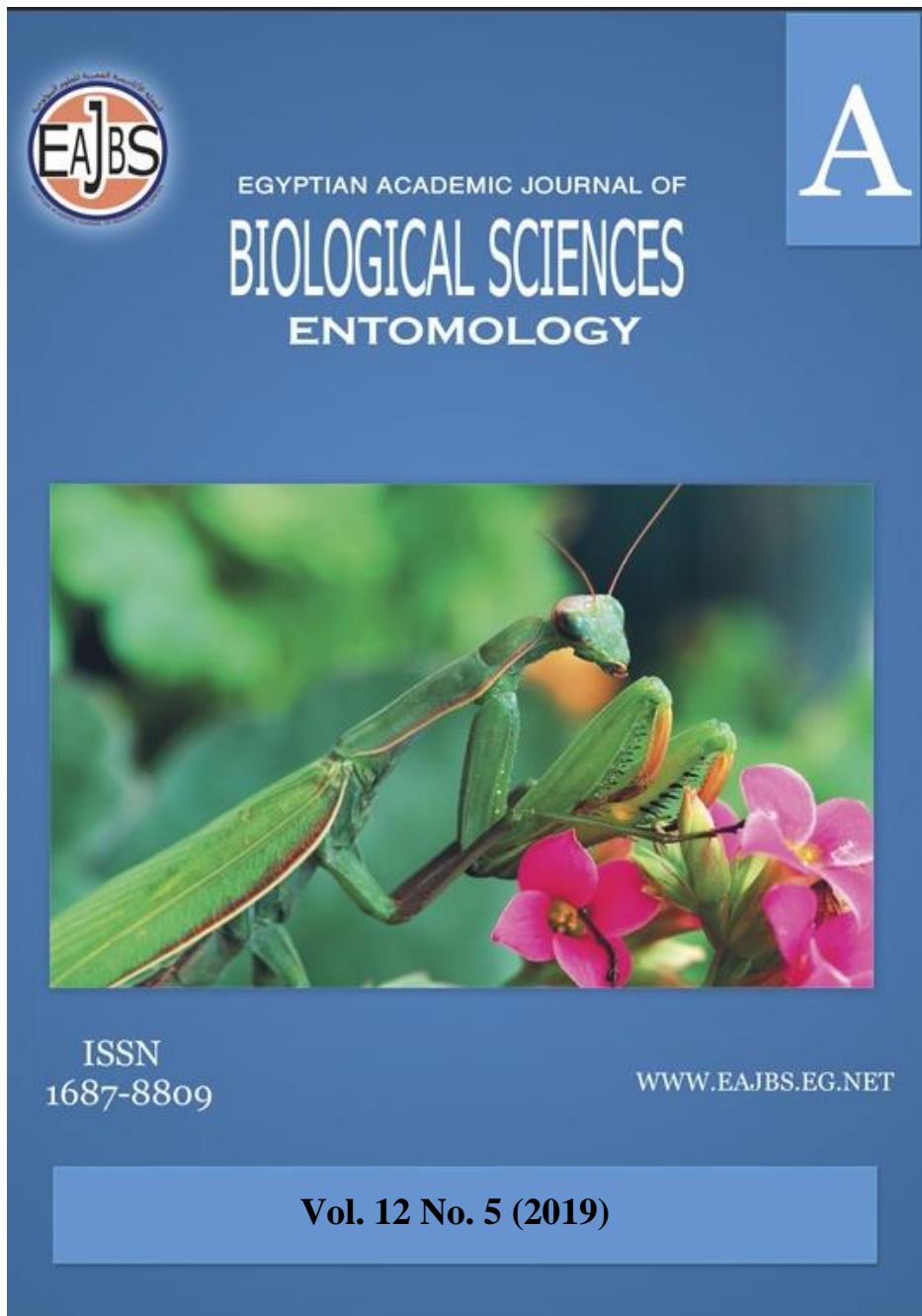
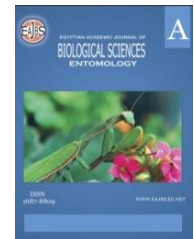


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Biochemical Comparative Study between Irradiated Adult *Tribolium confusum* and Adults Feed on Irradiated Flour

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ABSTRACT

Nowadays, radiation technique is widely used food preservation and pest control. Therefore, the present study was conducted to investigate the biochemical alterations in *Tribolium confusum* adults irradiated with the sterilizing dose (60 Gy) or fed on flour irradiated with the sterilizing dose for flour (3Kg). The results indicated a significant decrease in the nutritional contents (protein, lipids, and carbohydrates) in the studied samples when compared to control. The digestive enzymes (protease, lipase, and amylase) of *T. confusum* adults were estimated and they revealed that protease and amylase activities showed a significant decline in the gamma-irradiated adults, and a significant elevation in adults feed on irradiated flour. Moreover, lipase activity was non-significantly changed in the studied samples when compared to the control. In conclusion, it is noticeable that gamma irradiation of *T. confusum* more effective than flour irradiation but we can use food irradiation technique in combination with other control tools for controlling *T. confusum*.

INTRODUCTION

Common wheat is a staple diet for the majority of the world population. At the same time, insects, mites, rodents, and birds also feed on common wheat, its flour and other products during storage and processing.

The confused flour beetle, *Tribolium confusum* is one of the most destructive insect pests of flour, cereals, spices and other stored products. It characterized by a short life cycle under suitable conditions and its ability to fly for distribution (Simpanya *et al.*, 2001). Loss caused by insects is depending mainly on the shape and size of kernels, density, protein content, carbohydrates, and fats. The affected products become contaminated with faeces, and the increased humidity promotes molding. Moreover, the economic losses contain reduction in weight and quality of the product, accompanying of unpleasant smell that reduced its marketability. In addition, *T. confusum* is an allergen that may cause allergies in bakers (McGaughey *et al.*, 1990).

Currently, pests control depends on using fumigation with methyl bromide or hydrogen phosphide. However, methyl bromide has been categorized as an ozone exhauster, and its use has recently been banned in many countries (Hansen and Jensen, 2002). Therefore, it is important to use alternative safe methods. One such method is using of gamma radiation for flour preservation, since the Expert Committee of FAO/IAEA/WHO, has announced that

food irradiation with doses up to 10 kGy is safe and prevent food contamination (Agúndez-Arvizu *et al.*, 2006).

Moreover, Irradiation is a confirmed method for the direct control of stored wheat and flour insects in several countries (Brower and Tilton, 1983). Several studies had been done on the use of radiation to control stored-product insects (Khaghani *et al.*, 2010, Abbas and Nouraddin, 2011, Mansour, 2015) and they confirmed that gamma radiation could be used as a stored pest control method. The advantages of irradiation contain the absence of unwanted residues in the treated foods and no resistance developed in the insects (Lapidot *et al.*, 1991, Ahmed, 2001). Subsequently, the present work focuses on determining the effect of gamma irradiation on the nutritional contents (protein, lipids, and carbohydrates) and the digestive enzymes activities in irradiated *T. confusum* adult and also in other *T. confusum* fed on irradiated flour.

MATERIALS AND METHODS

Confused flour beetles were grown on flour in 2.5L jars and kept in a thermostat at the temperature of 27 ± 1 °C and relative air humidity of 40-60%, and the insects sieved 7 days later to make sure that the insects used in experiments were of a new generation.

Irradiation Technique:

Adults irradiated with 60 Gy and reared on normal flour and adults reared on irradiated flour with 3kGy for 3 months were used for this experiment

Irradiation made by using Co⁶⁰ gamma source at the National Center for Radiation Research and Technology (NCRRT, Cairo). The dose rate was 1.269KGy/h. at the time of the present investigation.

Experiment Design:

The study divided into 3 groups:

1. Twenty-five of newly emerged *T. confusum* were irradiated and placed in 25gm flour.
2. Twenty-five of newly emerged *T. confusum* fed on 25gm irradiated flour (3kGy).
3. Control group: twenty-five of newly emerged *T. confusum* fed on 25gm flour (unirradiated adults, unirradiated flour).

Three replicates of each group were prepared and placed in an incubator under fixed condition, 30 ± 2 °C, 65 ± 5 % RH.

Biochemical Studies:

The samples (irradiated adults, adults fed on irradiated flour for 3 months or the control) were homogenized in a cold glass homogenizer containing saline phosphate buffer, the contents were transferred to a new Eppendorf tube, and centrifuged at 4000 rpm for 10 minutes at room temperature, the supernatant was transferred to a new Eppendorf tube and kept frozen at -20 °C till required (Zaghloul, 2004).

The total protein was colorimetrically determined by the method according to Slater (1986), using kits purchased from Biodiagnostic Company, Dokki, Giza, Egypt. The method is dependent on the Biuret reaction. Total lipids were colorimetrically determined by the method according to Van Handel (1985), using kits purchased from Biodiagnostic Company, Dokki, Giza, Egypt. Total carbohydrates were extracted for assay according to Crompton and Birt (1967) and were estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubois *et al.* (1956).

The protease activity was measured as described by Tatchell *et al.* (1972), with some modifications, by measuring the increase in free amino acids split from substrate protein (albumin), during one-hour incubation at 30 °C according to the method described by Lee and Takabashi (1966). Lipase activity was colorimetrically determined by Spectrum diagnostic kit (www.spectrum-diagnostics.com). Amylase activity was determined according to the modifications of Amin (1998) to the method described by Ishaaya and Swirski (1976).

Statistical Analysis:

The data were statistically analyzed by Tukey Pairwise Comparisons test to examine the significant differences between the treatments using Minitab program.

RESULTS

Table (1) demonstrates that the gamma-irradiated *Tribolium confusum* adults exhibited a significant decrease in total protein and total lipid when compared to control and adults fed on irradiated flour. In addition, the total carbohydrate content of adults fed on irradiated flour revealed a non-significant change when compared to control and irradiated adult. However, there was a significant decline in the total carbohydrate in gamma-irradiated *T. confusum* adults in comparing to control.

Table (1): Effect of gamma irradiation on Total protein content, Total lipid content and Total Carbohydrate content of adult *Tribolium confusum* irradiated or fed on irradiated flour

Parameters Samples	Total protein content (g/100ml)	Total lipid content (g/100ml)	Total Carbohydrate content (mg/g b.wt)
Control	6.56±0.04 ^A	192.51±2.26 ^A	11.96±0.12 ^A
Gamma irradiated	4.97±0.08 ^C	162.79±1.15 ^B	11.41±0.07 ^B
Fed on irradiated food	5.99±0.03 ^B	196.01±1.15 ^A	11.75±0.06 ^{AB}

- Values represent the mean ± S.E of 3 replicates.
- Means with different letter are statistically significant at $p < 0.05$ (Tukey Pairwise Comparisons test).

Fig. (1) shows the protease, lipase and amylase activities of irradiated *T. confusum* adults or adults fed on irradiated flour. Protease activity of untreated control was significantly decreased to 52 ± 3 in gamma-irradiated adults; in contrast it significantly rises in adults fed on irradiated food in comparing to control. Lipase activity exhibited a non-significant decline in both irradiated adults and adults fed on irradiated flour when compared to control. Amylase activity decreased non-significantly in gamma-irradiated adults and increased significantly in adults fed on irradiated food comparing with control.

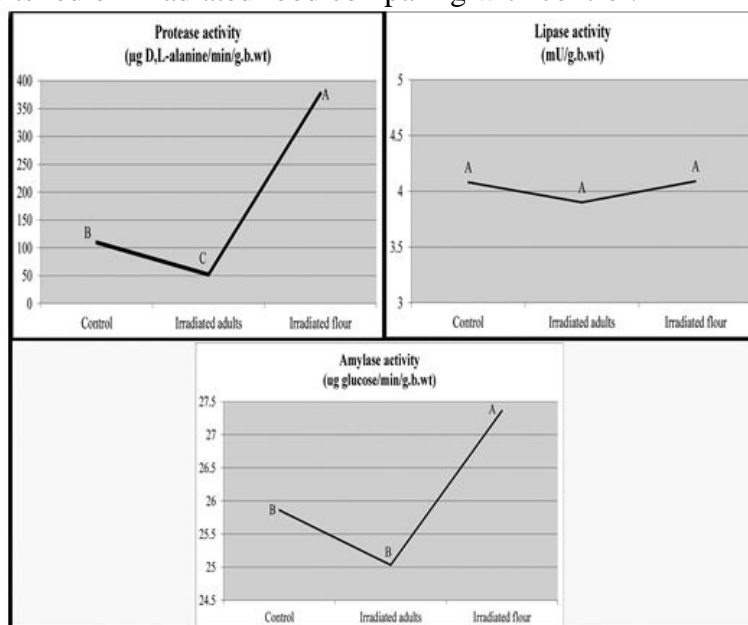


Fig. (1): Effect of gamma irradiation on protease, lipase and amylase activities of irradiated *Tribolium confusum* adults or adults fed on irradiated flour

- Values represent the mean ± S.E of 3 replicates.
- Means with different letter are statistically significant at $p < 0.05$ (Tukey Pairwise Comparisons test)

DISCUSSION

The present results implied a significant reduction in the total nutrient content (protein, lipids, and carbohydrates) and in protease and amylase activities in gamma-irradiated *Tribolium confusum* comparing to control.

Same decline in total nutrient contents were obtained by Amin *et al.* (1996) on gamma-irradiated *Culex pipiens*; Gabarty (2008) on F1 progeny in the 6th instar larvae of *A. ipsilon*; ElKholi (2009) on *Ceratitis capitata* irradiated as full-grown pupae with 90Gy, Gabarty (2013) on the 6th instar larvae of gamma-irradiated male pupae *Spodoptera littoralis*, Gabarty (2015) on the gamma-irradiated red palm weevil, Also, Sayed and Zahran (2017) on *Helicoverpa armigera* male irradiated as full-grown pupae.

The reduction in the enzymes is in consistent with Boshra (2007) on *Ephestia cautella*; Gabarty (2008) on F1 progeny in the 6th instar larvae of *A. ipsilon*. Also, Kheirallah and El-Samad (2016) suggested that the genotoxic effects of gamma radiation induced damaging effects on enzyme molecules.

The main effects of radiation on proteins are alteration in serological characteristics, decline or halt of protein synthesis, total or incomplete loss of function, variations of chemical structure, and conflicts in the transamination process. In addition, the main effects of radiation on carbohydrate presented in reduction of glucose absorption from intestines, degradation, chain breaks, and depolymerization of carbohydrate molecules especially polysaccharides. Regarding that, carbohydrate acts as a main source of energy and may be transformed to fats for storage and to amino acids (Chapman, 1998), therefore reduction of total carbohydrates declines main components in the insect body due to the stress stimulated in the body (Seyoum *et al.*, 2002)

Subsequently, this reduction is regarded to the genotoxic effect of gamma radiation that damaging the molecules (protein, lipid, carbohydrate, enzymes or DNA) by breaking the chemical bonds in them (Arvanitoyannis and Stratakos, 2010; Kheirallah and El-Samad, 2016), or by indirect action in which the molecule obtains energy from the free radicals resulted from water radiolysis in the body (El-Naggar, 2009).

As implied from the obtained results, there were a significant decrease total protein, protease and carbohydrate activities and a non-significant change in total lipids, total carbohydrate and lipase activity in adults feed on irradiated flour in comparing to control. However, this decline was significantly remarkable in gamma irradiated *T. confusum* adults. The same biochemical change observed by El-Degwi and Gareeb (2000) on *Trogoderma granarium* fed on different irradiated hosts; Machaiah and Pednkar (2002) on insects fed on gamma-irradiated legumes; Haiba and Abd-El Aziz (2008) on effects of potato irradiation on *Phthorimaea operculella*.

It is well known that the plant compounds (chemical, alkaloids or other secondary plant metabolites) can delay insect growth so considered as feeding inhibiting agents for many insects (Vigneron, 1978; Shekari *et al.*, 2008). So, the decline may regard to the change in the flour component after irradiation. Since Khattak and Klopfenstein (1989) reported changes in the amino acid profiles of gamma-irradiated wheat seed. Additionally, Haiba and Abd-El Aziz (2008) recorded a significant decrease in protein and carbohydrate contents of gamma-irradiated potato tubers.

In general, the results presented a biochemical reduction in gamma-irradiated *Tribolium confusum* and in adults fed on irradiated flour. Thus, we could recommend the introducing of flour irradiation in the integrated pest control programs to improve the quality of wheat flour.

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ARABIC SUMMARY

دراسات بيوكيميائية للمقارنة بين الحشرات الكاملة لخنفساء الدقيق المشعة بأشعة جاما والحشرات المغذاة على دقيق ممشع

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تم إجراء هذه الدراسة لتقييم تأثير الجرعة المعقمة من أشعة جاما (60 جراي) وتعقيم الدقيق المغذى عليه ب3 كيلو جراي على المحتوى الغذائي (بروتين – الدهون – الكربوهيدرات) وإنزيمات الهضم (بروتياز – ليباز – أميلاز) في الحشرات الكاملة لخنفساء الدقيق. أظهرت النتائج انخفاض معنوي في المحتوى الغذائي في العينات المدروسة بالمقارنة بالمجموعة الضابطة. أظهر كل من بروتياز و أميلاز إنخفاض معنوي في الحشرات المشعة بأشعة جاما وزيادة معنوية في الحشرات التي تغذت على دقيق مشع. علاوة على ذلك، أظهر ليباز تغيير غير معنوي في العينات المدروسة بالمقارنة بالمجموعة الضابطة. وفي الختام، لقد لوحظ أن تشعيع الحشرات الكاملة لخنفساء الدقيق أكثر فاعلية من تشعيع الدقيق لمكافحتها.