different (P, 0.05)

Fig 5: Impact of gamma radiation doses on alkaline phosphatase of *S. frugiperda* males



Means followed by different letters in each column are significantly different (P, 0.05)

Fig 6: Impact of gamma radiation doses on lactate dehydrogenase of *S. frugiperda* males

4. Discussion

According to Hamadah *et al.* [23], lactate dehydrogenase (LDH) is a crucial glycolytic enzyme that is found in almost all tissues and is frequently employed in toxicology, organ damage, and clinical chemistry to diagnose tissue and cells. It participates in the metabolism of carbohydrates and has been used as a criteria for indicating exposure to chemical stress. LDH is, also, a parameter

The amount of induced activity in the haemolymph of S. gregaria nymphs in the current study suggests generally an active energy metabolism in this crucial tissue as LDH is a key enzyme in the carbohydrate metabolism and related to energy production in the live cell. Additionally, it can be a sign that the Cori cycle's section in charge of the general recycling of lactate has been stimulated effectively [24]. Rendering to a phylogenetic analysis, insect chitinases and chitinase-like proteins can be divided into numerous families, as demonstrated by Arakane and Muthukrishnan [25]. Liu *et al.* [26] mention that when the *S. frugiperda* chitinase gene's expression was blocked, it affected the breakdown of chitin in the old epidermis and the development of new epidermis, and the concentration of chitin increased, which prevented the larvae from going through a normal moulting process. Between 12 and 48 hours, the chitinase activity and the expression of the CHI gene both drastically decreased. Merzendorfer and Zimoch [27] reported that chitin-containing structural remodelling is a prerequisite for insect development and morphogenesis. In several tissues throughout their bodies, insects manufacture chitin synthases and chitinolytic enzymes for this reason.

The findings corroborated those of Arthur *et al.* [28], who noted that radiation causes alterations in insect chromosomes that limit their ability to reproduce. However, irradiated insects may also experience a wide range of other negative impacts. Finding the radiation dose that will maximise sterility while minimising the negative effects on insect quality is therefore important, as is the 400 Gy level that induces total sterility.

In addition, the processes of cell proliferation and differentiation are impacted by low doses of gamma radiation, which also causes DNA damage, apoptosis, proteolytic breakdown, autophagy, and oxidative stress. Additionally, it affects how the immune system reacts and how the body develops. It also affects how proteins, lipids, fatty acids, amino acids, and hormones are metabolised, as well as how energy is used, which can modify how the cell cycle behaves [29-31].

Finally, we can decided that gamma radiation (100, 200, 300 and 400 Gy) could cause a novel protocol for control lepidopteran pests effectively. Variant and severe effects on *S. frugiperda* with 25% compare with control 59.1 mg/g.b.wt. Irradiation up to 400 Gy gamma radiation caused a significant lowering in the total proteins content with 41% (35.9±1.14 mg/g.b.wt) and also proteases enzymes with 28% (30.9±1.14 mg/g.b.wt) compare with control and alkaline phosphatase recorded high reduction with 52% (1952 mu/g.b.wt) compare with control 4068 mu/g.b.wt, however chitinaese increased to 40% an upturn in the activity. At the higher radiation doses "200, 300 and 400 Gy", a distinct decline was observed in the activities of all the enzymes. Developmental stages of *S. frugiperda*, survival of survivability, formation The pupation, adult emergence and normality of were significantly decrease after used gamma radation, takes advantage of compatible control methods against lepidopteran pests.

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