Genetic profile of gamma irradiated Locusta migratoria migratorioides: A futuristic eco-friendly control approach

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Abstract

The most common species of grasshopper in Africa is called *Locusta migratoria migrotaria (L.)*, and it is thought to pose a severe danger to agriculture worldwide. The *Locusta migratoria* species developed resistance to insecticides because of overuse, which also polluted the environment. As a result, opportunities to investigate new control strategies appeared. The purpose of the current study is to assess the effectiveness of gamma radiation in eradicating Locusta species and to look into the DNA alterations caused by radiation exposure in both male and female insects.

Males and female adults (around one-month-old) received radiation treatments of 10, 20, 30, and 40 Gy. DNA isolation and Start codon targeted polymorphism (SCoT-PCR) analysis were done, along with mortality percentage calculations. The death rate increased significantly over time as a result of gamma irradiations resulting in an estimated LD_{50} value for males and females of 33.94 and 51.55Gy, respectively. According to the SCoT research, the adults' radiation exposure resulted in the disappearance of some bands and the appearance of new additional bands. The similarity index was used to create a dendrogram. It was determined that irradiating the pest may be used as a technique to kill the insect and stop its spread. In addition, it resulted in certain genetic alterations within this species. Conclusions: The control of *L.* may be suggested by utilizing radiation technology, after further field studies.

Introduction

The African migratory locust, *Locusta migratoria migratoria* (L.), is an invasive pest with long-distance movement that is distinguished by sudden and erratic population outbreaks (Zhang and Yan 2000, Seino et al. 2010, Otuka 2012). Throughout the world, locusts are a severe agricultural pest that destroys crops and threatens food security and human livelihoods. As a result, it is seen as a serious threat to crops and global food security, so keeping control of it is essential (Gao et al. 2021; Muhamma et al. 2022).

This agricultural pest has primarily been controlled over time by the administration of several insecticides (Ma et al. 2004; Guo et al. 2011). In addition to leading to the emergence of resistance in L. populations, overuse of insecticides has also resulted in environmental pollution (Yang et al. 2009), which has sparked serious public concern and compelled experts to look for less dangerous substitutes for synthetic insecticides. Due to its high specificity, effectiveness, and systemic features, RNA interference (RNAi) has become a cutting-edge method for controlling insect pests (Joga et al. 2016).

Opportunities to implement control measures and strategies are easily overlooked, which can result in significant crop losses. Thus, identifying source sites and migratory paths is a crucial prerequisite for early monitoring, forecasting, and control of this pest.

Natural habitats are regularly subjected to man-made pollutants that present problems for the people living there. Numerous studies have shown that organisms that are exposed to an environmental stressor over an extended period, such as ionizing radiation, frequently suffer a direct cost (Zainullin et al. 1992; Mller and Mousseau 2006; Einor et al. 2016). Additional effects for the offspring of exposed parents have

been documented in laboratory and field research, including increased mutation rates (Carls and Schiestl 1999; Barber and Dubrova 2006; Natarajan 2006), elevated birth anomalies as well as epigenetic changes (Nomura 2006). Parents who are exposed to pollutants may be compelled to offer fewer resources of lower quality to their developing children (Mousseau and Fox 1998a; Mousseau et al. 2009). According to epigenetic studies, stressors experienced by parents can have an impact on their children's health and phenotypic expression (Jirtle and Skinner 2007; Skinner et al. 2010; Perera and Herbstman 2011).

In determining the evolutionary relationships between and among various species and cultivars, molecular markers are crucial. Studies of genetic diversity, comparative biology, physical traits, environmental factors, conservation, and phylogenic phenomena between plant species and cultivars frequently used DNA-based markers (Haq et al. 2014). Numerous molecular markers have been employed for phylogenetic study and cultivar identification (Bornet and Branchard 2004; Semagn et al. 2006).

It was thought that start codon targeted (SCoT) polymorphism was a new molecular marker. This marker was introduced by Collard and Mackill and focuses on the short start codon ATG found in plant genes (2009). It was distinguished from other DNA marker approaches such as random amplified polymorphic DNAs (RAPD) and ISSR by higher polymorphism and better marker resolvability, garnering its appeal for its superiority (Gorji et al. 2011). Compared to other molecular markers, SCoT markers are advantageous since they are simple to use, inexpensive, quick, and contain non-radioactive ingredients (Mulpuri et al. 2013). In comparison to RAPDs, ISSRs, and SSRs, SCoTs are employed more directly in the creation of marker-assisted breeding programs (Mulpuri et al. 2013). The conserved start codon in plant genes or the short region flanking the ATG translation beginning is of interest to the SCoT marker (Collard and Mackill 2009).

Tritordeum bergrothii L. Poaceae's DNA fingerprinting has been accomplished using SCoT markers (Cabo et al. 2014). Numerous investigations showed that start codon-focused markers have superior abilities for identifying polymorphisms and determining genetic variants across species than other random primers (Zeng et al. 2014; Tiwari et al. 2016). Many crop plant species, including rice (Collard and Mackill 2009), cowpea (Igwe et al. 2017), and Plantago, have been studied using SCoT markers (Rahimi et al. 2018).

The goal of this work is to evaluate the efficiency of gamma radiation in controlling *L.* and to investigate the induced changes in DNA in both male and female irradiated insects. This will be achieved by calculating the mortality percentage and analysing isolated DNA and Start codon targeted polymorphism (SCoT-PCR).

Materials And Methods

Locusta migratoria migratorioides rearing

Individuals of the migrating African locust were captured in Egypt's Abu Rawash hamlet for laboratory rearing. Insects are kept in hardwood breeding cages that measure 44 by 18 by 15 cm and have fine wires

for ventilation. They spend roughly 8 hours in the sunshine. Cadges were kept at a controlled temperature and relative humidity of 60 10% RH at 30 1°C. The cages were partially clothed to protect the locusts from the scorching heat. The cages were cleaned daily, and all locust stages were fed maize or alfalfa leaves (Hill and Taylor 1933). Cowpea (Igwe et al. 2017), Plantago (Collard and Mackill 2009), and (Rahimi et al. 2018).

Irradiation Process:

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. For each radiation dose and the control, 5 replicates were carried out (unirradiated male or female).

Mortality Percentages Calculation:

Males and females were separated into jars, and each day fresh food was provided. The number of dead males and females was counted after 5 days of irradiation, and the percentage of mortalities was computed. The lethal doses D_{50} and LD_{90} values were calculated using the LdPLine® program.

Dna Isolation And Start Codon Targeted Polymorphism (Scot-pcr) Analysis:

Fresh adults were used to obtain genomic DNA using the DNeasy insect micro kit (bio basic). A UV spectrophotometer was used to measure the absorbance ratios A260/A280, where DNA is pure when the ratio is between 1.8 and 2.0. Moreover, a qualitative assessment of DNA sample quality was carried out employing electrophoresis in 1% agarose gel with ethidium bromide (Williams et al. 1990).

In the molecular evaluation of *L. migratoria* adults' tissues, genomic DNA was employed as a template for PCR amplification with seven SCoT primers. Biobasic Com provided the SCoT primers. The 18-mer SCoT primers were all taken from Dataset I's highly expressed genes as described by Sawant et al (1999). The start codon ATG (+ 1, + 2, and + 3), "G" at position + 4, "C" at position + 5, and "A," "C," "C," and "A," respectively, at positions + 7, +8, + 9, and + 10, were fixed (5'——-ATGGCTACCA—-3') for SCoT primers design. The table contained a list of the primers' utilized sequences (1).

Following the procedures outlined by Xiong et al. (2011) and Fathi et al. (2013), the following amplification reactions for SCoT methods were carried out in a Techni TC-512 Thermal Cycler: The reaction was then stored at 4 ^{o}C after one cycle at 94 ^{o}C for 4 min, 40 cycles of 1 min at 94^{o}C, 1 min at an annealing temperature of 57 oC for 2 min at 72^{o}C, followed by 72^{o} C for 10 min.

On a 1.5% agarose gel, amplified products were loaded and separated using ethidium bromide and a ladder 50 bp marker from 50 bp to 1500 bp. The run took place in a tiny submarine gel BioRad for around 30 minutes at 100V.

Photos of DNA banding patterns were taken using the Bio-1D Gel Documentation system, and GelAnalyzer3 software was used to analyze them. This software scored clear amplicons as present (1) or absent (0) for each primer, and the results were recorded as a binary data matrix. According to Adhikari et al., DNA profiles for ISSR and SCoT methods were generated from this matrix (2015). The Molecular Distances MD (Dissimilarity) for the binary data matrix was calculated using the Dice coefficient (Nei and 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 12 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 Turkey Pairwise Comparisons test ($P \leq 0.05$).

Results

Figure 1 shows the accumulative mortality of *Locusta migratoria* migratorioides adults after 5 days of irradiation with different doses (10, 20, 30, and 40Gy). Compared to the mortality in controls (0%), gamma irradiation resulted in 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* recorded higher mortality than females in all irradiated doses.

The estimated LD_{50} values for males and females were 33.94 and 51.55Gy, respectively. Likewise, LD_{90} values of 161.13 and 207.95Gy were calculated for males and females, respectively. The tabulated resistance ratio exposed that adult females were more resistant to gamma radiation than adult males (Fig. 2; Table 2).

The fragments of DNA produced by 7 SCoT primers were amplified and separated using agarose gel electrophoresis as shown in figure (3). They ranged in size from 175 bp in SCoT 1 to 1185 bp in SCoT 12. The total number of scored bands varied from 1 (for SCoT 4 + SCoT 6) to 7 (for SCoT 15) (Table 3).

From Table (3) and Fig. (3), the analysis of SCoT 1, SCoT 4, SCoT 6, and SCoT15 discovered the detection and absence of polymorphism bands. In SCoT 1 there were 5 bands including 4 monomorphic, 1 polymorphic, and 1 unique band. The 2nd band (360bp) was absent in the control female locust. Regarding SCoT4, there were 5 bands distinguished by 4 polymorphic, 1 monomorphic band and 2 unique bands. The 5th band was detected in all tested groups, 1st band was absent in male insects (control and irradiated with 30 and 40 Gy), 2nd and 3rd bands were absent in the control male group only.

The 4th band was observed in the male group irradiated with 10Gy and females (control, 10,20, and 40 Gy irradiated groups). While SCoT 6 showed only 3 bands, 1 monomorphic band, and 2 polymorphic bands. The 1st band 425 bp was present in the male group irradiated with 30Gy, and female groups (control and irradiated with 30 Gy). SCoT15 recorded seven cleared bands. 5 monomorphic (215, 450, 500, 600, and 1185 bp), 2 polymorphic (335 and 114 bp), and 1 unique (335bp) band. The 2nd (1140bp) band was observed in male and female groups irradiated with 30 Gy, while the 6th band (335bp) was found in Female irradiated with a 10Gy group only. In addition, the generated patterns from SCoT 1, SCoT 4, and SCoT 6 discovered a difference in the genetic structure between normal (unirradiated) adult males and females. The total no. of the produced bands in males were 5, 1, and 1, while in females were 4, 5, and 3 bands for SCoT 1, SCoT 4, and SCoT 6, respectively. Using SCoT 4, SCoT 6, and SCoT 15 implied an alteration in the DNA of males and females *L. migratoria* after gamma irradiation, that there was a difference in the no. of the produced fragments.

The examined samples made 29 cleared bands, including 20 monomorphic, 9 polymorphic, and 4 unique bands. The number of monomorphic bands ranged from 1 (for SCoT 4 + SCoT 6); 2 for SCoT 8; 3 bands for SCoT 13; 4 bands for ScoT 1 and 16; 5 bands for SCoT 15 (Table 4). The presented data indicated that SCoT 4 yielded the highest polymorphic bands (4 bands and 80% polymorphism) among the tested samples (un-irradiated and irradiated males and females), followed by SCoT 6 (2 bands), then SCoT 15 (2 bands) then SCoT 1 which produced the fewer bands (1 band).

Based on the similarity index, a dendrogram was developed (Fig. 4). The greatest similarity index for SCoT 1 was obtained between the female control group and all other groups (0.88889). Regarding SCoT 4, such SI reached its lowest value (0.33333) between control and control male also female groups (control, those irradiated with 10, 20, and 30 Gy), while it reached its highest value (0.8889) between (control male and 01Gy irradiated group), (control male and females irradiated with 30 Gy), (male irradiated with 10 Gy and control female, also with females irradiated with 10, 20 and 40Gy). Concerning SCoT 6; the SI value ranged from 0.5 to 0.8 within the different groups. For SCoT 15, the highest IS values were 0.92308, 0.90909, and 0.83333) (Table 5). In addition, the estimated similarity index of SCoT 1, SCoT 4, and SCoT 15 showed a genetic diversity between normal (un-irradiated) males and females. Also, the tabulated results revealed that gamma radiation caused deviations in the DNA structure of males and females, which were obvious by different estimated similarities among the tested doses and the tested sexes after using SCoT 4.

Discussion

According to our knowledge, this is the first instance in which SCoT markers have been used to describe the genetic alteration brought on by gamma radiation in adult male and female *L*..

Due to the healthy proteins and minerals, they contain, locusts are utilized as edible insects; but, because of their horrible migration and the harm it is inflicting on food crops, governments are looking for ways to combat them. One of the most prevalent agricultural pests, the migratory locust, *L*., consumes a lot of

grass and harms crops. People have been moving into Egypt in greater numbers since the late 1990s, mainly in the southwest and west. From 2015 to 2019, outbreaks happened in Sharq El-Owinat and Toshka (Moustafa 2019).

Gamma radiation is the most invasive type of radiation produced by both natural and man-made sources and is made up of uncharged ionizing particles (IAEA-TECDOC-1363, 2003). Depending on the radiation dose received, ionizing radiation's biological effects can change. Gamma radiation causes errors at numerous molecular levels, including DNA, chromosomal abnormalities, cells, and proteins. Double strand breaks (DSBs) of DNA are the most prevalent sign (Rhee et al. 2012).

Numerous biological effects of parental radiation exposure have been documented in laboratory studies. These effects include chromosomal inversions (Sykes et al. 2006; Zeng et al. 2006), mitotic recombination in foetal cells (Liang et al. 2007), point mutations (Liang et al. 2007; Schilling-Tóth et al. 2011), increased DNA strand breaks in sperm (Schindewolf et (Nomura 1990, 2006; Barber et al. 2002).

The results of the current study on the effects of gamma radiation on adult male and female *L. migratoria migrotaria* showed that irradiation led to a progressive, significant rise in mortality along with an increase in radiation dose. This is in agreement with previous studies which revealed that the mortality of insects was dependent on the dose of gamma radiation (Sayed and Zahran 2017; Kiran et al. 2019; Sayed et al. 2020). This may be explained by the findings of Kinipling (1955), who reported that exposure to ionizing radiation causes somatic damage that interferes with the biological functions of the insects and induces genetic dominant fatal mutation with eventual insect sterilization (Ahmed 1992). In addition, exposure to ionizing radiation reduces the lifespan of insects and may hasten age (Baxter and Blair 1969). Furthermore, the data exposed that adult males were more sensitive to gamma radiation than adult females. The estimated LD_{50} value for males and females were 33.94 and 51.55 Gy, and the LD_{95} values were 161.13 and 207.95 Gy, respectively. This outcome was in agreement with Datkhile et al. (2009) who specified that female *Chironomus ramosus* was more tolerant to gamma irradiation than males.

Herein, there was a genetic diversity between normal (un-irradiated) males and females as presented by the similarity index of SCoT 1, SCoT 4, and SCoT 15. Likewise, 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 after using SCoT 4.

The primary cause of the mutation load in living things is oxidative DNA damage (Von Sonntag 1987). 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. 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 (El-Naggar 2009).

Although the residual-radionuclides have a low dose rate, the cumulative dose over time is likely to cause oxidative stress in exposed organisms, DNA damage, and an accumulation of unrepaired mutations (Bonisoli-Alquati et al. 2010a; Einor et al. 2016); and epigenetic effects that may amplify the effects of changes in DNA sequence (Kovalchuk and Baulch 2008; Ilnytskyy and Kovalchuk 2011).

Gamma-radiation caused some bands to appear and others to disappear throughout our investigation, resulting in variances in SCoT-PCR patterns across the various samples. In line with Hamed et al. (2009), El-said (2013), Rizk et al. (2017), Zahran et al. (2017), and Ali et al. (2018). Single and multiple strand breaks, point mutations caused by -rays, changed or oxidized bases, and band loss may all be associated with band loss (Esnault et al. 2010). 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).

Additionally, the outcomes of this research may be attributed to the fact that irradiation enhances the production of reactive oxygen species (ROS). Numerous biological processes, including the cell cycle, programmed cell death, development, and reproduction, are regulated by ROS, which affects gene-expression and transcription (Sarvajeet and Narendra 2010). Furthermore, it causes histones to separate from the chromatin strands (Volle and Dalal 2014). As a result of exposure to ionizing radiation, enzymes needed for DNA synthesis and repair, such as DNA polymerase, become inhibited. This could happen as a result of chance DNA-protein binding (Stobbe et al. 2002, Chalmers et al. 2010).

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).

Conclusion

It was observed that gamma radiation could alter the mature *L.* biosystem. Some DNA bands appeared and other vanished as a result of the exposure to it. As radiation exposure increased, mortality rates increased. Gamma radiation could therefore be employed as a management strategy to address this problem.

Declarations

Ethical Approval

All experiments were approved by National Research Centre- Medical Research Ethics - Committee (20-103)

Competing interests

All authors declare that there are no Competing Interests and Funding. All authors read and approved the final manuscript

Authors' contributions

All authors equally participated in this work

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

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Tables

Tables 1-5 are not available with this version.

Figures

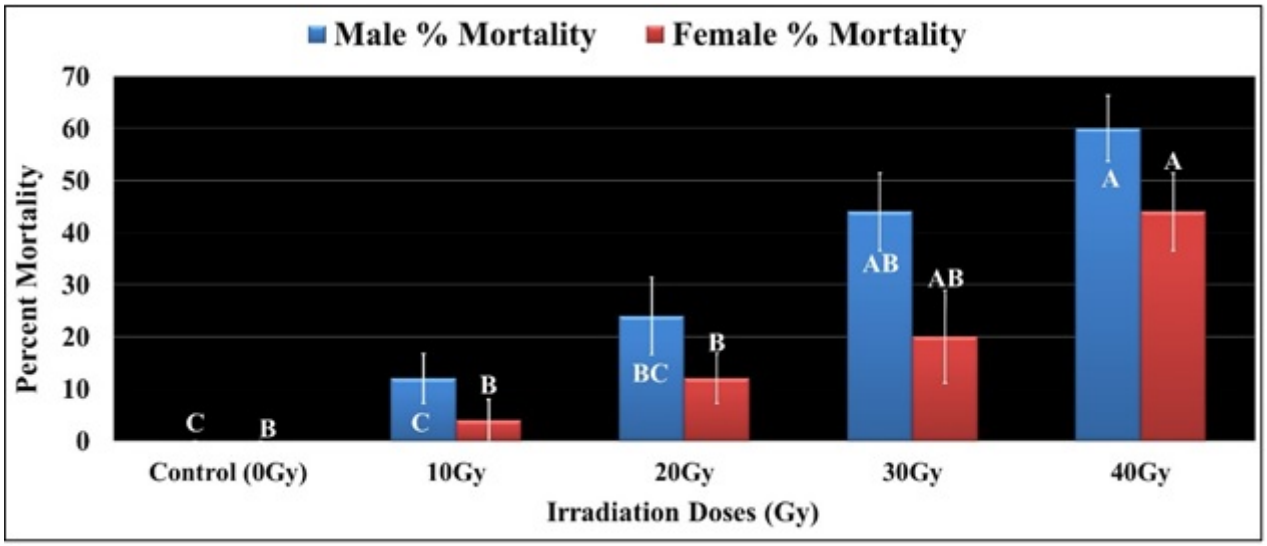

Figure 1

Accumulative mortality of *Locusta migratoria migratorioides* adults after irradiation for5 days with different doses

· Values represent the mean±SE of 5 replicates each of 5 adults.

· Means in the same group (male or female) that do not share a letter are significantly different (Tukey Pairwise Comparisons).

· For male: F=16.59, P=0.000; for female: F=8.64, P=0.000.

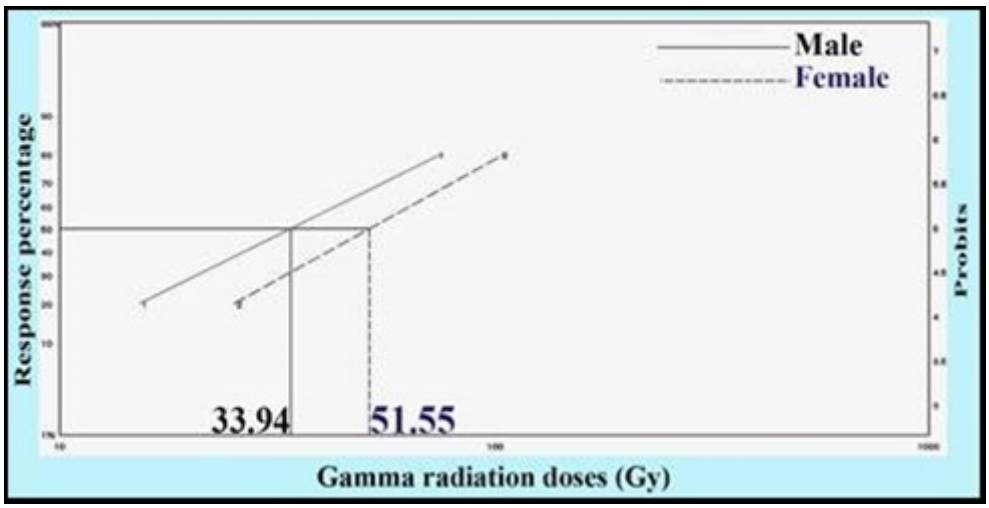

Figure 2

LD_{50} values of γ radiation after 5-days irradiation of *Locusta migratoria* adults.

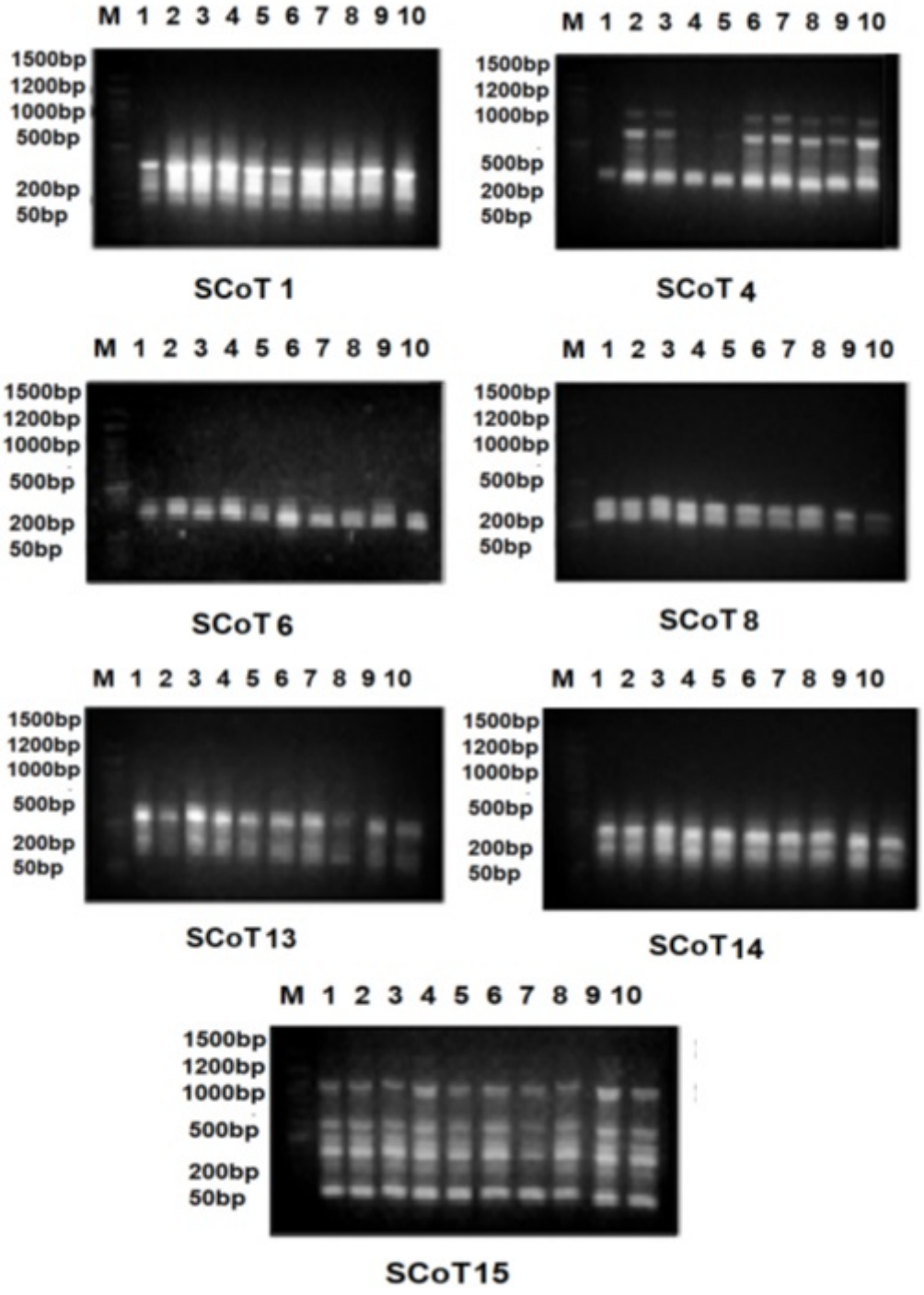

Figure 3

Agarose-gel electrophoresis of SCoT product generated with the primers in control and irradiated adults of *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

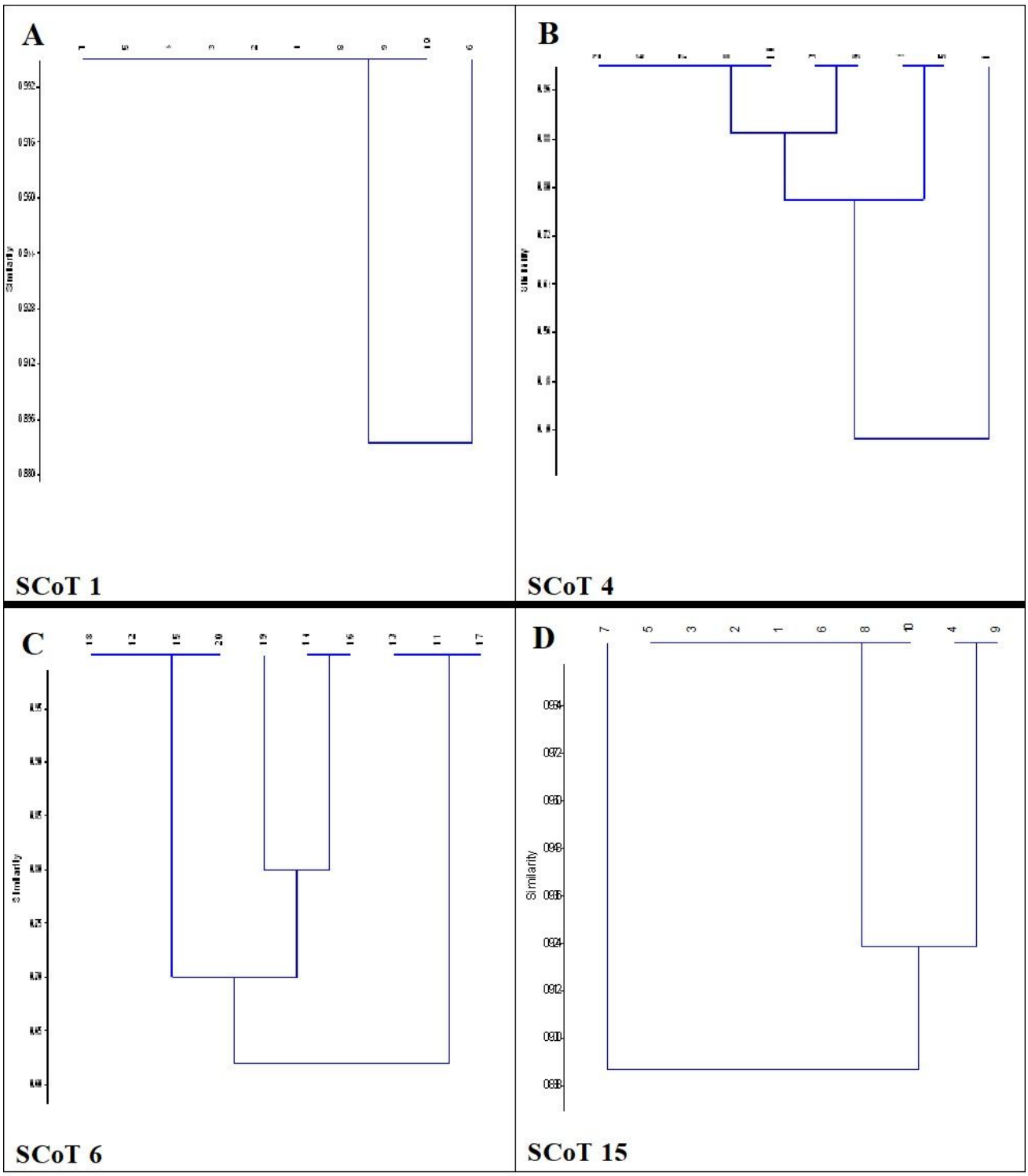

Figure 4

Dendrogram analysis using SCoT data for cDNA from un-irradiated and irradiated *Locusta migratoria* adults