Genetic Profile of Gamma Irradiated *Locusta migratoria migratorioides*: A Futuristic Eco-friendly Control Approach

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Abstract

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Locusta migratoria migratorioides (L.) is the most common species of grasshopper in Africa, and it causes severe crop losses worldwide. The *L. migratoria* species developed resistance to insecticides because of overuse, which also polluted the environment. The purpose of the current study is to assess the effectiveness of gamma radiation to control this pest and to estimate the DNA alterations caused by radiation exposure to both male (♂) and female (♀) insects. Pest mortality rate of male and female adults were measured following exposure to radiation doses of 10, 20, 30, and 40 Gy, and Start codon targeted polymorphism (SCoT-PCR) analysis was carried out. The results obtained revealed that the mortality rate increased significantly with increasing gamma radiation dose and males were more tolerant than females with LD_{50} of 33.94 and 51.55 Gy, for males and females, respectively. According to the SCoT assay, the adults' irradiation resulted in genomic alterations as witnessed by the the disappearance of some bands and the appearance of new additional bands, with slight differences between males and females. It can be concluded that the utilization of radiation technology could be a potential approach for the control of *L. migratoria migratorioides* after further field studies.

Keywords: African migratory Locust, Gamma radiation, Mortality, DNA, SCoT-PCR.

Introduction

The African migratory locust, *Locusta migratoria migratorioides* (L.), is an invasive pest with long-distance migration. Throughout the world, locusts are known to be pests that causes severe losses to agricultural crops and threatens food security and human livelihoods (Muhammad *et al.*, 2022). This pest has primarily been controlled over time by the application of several insecticides (Ma *et al.*, 2004). The wide use of chemical insecticides led to increased environmental pollution and development of insecticides resistance in insect pests, which encouraged the search for less dangerous approaches (Guo *et al.*, 2011). The use of irradiation procedures appeared to be a promising as it works well against the majority of insects at dosages that don't reduce the quality of goods (Follet, 2004), don't develop insect resistance or leave a residue on foods (Kwon *et al.*, 2004). Radiation causes genetic changes in insects that can reduce their life span. Dushimirimana *et al.* (2010) reported that exposure to 4Gy of ionising radiation caused destruction of the epithelium layer of the desert locust; *Schistocerca gregaria* male midgut 9-days after treatment and increased mortality of all insect stages in two weeks. Additionally, applying radiation can cause genomic abnormalities that can lead to increased pest mortality (Rhee *et al.*, 2012).

Numerous molecular tests, such as the polymerase chain reaction (PCR) or the start codon targeted (SCoT) polymorphism, were employed to explore the genomic changes made by irradiation (Collard & Mackill, 2009).

The goal of this work is to evaluate the efficiency of gamma radiation in controlling *L. migratoria migratorioides* and to investigate the induced changes in DNA in both male and female irradiated insects.

Materials and Methods

Rearing and irradiation of *Locusta migratoria migratorioides*

Individuals of the migrating African locust were captured in Egypt's Abu Rawash hamlet for laboratory rearing according to the method of Hill & Taylor (1933). Five adults (males or females) in each jar were exposed to doses of 10, 20, 30, and 40 Gy using a Cobalt-60 gamma cell at the National Centre for Radiation Research and Technology in Cairo (NCRRT), which has a dose rate of 0.766KGy/h. Unirradiated male or female adults were used as control. Five replicates were used for each treatment. All experiments were approved by National Research Centre, Medical Research Ethics Committee (20-103).

Mortality assessment

Males and females were placed in separate jars, and each day fresh food was provided. The number of dead males and females was counted 5 days after irradiation, and the mortality rate was computed. The lethal doses LD_{50} and LD_{95} values were calculated using the LdPLine® software.

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DNA isolation and start codon targeted polymorphism (SCoT-PCR) analysis

Fresh (unirradiated) and irradiated adults were used to obtaining genomic DNA using the DNeasy insect micro kit (bio basic), which was employed as a template for PCR amplification with seven SCoT primers, which were all taken from Dataset I's highly expressed genes as described by Sawant *et al.* (1999). The sequences of the utilized primers are listed in Table 1. The PCR procedure was carried out according to the method of Xiong *et al.* (2011). The Molecular Distances MD (Dissimilarity) for the binary data matrix was calculated using the Dice coefficient (Nei & Li, 1979), and the Agglomerative Hierarchical Clustering (AHC) analysis, which was derived from the Unweighted Pair-Group Average (UPGMA) method, was carried out using the XLSTAT.7 program. Since the SCoT 1, 4, 6, and 15 primers were the only ones for which similarity indices were estimated, those bands were produced by separate primers.

Statistical analysis

Minitab program was used to analyze the mortality percentages results using ANOVA, which were significantly compared by Tukey Pairwise Comparisons test ($P \leq 0.05$).

Table 1. Sequence of primers used for SCoT-PCR analysis.

Primer name	Sequences (5` to 3`)
SCoT 1	ACG ACA TGG CGA CCA CGC
SCoT 4	ACC ATG GCT ACC ACC GCA
SCoT 6	CAA TGG CTA CCA CTA CAG
SCoT 8	ACA ATG GCT ACC ACT GAG
SCoT 13	ACC ATG GCT ACC ACG GCA
SCoT 14	ACC ATG GCT ACC AGC GCG
SCoT 15	CCA TGG CTA CCA CCG GCT

Results and Discussion

The accumulative mortality of *Locusta migratoria migratorioides* adults after 5 days of irradiation with different doses (10, 20, 30, and 40 Gy) are shown in Figure 1. Gamma irradiation led to a progressive significant increase in mortality along with the increase of the radiation dose for both males and females. Furthermore, the obtained results exposed that males *L. migratoria* were more sensitive (higher mortality) than females in all irradiated doses.

The estimated LD_{50} values for males and females were 33.94 and 51.55 Gy, respectively. Likewise, the estimated LD_{95} values against males and females were 161.13 and 207.95 Gy, respectively. The tabulated resistance ratio exposed that adult's female were more resistant to gamma radiation than adult males (Figure 2, Table 2).

The results of the current study on the effects of gamma radiation on adult male and female *L. migratoria migratorioides* showed that irradiation led to a progressive, significant increase in mortality rate in response to the increase of radiation dose. This is in agreement with previous studies which revealed that insect mortality was dependent on the dose of gamma radiation (Sayed & Zahran, 2017; Tabikha, 2022). Kinipling (1955), reported earlier that exposure to ionizing radiation causes somatic damage that interferes with the biological functions of the insects, induces genetic dominant fatal mutation with eventual insect sterilization and reduces the lifespan of insects. Furthermore, the results obtained showed that adult males were more sensitive to gamma radiation than adult females. This outcome was in agreement with Datkhile *et al.* (2009) who specified that female *Chironomus ramosus* was more tolerant to gamma irradiation than males.

The DNA fragment amplified by 7 SCoT primers and separated using agarose gel electrophoresis (Figure 3) ranged in size from 175 bp in SCoT 1 to 1185 bp in SCoT 12. The total number of amplified bands varied from 1 (for SCoT 4 + SCoT 6) to 7 (for SCoT 15) (Table 3).

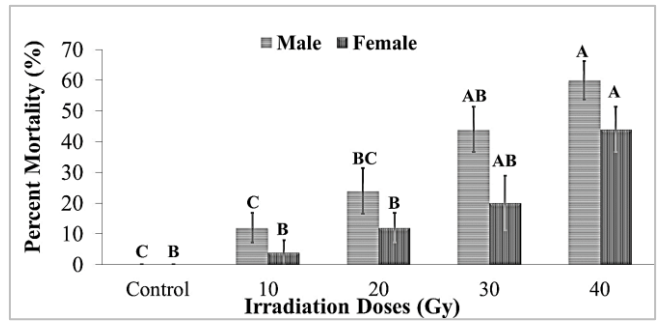

Figure 1. Accumulative mortality of *Locusta migratoria migratorioides* adults 5 days after applying different doses of gamma irradiation. Values represent the mean±SE of 5 replicates each of 5 adults. Means (for males or females) marked with the same letters are not significantly different at P=0.05 (Tukey Pairwise Comparisons).

Table 2. LD_{50} and LD_{95} levels, resistance ratio, slope values, and toxicity index of gamma radiation against *Locusta migratoria* adults.

	Insect sex	
	Male	**Female**
LD_{50}	33.94 (29.65–40.86)	51.5 (42.74–71.27)
LD_{95}	161.13 (106.5–327.39)	207.95 (126.75–530)
Slope	2.432±0.33	2.715±0.43
Resistance ratio	1	1.518
Toxicity Index	100	65.855

Toxicity index and resistance ratio were calculated in comparison with accumulative mortality of adults' males based on LD_{50}.

Different levels of polymorphism were evident when different primers were used (Table 3 and Figure 3). When SCoT 1 primer was used, 5 bands were produced, 4 monomorphic and 1 polymorphic. With SCoT4 primer, 5 bands were observed, 4 polymorphic and 1 monomorphic. When SCoT 6 primer was used, a 425bp band was amplified in the male group irradiated with 30 Gy, and the female groups (control and irradiated with 30Gy). Using the SCoT15 primer amplified seven clear bands, 5 monomorphic (215, 440, 500, 600, and 1185 bp) and 2 polymorphic (335

and 1140 bp). In addition, the generated patterns from SCoT 1, SCoT 4, and SCoT 6 primers identified a difference in the genetic structure between normal (unirradiated) males and females. When insects were irradiated with 40 Gy, the total no. of amplified bands in males were 5, 3, and 3, whereas in females were 5, 5, and 2 bands for SCoT 1, SCoT 4, and SCoT 6 primers, respectively. Using SCoT 4, SCoT 6, and SCoT 15 primers showed an alteration in the DNA of males and females of *L. migratoria* after gamma irradiation, with a difference in the no. of the produced fragments.

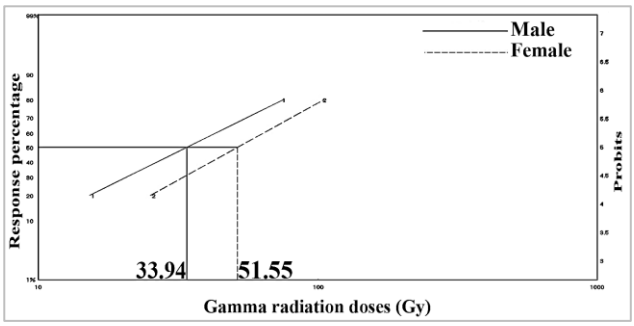

Figure 2. LD_{50} values of *Locusta migratoria* adults 5 days after irradiation with gamma rays.

Based on the similarity index (SI) (Table 4), a dendrogram was developed (Figure 4). The highest IS values (0.92308) was estimated between 30 Gy irradiated male or female and other treatments when SCoT 15 primer was used. Whereas, the lowest value (0.3333) was obtained with SCoT 4 primer when comparing control male with 10 Gy irradiated male, control female, 10 Gy irradiated female or 20 Gy irradiated female.

According to our knowledge, this is the first instance in which SCoT markers are used to describe the genetic alteration caused by gamma radiation in insect pests. Herein, the present results revealed that gamma radiation caused changes in the DNA structure of males and females, which were apparent by different estimated similarities among the tested doses and the tested sexes. Gamma-radiation caused some bands to appear and others to disappear, resulting in variances in SCoT-PCR patterns across the various samples. This was in line with what has been reported by Zahran *et al.* (2017), and Ali *et al.* (2017). The formation of new bands may be caused by variations in oligonucleotide primer sites as a result of DNA changes and other mutations (Dhakshanamoorthy *et al.*, 2011).

The primary cause of the mutation load in living things is oxidative DNA damage. Indirect action, which requires energy transfer from another molecule, and direct action, which ionizes the target molecule directly, are the two ways that ionizing radiation can alter biochemistry and DNA. These mechanisms are well-defined in biological systems where water is a key component. Proteins, carbohydrates, lipids, and enzyme molecules make up the biological system of an insect's essential nutrients. Any alteration to this component consequently has an impact on the biological system and adult functioning (Ravi *et al.*, 2017).

The recorded difference in female and male mortality by gamma radiation was clarified by the difference in generated fragments by SCoT-PCR. That variance might be due to the basic difference between the germ cells of males and those of females besides the genetic variation between them, therefore, they vary in their response toward gamma radiation (Limohpasmanee *et al.*, 2017).

Table 3. Polymorphism rate in irradiated *Locusta migratoria* adults reflected in the generated bands by SCoT primers.

Primer name	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism rate (%)
SCoT 1	5	4	1	20
SCoT 4	5	1	4	80
SCoT 6	3	1	2	66.66
SCoT 8	2	2	-	-
SCoT 13	3	3	-	-
SCoT 14	4	4	-	-
SCoT 15	7	5	2	28.57
Total	29	20	9	31.03

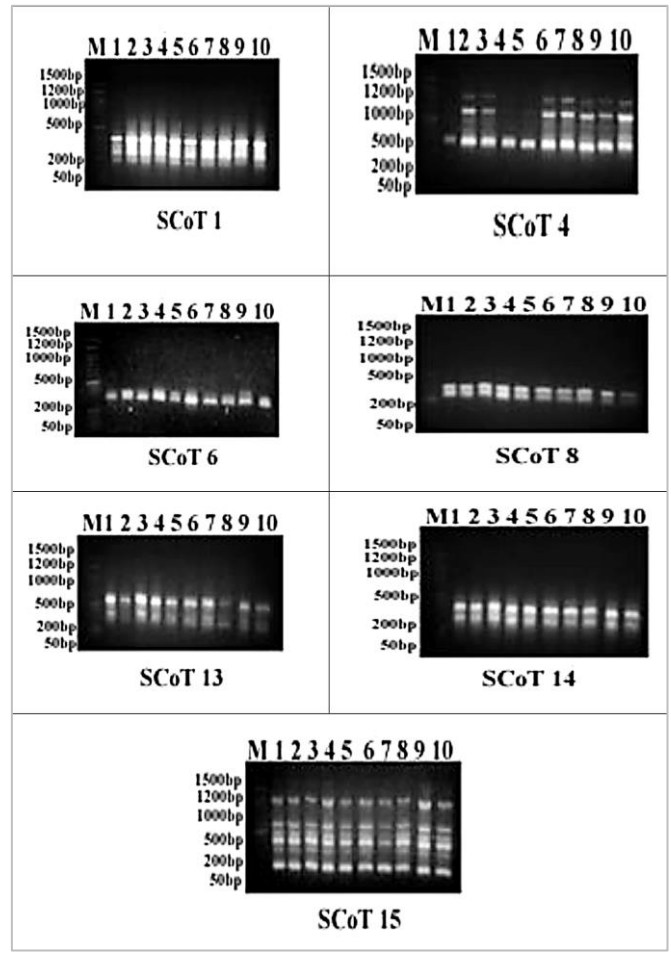

Figure 3. Agarose-gel electrophoresis of SCoT product generated with the primers in unirradiated and irradiated *Locusta migratoria* adults. (1) Control male, (2) Male irradiated with 10Gy, (3) Male irradiated with 20Gy, (4) Male irradiated with 30Gy, (5) Male irradiated with 40Gy, (6) Control female, (7) Female irradiated with 10Gy, (8) Female irradiated with 20Gy, (9) Female irradiated with 30Gy, (10) Female irradiated with 40Gy.

Table 4. Similarity index using SCoT data from control and irradiated *Locusta migratoria* adults.

Group	1	2	3	4	5	6	7	8	9	10
SCoT 1										
1	1									
2	1	1								
3	1	1	1							
4	1	1	1	1						
5	1	1	1	1	1					
6	0.88889	0.88889	0.88889	0.88889	0.88889	1				
7	1	1	1	1	1	0.88889	1			
8	1	1	1	1	1	0.88889	1	1		
9	1	1	1	1	1	0.88889	1	1	1	
10	1	1	1	1	1	0.88889	1	1	1	1
SCoT 4										
1	1									
2	0.33333	1								
3	0.4	0.88889	1							
4	0.5	0.75	0.85714	1						
5	0.5	0.75	0.85714	1	1					
6	0.33333	1	0.88889	0.8	1	1				
7	0.33333	1	0.88889	1	0.5	1	1			
8	0.33333	1	0.88889	0.5	0.8	0.66667	1	1		
9	0.4	0.88889	1	0.8	0.8	0.66667	0.5	1	1	
10	0.33333	1	0.88889	0.8	0.8	0.66667	1	0.5	1	1
SCoT 6										
1	1									
2	0.66667	1								
3	1	0.66667	1							
4	0.5	0.8	0.5	1						
5	0.66667	1	0.66667	0.8	1					
6	0.5	0.8	0.5	1	0.8	1				
7	1	0.66667	1	0.5	0.66667	0.5	1			
8	0.66667	1	0.66667	0.8	1	0.8	0.66667	1		
9	0.66667	0.5	0.66667	0.8	0.5	0.8	0.66667	0.5	1	
10	0.66667	1	0.66667	0.8	1	0.8	0.66667	1	0.5	1
SCoT 15										
1	1									
2	1	1								
3	1	1	1							
4	0.92308	0.92308	0.92308	1						
5	1	1	1	0.92308	1					
6	1	1	1	0.92308	1	1				
7	0.90909	0.90909	0.90909	0.83333	0.90909	0.90909	1			
8	1	1	1	0.92308	1	1	0.90909	1		
9	0.92308	0.92308	0.92308	1	0.92308	0.92308	0.83333	0.92308	1	
10	1	1	1	0.92308	1	1	0.90909	1	0.92308	1

1: Control male, 2: Male irradiated with 10Gy, 3: Male irradiated with 20Gy, 4: Male irradiated with 30Gy, 5: Male irradiated with 40Gy, 6: Control female, 7: Female irradiated with 10Gy, 8: Female irradiated with 20Gy, 9: Female irradiated with 30Gy, 10: Female irradiated with 40Gy

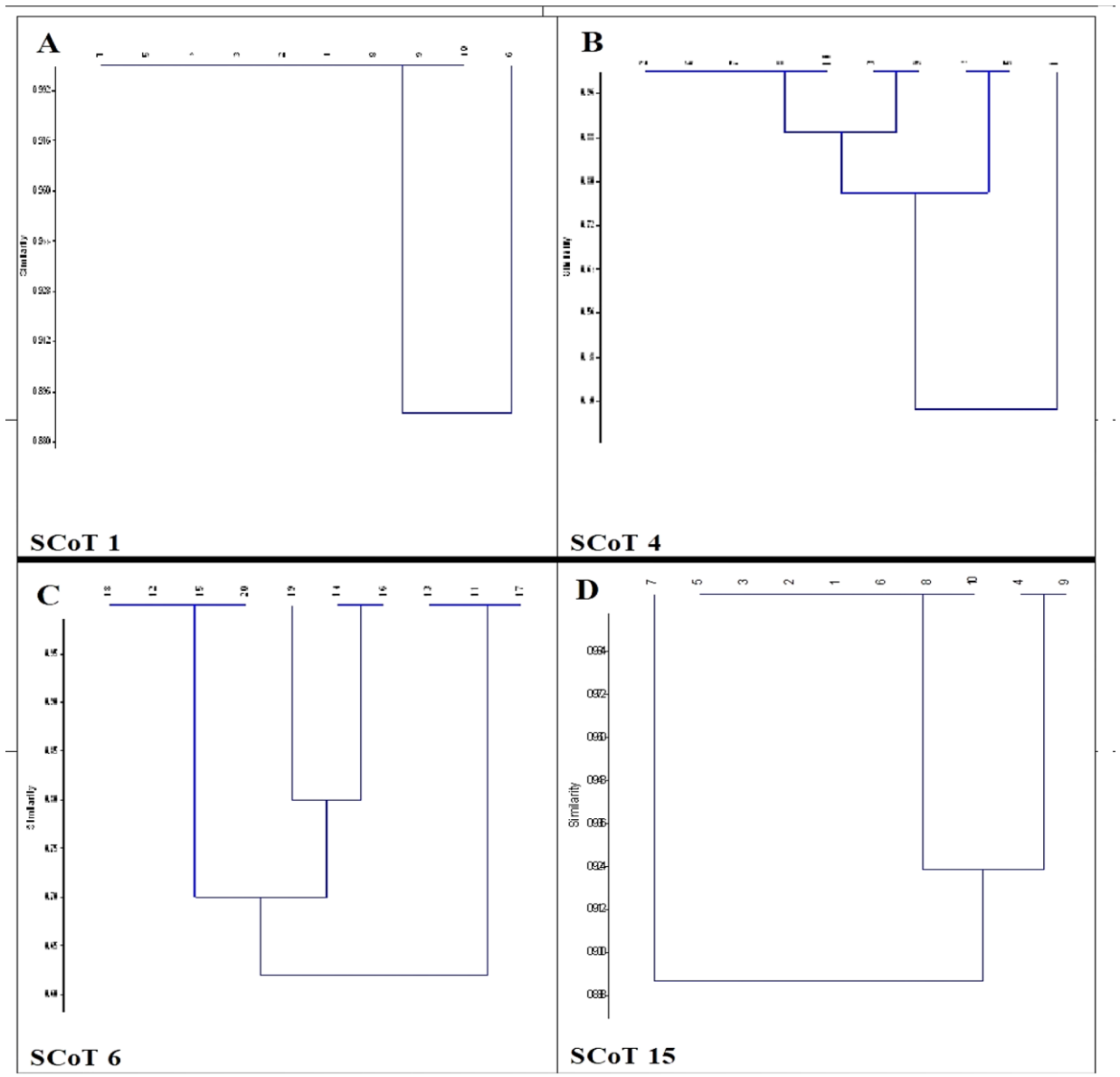

Figure 4. Dendrogram analysis using SCoT data for cDNA from unirradiated and irradiated *Locusta migratoria* adults. (1) Control male, (2) Male irradiated with 10Gy, (3) Male irradiated with 20Gy, (4) Male irradiated with 30Gy, (5) Male irradiated with 40Gy, (6) Control female, (7) Female irradiated with 10Gy, (8) Female irradiated with 20Gy, (9) Female irradiated with 30Gy, (10) Female irradiated with 40Gy.

الملخص

علي، هناء م.، زينب فتحي، س.س. إبراهيم و ر.م. سيد. 2024. صورة وراثية عن الجراد المهاجر المشعع بأشعة جاما: نهج مستقبلي للمكافحة وصديق للبيئة. مجلة وقاية النبات العربية، 42(1): 75−81. https://doi.org/10.22268/AJPP-001212

يُعدّ *Locusta migratoria migratorioides* L. أكثر أنواع الجراد المهاجر شيوعاً في إفريقيا، ويُعتقد أنه يشكل خطراً شديداً على الإنتاج الزراعي في جميع أنحاء العالم. وبسبب الإفراط في استخدام المبيدات الحشرية، طوّرت هذه الآفة مقاومةً للمبيدات المستخدمة، كما أنها عامل مهم في تلوث البيئة. هدفت الدراسة الحالية إلى تقييم فعالية أشعة جاما في القضاء على هذه الحشرة وتقييم التغير في الحمض النووي نتيجه التعرض للإشعاع في كل من جنسي الحشرة (الذكور والإناث). تم تعريض الذكور والإناث (الحشرات البالغة) لجرعات إشعاعية مختلفة 10، 20، 30 و 40 جراي، ثم حسبت نسبة الموت لكل من الذكور والإناث، كما تم إجراء تحليل تعدد أشكال البنية الوراثية المستهدفة (SCoT-PCR). أظهرت النتائج أن معدل الموت زاد بشكل ملحوظ بزيادة الجرعات الإشعاعية، وتبيّن أن الذكور أكثر حساسية من الإناث، حيث كانت الجرعة المميتة النصفية (LD_{50}) للذكور والإناث 33.94 و 51.55 جراي، على التوالي. وفقاً لتقييم SCoT، أدى تشعيع بالغات الحشرة إلى اختفاء

بعض قطع الحمض النووي وظهور شرائط إضافية جديدة، كما وجدت إختلافات طفيفة بين الذكور والإناث. وعليه، يمكن الاستنتاج حسب هذه الدراسة أنه يمكن اقتراح مكافحة الجراد المهاجر من خلال إستخدام تكنولوجيا الإشعاع، ولكن بعد مزيد من الدراسات الميدانية.

كلمات مفتاحية: الجراد الفريقي المهاجر، تشعيع بأشعة جاما، الوفيات، DNA، SCoT-PCR.

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