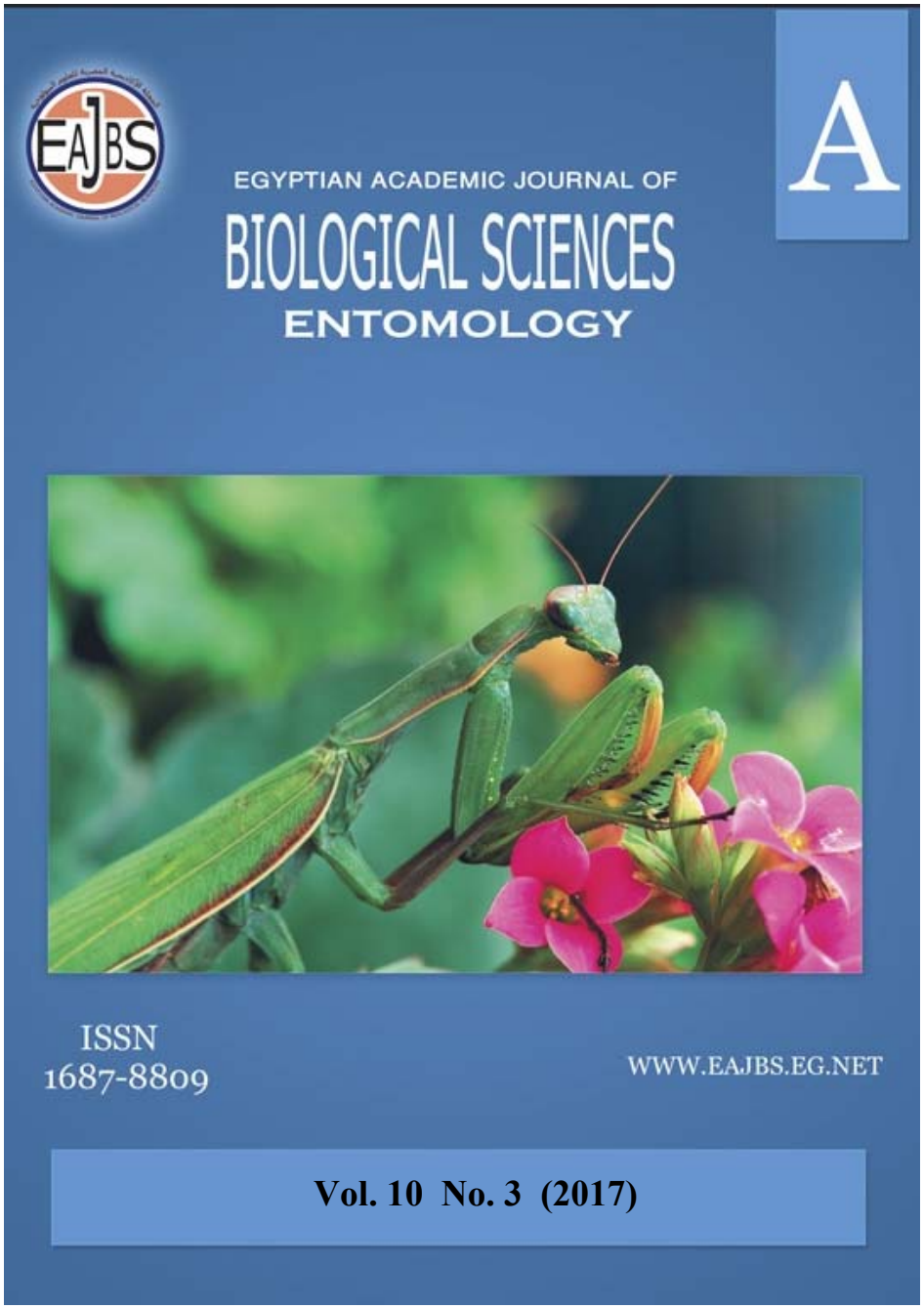


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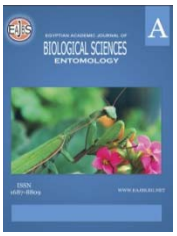

# Biological and Biochemical Alterations Associated with Sterility Induced by Gamma Radiation on Parents and First Generation Males of *Helicoverpa armigera* (Hübner)

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## ABSTRACT

The efficiency of substerile gamma radiation dose on biological, biochemical analysis of the essential nutritive components (total protein, lipids and carbohydrate concentrations), and the sexual hormones (free testosterone and follicle stimulating hormone) of male *Helicoverpa armigera* (parent generation P and first generation $F_1$) irradiated as full grown male pupae were studied. The results revealed that there was a significant decrease in emergence percentage of P and $F_1$ generations with increasing radiation dose, which noted that the sex ratio of P and $F_1$ generations were in favour of male. The fecundity and fertility of P and $F_1$ were decreased. However, the sterility percentage of P and $F_1$ generations was increased by increasing the radiation dose which was more pronounced in $F_1$ than in P. A negative significant correlation was found between both of the larval duration and pupation percentage of $F_1$ and the increase of radiation dose. The results indicated a significant decrease in the essential nutritive components (total protein, lipids, and carbohydrate concentrations) and the sexual hormones (free testosterone and follicle stimulating hormone). Furthermore, the reduction was more in $F_1$ than in P males. In general, it was obvious that the substerile gamma radiation doses enhanced an inherited sterility in *H. armigera*. Therefore, it could be concluded that inherited sterility of *H. armigera* may contribute to reduction of insecticides application or may integrate with other safe control methods in controlling *H. armigera*.

## INTRODUCTION

*Helicoverpa (Heliothis) armigera* (Hübner) is a highly polyphagous agricultural pest causing severe loss to many economically important crops over the world (Mironidis and Savopoulou-Soultani, 2008). Females lay eggs on the flowering and fruiting structures of these crops, where voracious larval feeding leads to substantial economic loss (Liu *et al*., 2004). The outbreak of this pest has been attributed to the development of insecticide resistance and the use of broad spectrum insecticides, which are known to have a detrimental effect on populations of its natural enemies and nutritional and bioclimatic factors in host plants (Naseri *et al*., 2009). Therefore, it is necessary to design an alternative program for controlling this pest.

Among several means of new pest control technology is the use of insects to control themselves by releasing of sterilized insects into wild populations which resulting in a significant reduction in them population (Knipling, 1955).

But the major problem is that lepidopterous species require high doses of irradiation to induce complete sterility that affect mating competitiveness and biology of the resulting adult (North, 1975). Therefore, the favored alternative to use fully sterile moths is the use of substerilizing doses of radiation to produce sexually competitive moths as the $F_1$ offspring of irradiated lepidopterous species are often partially or completely sterile; thus, reproduction is suppressed. This phenomenon is known as $F_1$ sterility or inherited sterility. Previous studies by Rizk and Mikhaiel (2008) on *Sitotroga cerealella*, Amin *et al*. (2010) on *Earias insulana*, and El-said (2013) on *Spodoptera littoralis*) stated that inherited sterility by radiation is an ideal tool for pest suppression.

Some hormones are associated with the sterility and can be used as a biomarker for monitoring sterilization of the irradiated male before its release in the field. Testosterone is a male sex hormone that is important for sexual and reproductive development and reported as a rapid monitor for measurements of medfly male sterility before release in the field in a sterile insect technique (SIT) program (Shoman *et al*. 2000). Also, Ibrahim (2012) stated that testosterone level was decreased by gamma irradiation of *S. littoralis* pupae. Similarly, follicle-stimulating hormone (FSH) is glycoprotein polypeptide hormones secreted by gonadotrope cells; that stimulates primary spermatocytes to undergo the first division of meiosis, to form secondary spermatocytes and the diminished secretion of FSH can result in failure of gonadal function (Boulpaep and Boron, 2005).

Moreover, any disturbance in the main nutritive components (proteins, lipid, and carbohydrates) of the biological system leads to the disorder in its performance.

Subsequently, this study was conducted to investigate the possibility of inducing inherited sterility in *H. armigera* by gamma radiation. Also, exploring some associated physiological alteration (total body content of proteins, carbohydrates, and lipids) and hormonal variation (testosterone and follicle-stimulating hormone).

## MATERIALS AND METHODS

***Rearing and Maintenance of Helicoverpa armigera:***

A field colony of the American bollworm, *H. armigera* was collected from the area of Menoufia Governorate, reared in the laboratory under constant conditions of 27±1ºC and 70±5% RH on lettuce leaves (Khidr, 1982).

***Irradiation Process:***

Full grown male pupae were irradiated with substerile doses (100 and 150Gy). Non-irradiated ones were used as control. Irradiation process was conducted by Gamma cell unit ($Co^{60}$ source), located at National Center for Radiation Research and Technology, Cairo, with a dose rate of 2.08 kGy/h.

***Biological Studies:***

The adult emergence, adult malformation, and sex ratio of male parent generation (P) were recorded. To investigate parent sterility, males P were paired with un-irradiated females to obtain $F_1$ generation. The fecundity, fertility, larval duration, % pupation, adult emergence, and adult malformation at $F_1$ generation were determined. Also, fertility and sterility of males $F_1$ were estimated by paring $F_1$ with un-irradiated females. Percent of sterility was calculated according to Guirguis (1979). Each treatment and control were replicated five times.

***Biochemical Determination:***

The newly emerged adults of male P and $F_1$ generations were homogenized in saline (1g/ml) and centrifuged in cold at 6000 rpm for 10 min. Total protein was

determined calorimetrically by the method according to Slater (1986) using kits purchased from Biodiagnostic Comp., Dokki, Giza, Egypt. Total lipids were determined calorimetrically by the method according to Van Handel (1985) using kits purchased from (Biodiagnostic Comp., Dokki, Giza, Egypt). Total carbohydrates contents of the total body tissues were determined calorimetrically according to Singh and Sinha (1977). Testosterone and follicle-stimulating hormone levels were estimated in total body homogenate according to Uçkan *et al*. (2012). Testosterone was analyzed in P and $F_1$ testes using ELISA kit (diagnostic Automation INC, USA). Follicle-stimulating hormone level (FSH) was determined using AccuDiag™ FSH ELISA kit.

***Statistical Analysis:***

A graphpad instat software© 1994 (version 2.05a) was used to carry out all statistical tests. Values are expressed as means ± S.E. mean for all data. Data were analyzed by one-way analysis of variance (ANOVA), followed by a Tukey's multiple range test.

## RESULTS

Data in Table (1) represented that there was a significant decrease in emergence percentage of irradiated full grown male pupae of *Helicoverpa armigera* with increasing radiation dose (81.7 and 63.3%). while, the sex ratio comparing with control (male:female) was increased in favor of male (53.4 and 83.33%).

Table 1: Effect of gamma radiation on emergence percentage and sex ratio of *Helicoverpa armigera* parents irradiated as full grown pupae.

| Dose (Gy) | % Emergence | Sex ratio (%) | |
|---|---|---|---|
| | | Male | Female |
| **0** | 99.6 | 48 | 52 |
| **100** | 81.7*** | 53.4 | 46.6 |
| **150** | 63.3*** | 83.33 | 16.66 |

- *** Statistically significant at p<0.001 with reference to control (Tukey's multiple range test).
- Values represent the mean of 5 replicates for each treatment.

Data presented in Figure (1) revealed that the fertility percentage of the *H. armigera* P and $F_1$ generations was significantly decreased (p<0.001) by increase of the radiation dose.

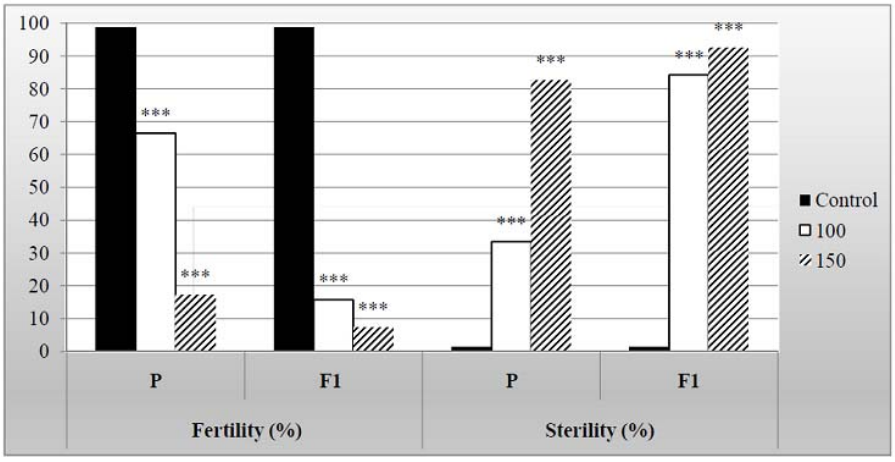


Figure 1: Effect of gamma radiation on fertility and sterility percentage of *Helicoverpa armigera* P and $F_1$ males paired with un-irradiated females.

- *** Statistically significant at p<0.001 with reference to control (Tukey's multiple range test).
- Values represent the mean of 5 replicates for each treatment.

On the other hand, sterility percentage was significantly increased ($p<0.001$) after pupae irradiation, as compared to control.

Moreover, the sterility percentage of P and $F_1$ generations was increased (33.4 and 82.6%) and (84.2 and 92.6%) for P and $F_1$ generations, respectively, compared to 1.2% in control. In addition, the decrease in fertility and sterility percentages in $F_1$ generation was more pronounced than in P generation.

Table (2) showed that the $F_1$ larval duration was significantly increased ($p<0.001$) by increasing the irradiation dose (37.7 and 38.5 days) comparing with control (28.47 days). Also, a significant negative correlation was found between the pupation percentage and the increase of radiation doses. The same trend was observed with adult emergence $F_1$ generation and the radiation doses. The sex ratio of $F_1$ adults' was in favor of males with increasing radiation dose (56.5 and 88.88), (43.5 and 11.11) for males and females at 100 and 150Gy in comparison with (49.89 and 50.1) for males and females in control.

Table 2: Effect of gamma radiation on $F_1$ progeny of *Helicoverpa armigera* from male parents irradiated as mature pupae.

| Radiation Doses (Gy) | Larvae Duration | % Pupation | Adults | | |
|---|---|---|---|---|---|
| | | | Emergence | Sex ratio | |
| | | | | Male | Female |
| **Control** | 28.47 ± 0.37 | 99.57 | 99.6 | 49.89 | 50.1 |
| **100** | 37.7 ± 0.15*** | 77.4*** | 85.5*** | 56.5 | 43.5 |
| **150** | 38.5 ± 0.74*** | 41.4*** | 51.5*** | 88.88 | 11.11 |

- Legends as table (1).

Data illustrated in Table (3) demonstrate the variation in total protein, lipids, and carbohydrate contents of total body tissue of *H. armigera* P and $F_1$ generations adult males' un-irradiated and irradiated as P generation full grown pupae. The results indicated a significant decrease ($p<0.001$) in the total protein, lipid, and carbohydrate concentration in both P and $F_1$ generations males with the increasing of radiation doses. Where that, $F_1$ males showed lower levels of total protein, lipid, and carbohydrate concentration than P males.

Table 3: Effect of gamma radiation on total protein, lipids and carbohydrate contents on total body tissue of *H. armigera* P and $F_1$ generations' male adults:

| doses (Gy) | Total protein Conc. (g/100ml) | | Total Lipid Conc. (mg/100ml) | | Total carbohydrate conc. (g/100ml) | |
|---|---|---|---|---|---|---|
| | **P** | **$F_1$** | **P** | **$F_1$** | **P** | **$F_1$** |
| **Control** | 3.34 ±0.16 | | 18.72 ±0.93 | | 0.409±0.02 | |
| **100 Gy** | 2.41±0.2*** | 0.81±0.01*** | 14.83±1.19*** | 13.57±0.87*** | 0.327±0.01*** | 0.271±0.09*** |
| **150 Gy** | 1.68±0.13*** | 0.48±0.03*** | 10.91±1.8*** | 9.64±0.3*** | 0.243±0.01*** | 0.187±0.02*** |

- Legends as in Table (1).

As shown in Figure (2), the free testosterone concentration of gamma irradiated parents males (P) and $F_1$ males was significantly decreased ($P<0.001$) (252.41 and 122.39) and (211.41 and 98.8) in comparison with control (362.2). Furthermore, the reduction was more in $F_1$ males than in P males. Similarly, P and $F_1$ males showed the same trend in FSH concentration (6.5 and 4.8) and (5.17 and 2.04) for 100 and 150Gy, respectively, when compared with control (9.65) Figure (3).

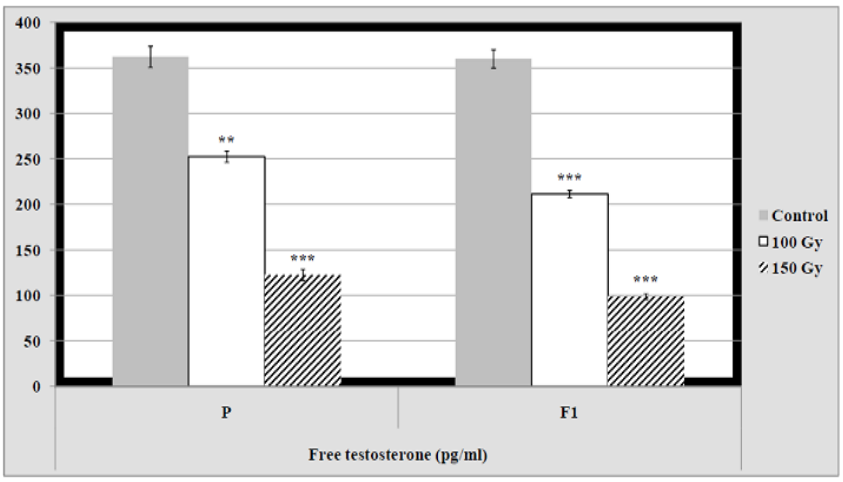


Figure 2: Effect of gamma radiation on free testosterone in *H. armigera* P and $F_1$ male.

- *** Statistically significant at p<0.001 with reference to control (Tukey's multiple range test).
- Values represent the mean ± SE of 5 replicates for each treatment.

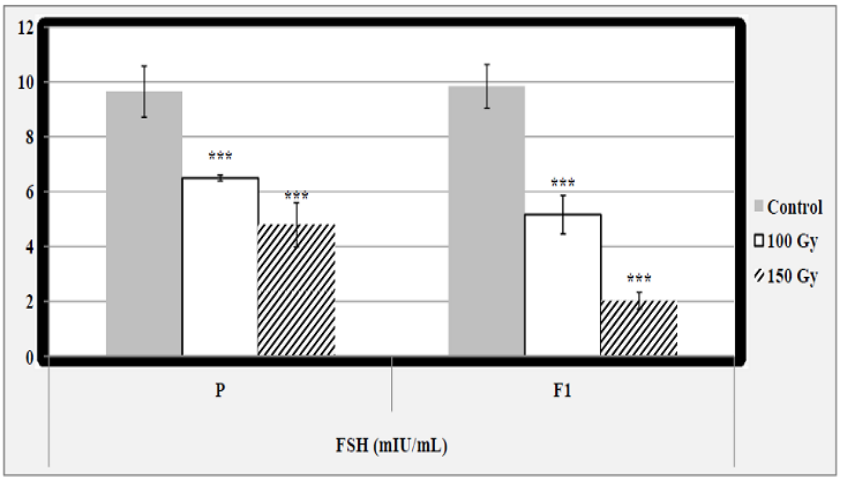


Figure 3: Effect of gamma radiation on follicle-stimulating hormone levels (FSH) in *H. armigera* P and $F_1$ male.

- *** Statistically significant at p<0.001 with reference to control (Tukey's multiple range test).
- Values represent the mean ± SE of 5 replicates for each treatment.

## DISCUSSION

*Helicoverpa armigera* (Hüb.) is one of the most important economic insect pests in Egypt (Ibrahim *et al.*, 1974). The extensive and continuous use of synthetic pesticides in control of agricultural pests has created many problems such as; resistance, residues, and elimination of natural enemies of pests. Irradiation techniques seem to offer substitutionaly phytosanitary solutions that are desirable in many aspects and more reliable than chemical control (Baxter and Blair, 1969).

As implied from the obtained results, there was a significant reduction in the adult emergence and increased the male ratio after full grown pupae irradiation with 100 and 150Gy. In contrast, gamma irradiation doses used significantly increase sterility of P and $F_1$ generations which was more remarkable in $F_1$ generation of irradiated pupae with 150Gy. This observation was in agreement with LaChance *et al.* (1973) who stated that the $F_1$ males are more sterile than their irradiated parents.

In addition, irradiated *H. armigera* pupae significantly increased the larval duration and significantly decreased larval pupation and $F_1$ generation adult emergence of their progeny. The sex ratio of the emerging $F_1$ adults was also in favor of males, and more males were obtained than in P and controls. The aforementioned incidents were similarly reported by Ibrahim (2012) on *Spodoptera littoralis* and Salem *et al*. (2014) on *Agrotis ipsilon*.

The reduction in fertility and fecundity by increasing the irradiation dose was previously explained and discussed by Mansour (2002) who stated that the percentage of sperm transfer decreased as the dose was increased. The *H. armigera* sterility may be regarded to the lack of eupyrene sperm transferred by irradiated males during mating (Henneberry and Clayton, 1981). Also it may be related to the transmission of various complexes of chromosome translocations stimulated by gamma radiation (Tothová and Marec, 2001). It is well known that irradiation shortens life span of insects possibly by accelerating senescence (Baxter and Blair, 1969). This fact explained the reduction in P progeny (larval duration and pupation).

The results of the present investigation revealed that $F_1$ sterility was more than P. This is in parallel with Knipling (1970) and LaChance *et al*. (1973), who reported that the $F_1$ progeny was either fully or partially sterile depending on radiation doses received by the male parent. The $F_1$ progeny was more sterile than the treated P male parent regardless of the dose to the male parent,

As observed from the obtained results, there was a significant reduction in total content of tissue proteins, lipids, and carbohydrates of P and $F_1$ in *H. armigera* adults' male irradiated as full grown pupae with 100 and 150Gy. This decrease was positively correlated to the radiation dose increase.

Similar reduction in total protein, carbohydrate, and lipid contents was recorded in *Agrotis ipsilon* larvae of irradiated pupae with 100Gy (Gabarty, 2008). Also, El-Kholy (2009) detected the same decrease in total proteins and carbohydrates content of *Ceratitis capitata* irradiated as grown pupae with 90Gy, but she described an increase in the total lipid content. This difference in lipid result may be due to the difference in insect species, as response to irradiation varies with the insect species, life stage and the absorbed dose received by the insect (Follett and Griffin, 2006). This result was in accordance with Tribe and Webb (1979) who declared that irradiation may cause protein denaturation, which may impair enzyme activity.

It is obvious from the results of that the exposure of full grown pupae of *H. armigera* to gamma radiatin doses significantly reduced the free testosterone and follicle-stimulating hormone levels (FSH) in P and $F_1$ adult males. This reduction was negatively correlated with given dose. This was formerly reported by Hazaa (2007) on *Spodoptera littoralis*, El-Kholy (2009) on *C. capitata*, and Ibrahim (2012) on *S. littoralis*.

The reduction in the nutritive components (protein, lipids, and carbohydrate) and the sexual hormones (free testosterone and FSH) may be attributed to the direct action of gamma radiation that breaks the chemical bonds within molecules (Arvanitoyannis and Stratakos, 2010). On the other hand, the reduction may be a result of the indirect effect of gamma ray by a significant excess of free radicals (water radiolysis) which lead to increase the oxidative stress in body fluid and tissues, physiological alterations, cell damage, chromosomal aberration, and faulty translation of genetic materials (El-Naggar, 2009). Also, the highly significant reduction in the sexual hormones might be due to the testes damage by the indirect effect of radiation as mentioned by Hazaa *et al*. (2009); where they stated that gamma radiation induced histopathological changes in the testes of *S. littoralis* larvae and adults including

necrosis of spermatocytes, retardation of sperm maturation, bursting of sperm bundles, and the vacuolated area resulting from depletion of spermatogonia that increased in size.

In general, it was obvious that substerile gamma radiation doses (100 and 150Gy) enhanced inherited sterility in *H. armigera*; which presented in reduction of P and $F_1$ adult emergence, increased P and $F_1$ sterility and decreased life span of P progeny. Also, it significantly lowered the main nutrient components (protein, lipids, and carbohydrate) and the sexual hormones (free testosterone and FSH) and this reduction confirms the biological result of P and $F_1$ sterility.

Therefore, it could be concluded that inherited sterility by gamma radiation may contribute to reduction of insecticides applications in controlling *H. armigera*.

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## ARABIC SUMMERY

### التغيرات البيولوجية والكيميائية الحيوية المرتبطة بالعقم المستحدث بأشعة جاما في الآباء والجيل الأول الذكور *لهيليكوفيربا أرميجيرا*

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قسم بحوث المنتجات الطبيعية – المركز القومي لبحوث وتكنولوجيا الإشعاع – هيئة الطاقة الذرية القاهرة – مصر.

يقصد بهذا البحث دراسة كفاءة جرعات تحت معقمة من أشعة جاما على بعض النواحي البيولوجية والمكونات الغذائية الأساسية (البروتين الكلي والدهون وتركيزات الكربوهيدرات) وبعض الهرمونات الجنسية (التستوستيرون الحر والهرمونات تحفيز الجريب) في ذكور *هيليكوفيربا أرميجيرا* (جيل الآباء P والجيل الأول $F_1$ ) المشعة كعذارى كاملة النمو. أظهرت النتائج وجود انخفاض معنوي في نسبة خروج غراشات كل من جيل الآباء والجيل الأول مع زيادة الجرعة الإشعاعية. كما وجد انخفاض في خصوبة ذكور الجيلين وبذلك تم زيادة نسبة العقم بزيادة جرعة الإشعاع، والتي كانت أكثر وضوحا في ذكور الجيل الأول ($F_1$) عن ذكور الآباء (P). لوحظ أيضا وجود علاقة عكسية معنوية بين كل من عمر اليرقات ونسبة التعذير للجيل الأول $F_1$ مع زيادة الجرعة الإشعاعية لعذارى الآباء. أشارت النتائج إلى انخفاض معنوي في المكونات الغذائية الأساسية (البروتين الكلي والدهون وتركيزات الكربوهيدرات) وكذلك الهرمونات الجنسية (هرمون التستوستيرون الحر والهرمون المحفز للجريبات). وكان الانخفاض أكثر في ذكور الجيل الأول $F_1$ من ذكور الآباء P. كان من الواضح أن الجرعات الإشعاعية عززت العقم الموروثة في H ذكور *هيليكوفيربا أرميجيرا*. لذلك، يمكن أن نستنتج أن العقم الموروثة لذكور *هيليكوفيربا أرميجيرا* قد تسهم في الحد من تطبيق مبيدات الحشرات أو قد تتكامل مع أساليب التحكم الآمنة الأخرى في مكافحتها.