Egyptian Journal of Phytopathology, Vol. 51, No. 2, pp 14-29 (2023)
DOI 10.21608/ejp.2023.221669.1098

ORIGINAL PAPER

Influence of Peanut Seed Exposure to Microwave and X-ray Radiations on Sclerotium Stem Rot and Effects on Yield Improvement, and RAPD-PCR Mutagenic Analysis of Peanut Plants

Ahmed, H.A.M.*[1] and Amein, K.A.[2]

Received: 8 July 2023 / **Accepted**: 29 July 2023 / **Published online**: 31 July 2023.

ABSTRACT

Peanut seeds were exposed to microwave and X-ray radiation before planting in artificially infested soil with S. rolfsii under a greenhouse and natural field conditions. Results showed a high reduction of stem rot and improvement of yield and seed oil content of peanut following seeds exposed to X-ray at 45KeV for 5 sec and MR or 30 sec compared to the control-(non-irradiated seeds). Based on the RAPD-PCR mutagenic analysis of DNA peanut plants using eight ten-mer (random primers used for molecular polymorphism analysis) random oligonucleotide primers following seed irradiation, six new DNA bands, and no missing bands were detected with mutagenic frequency (20%) in plants raised from seeds exposed to MR for 30 sec. In addition, four new DNA bands were detected, and only one missing band was detected with mutagenic frequency (18.51%) in plants generated from seeds exposed to X-ray at 45 KeV for 5 sec. In contrast, the most noticeable changes were the increased number of missing DNA bands by increasing the X-ray exposure dose, where the number of missing bands was increased from 1 in 45 KeV to 3 bands in 75 KeV and 6 bands in 95 KeV. Therefore, these findings demonstrated that seed exposure to X-rays at the low dose of 45 KeV for 5 sec effectively controlled Sclerotium stem rot of peanut but caused more severe plant injuries at high radiation doses.

Keywords: Peanut, *Arachis hypogaea*, *Sclerotium rolfsii*, Radiation, Mutagenicity, RAPD-PCR Marker

***Correspondence: Ahmed, H.A.M.**

E-mail: hudafatah@arc.sci.eg

Hoda A.M. Ahmed

https://orcid.org/0000-0003-4909-408X

1- Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

Karam A. Amein

https://orcid.org/0000-0003-4557-4035

2- Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt.

INTRODUCTION

Peanut (*Arachis hypogaea* L.), also known as groundnut, is a member of the family Fabaceae and is produced in more than 82 countries worldwide. It is an economically important oilseed crop with a high nutritional value of oils, proteins, calories, and vitamins. In Egypt, it is one of the most exported and locally consumed oil crops. The cultivated area in Egypt is estimated to be about 170 thousand feddans, with an average of 23 ardebs/feddan, with a total productivity of about 3.9 million ardebs, equal to about 293 thousand tons of peanut pods (FAOSTAT, 2021). Unfortunately, the peanut crop is subjected to a relatively large number of dangerous fungal diseases during its growth, causing severe losses in productivity. Among the fungal diseases, stem rot caused by *Sclerotium rolfsii* Sacc. is a potential threat to peanut production and causes severe damage during any stage of crop growth. Yield losses caused by *S. rolfsii* can reach up to 80% due to the infections of stems, roots, pegs, and pods of groundnut in the soil (Yan *et al*. 2021). *S. rolfsii* not only reduces the yield of groundnut but also degrades the quality of peanut kernels.

Sclerotium rolfsii Sacc (teleomorph *Athelia rolfsii*), is a soil-borne fungal pathogen, commonly occurs in the world's tropics, sub-tropics, and other warm temperate regions, causing root rot, stem rot, wilt, and foot rot on more than 500 plant species (Priya *et al.*, 2013). Stem rot, also known as white mold, southern stem rot, southern blight, and Sclerotium rot, is prevalent worldwide in almost all peanut-growing areas. Control of *S. rolfsii* is difficult due to its host range and survival of sclerotia for several years in soil and plant residues (Cilliers *et al.*, 2003). The use of synthetic fungicides generally achieves effective management of crop diseases. However, these fungicides pollute the environment, soil, and water, causing deleterious effects on human health and the biosphere (Sivaprakasam, 1994). Therefore, environmentally safe and economically alternative approaches for controlling plant

diseases, including seed treatments using physical methods such as radiation exposure, are essential in agriculture.

Microwave radiation (MR) is a form of electromagnetic radiation, and many conducted studies have shown different responses by plants exposed to electromagnetic radiation. The MR has been used in agriculture since the mid-twentieth century and is considered one of the best non-chemical methods to control plant fungal pathogens efficiently (Kalinin *et al.*, 2005). The MR may directly disrupt microbial cells (Yoshida and Kajimoto, 1988), although this effect has not been confirmed yet. However, it has suppressed pathogens' development and growth, using heat to kill plant pathogens (Grondeau*et al*., 1994). On the other hand, the affected plants were taller and heavier when exposed to radiation (Martínez *et al*., 2003).

Moreover, microwave seed treatment stimulates early germination and increases seedling vigor (Tylkowska *et al*., 2010). To date, there is no data to explain the effects of MR on pathogenic fungi colonizing seeds. However, due to its ability to generate heat rapidly, a short period of radiation exposure is needed for seed or pathogen treatment with radiation (Adu *et al*., 1995). Also, non-ionizing electromagnetic (microwave) radiation affects biological systems at the whole organism, tissue, cell, and molecular level (Roux *et al.,* 2006 and Hamada, 2007). Microwaves in a microwave oven cause rotation in dielectric molecules such as water under an electromagnetic field, which causes the system to heat up. This movement destabilized biomolecules such as DNA (Pavel *et al*., 1998 and Hamada, 2007). Also, Jangid *et al*. (2010) found that microwave seed treatment induced mutations and altered gene expression in the moth bean (*Vigna aconitifolia*) plant.

X-ray radiation is valuable in diagnosing and treating many plant injuries and diseases. Moreover, radiation treatment is considered an excellent tool for sterilization, preservation of food, and other different food engineering processes, which benefits human society (Alariqi *et al.,* 2006). In agriculture, various control methods are used to avoid yield losses due to infection by soil-borne pathogens, such as radiation (Spadaro and Gullino, 2005). In this respect, X-ray is commonly used to observe and quantify the soil environment, including plant root development (Tracy *et al*., 2012), fungal influence (Martin *et al*., 2012), and the influence of microbial activity (Nunan *et al*., 2006). Moreover, X-ray dose influences plant root growth, size and weight, and fungal or microbial activity. The influence of X-ray doses on plants is still under study (Al-Enezi *et al*., 2012). However, ionizing radiation, including X-ray, alters plant growth and development by inducing genetic and morphological changes (De Micco *et al*., 2011 and Arena *et al*., 2014). Macromolecules, particularly DNA, are critical targets of X-ray (Al-Enezi and Al-Khayri, 2012). The impact of ionizing radiation on DNA is well-documented in various plant species (Esnault *et al.,* 2010 and Arena *et al.,* 2017). The DNA may be directly or indirectly damaged due to the overproduction of free radicals, resulting in single and double DNA breakage (Yokota *et al.,* 2007). These breaks are the primary source of chromosomal aberrations, which determine alterations in gene expression and subsequent changes in plant structure and function (Kovalchuk *et al.*,2004). In many plant species, exposure to X-rays leads to gene alterations (Kranz *et al.*, 1994 and Naito *et al*., 2005) and chromosomal aberrations (Kovalchuk *et al*., 2007 and Watanabe *et al*., 2009). Moreover, X-rays negatively influenced DNA synthesis (Evans and Scott, 1964) and RNA and protein synthesis (Roy *et al*., 1972).

Random amplified polymorphic DNA (RAPD)-PCR primers (markers) were used to evaluate if ionizing radiation can trigger mutagenesis in plant cells (Dhakshanamoorthy *et al*., 2011; El-Sherif *et al.*, 2011; Abdel Haliem *et al.*, 2013 and Arena *et al*., 2017). It was reported that the RAPD-PCR analysis has the potential to detect various types of DNA damage and mutations, even those that do not reveal alterations phenotypically (Atienzar and Jha, 2006). Different RAPD-PCR profiles are proxies of genetic differences between plant samples since other DNA profiles are correlated to different base compositions in some annealing sites of the DNA samples (Al-Enezi and Al-Khayri, 2012). Also, Arena *et al.* (2017) irradiated mature tomato plants with different doses of X-rays to investigate possible variations in leaf morpho-anatomical traits, photosynthetic efficiency, and genomic tomato DNA. The results of RAPD-PCR analysis showed that the X-ray induced mutagenic effects in the L2 leaves of dwarf tomato even at low doses, despite the absence of severe phenotypic alterations.

Peanuts are considered a self-pollinated species, and their embryo is easily exposed to mutagens and has 6-8 primordia with buds in its axil, making several potential targets available (Ashri, 1976), and thus it is suited for mutagenesis studies.

The present work's objective was to control Sclerotium stem rot of peanut and improve the yield by exposing peanut seeds to Microwave radiation (MR) and X-ray radiation planting under greenhouse and field conditions to induce mutations to improve plant characteristics and increase the genetic variability of peanuts. Another objective was to study the RAPD-PCR mutagenic analysis of peanut plants raised from seeds exposed to radiation**.**

MATERIALS AND METHODS

Isolation, purification of the associated fungi:

The causal pathogen of stem rot in peanut, was isolated from naturally infected plants showing typical stem rot symptoms Fig (1) collected from different locations in Assiut Governorate, Egypt. Diseased peanut plant samples were thoroughly washed with tap water, and the root and stem were cut into small pieces. Then pieces were surface sterilized with 0.5% sodium hypochlorite (SH) solution for 3 min, rinsed in three changes of sterile distilled water (SDW), and dried between two sterilized filter papers. Pieces were then placed in Petri dishes (9.0 cm) containing Potato Dextrose Agar (PDA) medium supplemented with streptomycin sulfate (200 mg L^{-1}). The plates were incubated at 28±2°C for 7 days and examined daily for promoted fungal growth. Purification of the isolated fungi was carried out using the hyphal tip technique (Rangas Wamy, 1972). Then the obtained fungal isolates were identified based on their morphological characteristics described by Domsch *et al*. (2007). All *S. rolfsii* isolates were re-cultured on the PDA slants in test tubes and then kept at 5°C for further studies.

Fig. (1): Symptoms of peanut stem rot, stem lesions are formed near the plant's crown, starting at points of contact with the soil, and are color brown, may have a canker-like appearance and are covered with white mycelium (A). Distinct lesions are formed on pods during the early stages of infection, but later pods are wholly consumed with dry brown rot, rotted pods are thin and brittle, and seeds may be stained, rotted, or absent (B).

Pathogenicity test:

The pathogenic capabilities of *S. rolfsii* isolates were determined on the peanut Giza 6 cultivar under open greenhouse conditions at the Experimental Farm of Arab-El-Awamer Research Station, Assiut governorate, Egypt.

The inoculum of each tested isolate of *S. rolfsii* was prepared by placing two equal agar disks (0.6 cm) taken from the 7-day-old culture into 500 ml glass bottles tightly closed with cotton plugs containing autoclaved 200 g moistened wheat grains and washed sand medium (3:1 weight, respectively), according to Rubayet and Bhuiyan (2016). Then bottles were incubated at 28±2 °C for 2 weeks. Formalin-sterilized 30 cm plastic pots were filled with formalin-sterilized clay-loam soil (5 kg per pot), infested with 3% inoculum (150 g per pot) of each tested isolate, and then slightly irrigated every other day for a week. Pots treated with the same amount of non-inoculated wheat grains and washed sand medium were served as

control. Seeds of peanut Giza 6 cv. were disinfected by dipping in 0.5% sodium hypochlorite solution for 3 min, rinsed three times in SDW for 3 min, and then sown at a rate of 6 seeds per pot. Four pots as replicates of each tested fungal isolate in a completely randomized experimental design were used. Pots were checked daily and irrigated when necessary.

Stem rot symptoms were checked daily. After 35 days and up to 2 weeks before harvesting, peanut plants were uprooted and examined for root and stem colonization by *S. rolfsii*. The percentage of disease incidence (DI) of peanut stem rot was calculated according to the following formula:

$$\text{Disease incidence \%} = \frac{\text{Number of infected plants}}{\text{Number of planted seeds}} \times 100$$

The peanut's stem rot pathogen, *S. rolfsii*, was also re-isolated from the infected peanut root and stem tissues.

Peanut seed treatment with MR:

Seeds were exposed to MR after randomly sampling 20 peanut Giza 6 cv. in the test tube. Then each seed sample was individually exposed to a household microwave oven (Type Zanussi, 230 V, 50 Hz,2450 MHz, 1100 W, China) for 20, 30, and 40 sec. Before starting, the seed coat reduction was prevented by placing 150 ml water into a glass beaker (Reddy *et al*., 2000). Then the SDW was kept in the oven when the sample was introduced to avoid increasing temperature through seed exposure.

Peanut seed treatment with X-ray

Seeds of peanut Giza 6 cv. were treated with X-ray at the Department of Radiation, National Cancer Institute, Egypt, according to the method described by Kumar and Singh (1996). Each seed sample 20 peanut was divided into three groups and put in clean Petri dishes. Seed samples were exposed to X-rays at 45, 75, and 95 KeV (Kilo Electron Volts) each for 5 sec at room temperature.

Effect of exposing peanut seeds to MR and X-ray on control of stem rot disease:

A- Greenhouse experiment:

A pot experiment was conducted under open greenhouse conditions during 2019 growing season at the Experimental Farm of Arab-El-Awamer Research Station, Assiut governorate, Egypt. Peanut seeds of Giza 6 cv. irradiated with microwave and X-ray were planted in sterilized 30 cm plastic pots containing infested soil with the aggressive isolate of *S. rolfsii* inoculum. Each pot was sown with 4 seeds, and four pots as replicates of each treatment were used. A randomized complete block design was used in this experiment to arrange every pot on a screen house bench, and each pot was given regular watering to maintain the required moisture. In the control treatment, seeds not exposed to radiation were sown in pots containing infested soil with *S. rolfsii* inoculum. After 35 days, the percentage of disease incidence (DI) of peanut stem rot was calculated, as mentioned before.

B- Field experiment:

A field experiment was conducted throughout two successive seasons (2019 and 2020) in a naturally infested soil with *S. rolfsii* to determine the impact of peanut seed exposure to MR and X-ray on controlling stem rot incidence and peanut yield as well as some plant growth characteristics and seed oil content at the Experimental Farm of Arab-El-Awamer Research Station, Assiut governorate, Egypt. The soil quality was the field soil is sandy loam, (Ahmed and Abdel-Gayed, 2017)

Peanut seeds were exposed to MR and X-ray radiation only in the first year of this experiment to induce mutations for improving plant characteristics and increasing the genetic variability of peanuts before planting in 2019 season. Peanut plants grown in the first year from the irradiated seeds were utilized for producing seed material used in the experiment in the second year of 2020.

Field plots with dimensions of 4×6 m and 70 cm between rows and five rows in each plot were used. Irradiated Giza 6 cv. seeds were sown in hills with 20 cm space between hills in the plot rows, and non-irradiated seeds were sown as a control. The experiments were set up in a randomized complete block design with 3 replicates and seven treatments. All cultural practices recommended for peanut production were followed. At 120 days after planting, before harvesting, peanut plants were uprooted, before harvesting and examined for root and stem colonization by *S. rolfsii*.

For investigating the effects of exposing peanut seeds to MR and X-ray on yield components, and seed oil percentage, five plant samples from each plot were randomly selected at harvest time (after 120 days from planting) to examine the following plant characteristics: number of pods/plant and seeds/plant, as well as weight of 100-pods and 100-seeds (g) and percent of seed oil content. Also, any changes in the appearance of plant leaves, pods, and seeds produced from seeds exposed to MR and X-ray were recorded.

Random amplified polymorphic DNA (RAPD) mutagenic analysis of peanut plants following seed exposure to MR and X-ray:

A- DNA extraction:

Total genomic DNA extracts were isolated from peanut plants (28-day-old) generated from seeds exposed and not exposed to MR and X-ray using the CTAB isolation protocol described by Murray and Thompson (1980) and Kumar *et al.* (2003) with some modifications.

B- RAPD-PCR analysis:

RAPD-PCR analysis was performed according to the protocol described by Williams *et al*. (1990). Eight ten-mer random oligonucleotide primers and their sequences were used in this study (Table ,1). The conditions of the PCR reaction were optimized, and mixtures (25 µl total volume) were composed of 11 µl dH_2O, 3 µl 10X reaction buffer, 3 µl dNTP's mix, 2 µl primer, 4 µl $MgCl_2$, 0.3 µl Taq DNA polymerase and 1µl template DNA (25 ng/µL). Amplification conditions were carried out in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cam-bridge Ltd, Duxford, and Cambridge, UK) with the following specifications: Initial denaturation for 5 min at 94ºC (1st step), 40 cycles of 1 min at 94ºC, 1 min at 34ºC and 2 min at 72ºC (2nd step), 10 min at 72ºC, and a final hold at 4 ºC were applied. The amplified products were separated into 1.4% agarose gel. Electrophoresis was carried out under a constant voltage of around 80V for approx. 3-3.5 h. The banding patterns were then visualized on a trans illuminator (Ultra-Violet Product, Upland, CA, USA).

Table (1): Primer codes and sequences used for the RAPD-PCR analysis in this study.

Primer code	Sequence (5`----3`)
OPU-7	CCTGCTCATC
OPAB-4	GGCACGCGTT
OPE-5	TCAGGGAGGGT
OPA-3	AGTCAGCCAC
OPA-2	TGCCGAGCTG
OPA-15	AGATGCAGCC
OPC-18	TGGGGGACTC
OPY-5	GGCTGCGACA

Statistical analysis:

We achieved analyses of variance using the MSTATC computer program. The least significant difference was calculated at $P \leq 0.05$, according to Gomez and Gomez, (1984).

RESULTS

Pathogenicity test:

The pathogenic capabilities of ten isolates of *S. rolfsii* that were obtained from naturally infected peanut plants showing typical stem rot symptoms collected from different locations of Assiut Governorate and tested in the pot experiment on peanut Giza 6 cv. are shown in Table (2). Results show that all tested *S. rolfsii* isolates were pathogenic to peanut plants, causing DI% of stem rot ranging from 20.83 to 87.49%. The highest stem rot was recorded with isolate No.8 (87.49%) isolated from Al-Ganaem county followed by isolate No. 9 of Abnoub county (74.99%). While the lowest DI% was recorded with isolate No. 6 (20.83%) of AboTeeg county followed by isolate No. 5 (24.99%) of El-Badari. The more pathogenic isolate (No.8) was selected for the greenhouse experiment.

Table (2): Pathogenic capabilities of 10 isolates of *S. rolfsii* to cause stem rot on peanut Giza 6 cv. collected from Assiut governorate under the open greenhouse conditions.

No.	*S. rolfsii* isolate	DI%
1	Dayrout	54.16*
2	Al-Qosiah	41.66
3	Manfalout	45.83
4	Assiut	37.49
5	El-Badari	24.99
6	Abo Teeg	20.83
7	Sahel Seliem	29.16
8	Al-Ganaem	87.49
9	Abnoub	74.99
10	Sedfa	50.00
Control	-	0.00
L.S.D. at 0.05		1.53

*Values of stem rot disease incidence are the means from 24 plants over four replicates.

Effect of exposing peanut seeds to MR and X-ray on control of stem rot disease:

A- Greenhouse experiment:

Data in Table (3) and Fig. (2) show a significant decrease in the DI% of peanut stem rot caused by *S. rolfsii* was recorded in plants that raised from seeds exposed to MR and X-ray compared to the control of those that generated from not-exposed seeds. The most effective treatment for decreasing the DI% of stem rot

was recorded in plants grown from seeds exposed to X-ray at 45 KeV for 5 sec and MR for 30 sec, where the DI% was 12.5 and 18.75%, respectively, compared with 81.25% of control. On the other hand, seeds exposed to MR for 40 sec and X-ray at 95 KeV for 5 sec provided the least peanut protection against *S. rolfsii* infection (DI% was 31.25 and 37.5%, respectively). At the same time, X-ray at 75 KeV for 5 sec and MR for 20 sec was moderately efficient for reducing infection by *S. rolfsii*, where DI% was 25% in both treatments.

B- Field experiments:

Under field conditions and natural soil infestation with *S. rolfsii* over the conducted two trials in 2019 and 2020, a significant decrease in the DI% of peanut stem rot was recorded in the plants raised from seeds exposed to MR and X-ray compared to the control of those developed from not exposed seeds, (Table, 4). The results indicated that peanut seeds irradiated by X-ray at 45 KeV for 5 sec and MR for 30 sec have proved to be the most effective treatment in controlling the stem rot through 2019 and 2020 growing seasons, with DI% of (4.10 and 3.70%) and (6.96 and 6.04%), respectively compared to the control, not exposed seeds in both seasons (42.76 and 39.35%). The mean DI% value of peanut stem rot over the two seasons of both radiation treatments was 3.90 and 6.50%, respectively. In contrast, peanut seeds exposed to MR for 40 sec and X-ray at 95 KeV for 5 sec provided the least protection against *S. rolfsii* infection, where DI% was (11.46 and 10.46%) and (19.71 and 18.51%) in both seasons, respectively. The mean DI% of peanut stem rot over the two seasons of both treatments were 10.96 and 19.11%, respectively. On the other hand, irritated seeds to X-ray at 75 KeV for 5 sec and MR for 20 sec were moderately efficient treatments for controlling infection by *S. rolfsii*, where DI% value of stem rot of both radiation treatments in both seasons were (12.77 and 11.10%) and (9.97 and 8.64%), respectively with mean DI% over the two seasons of 11.94 and 9.31%.

Table (3): Effect of exposing peanut seeds to microwave and X-ray radiation on control of stem rot disease caused by *S. rolfsii* under the open greenhouse conditions.

Time of Microwave	DI% of stem rot	Dose of X-ray	DI% of stem rot
0 sec	81.25	0 KeV	81.25
20 sec	25.00	45 KeV	12.50
30 sec	18.75	75 KeV	25.00
40 sec	31.25	95 KeV	37.50
L.S.D. at 5% =	27.79		28.88

*Values of stem rot disease index are the means from 16 plants over four replicates.

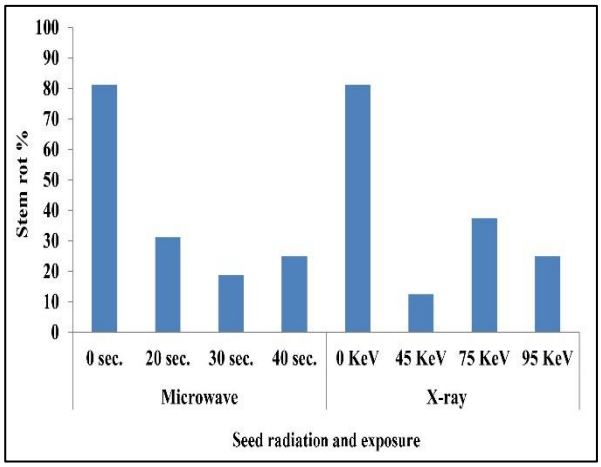

Fig. (2): Effect of exposing peanut seeds to microwave and X-ray radiation on control of stem rot disease caused by *S. rolfsii* in the open greenhouse:

Table (4): Effect of exposing peanut seeds to microwave and X-ray radiations on control of stem rot disease caused by *S. rolfsii* in field through the 2019 and 2020 growing seasons.

Radiation exposure							
	X-ray				Microwave		
Dose of X-ray	Season		Mean	Time of dose	Season		Mean
	2019	2020			2019	2020	
0 KeV	42.76	39.35	41.05	0 sec	42.76	39.35	41.05
45 KeV	4.10	3.70	3.90	20 sec	9.97	8.64	9.31
75 KeV	12.77	11.10	11.94	30 sec	6.96	6.04	6.50
95 KeV	19.71	18.51	19.11	40 sec	11.46	10.46	10.96
L.S.D. at 5%	3.90	3.60	3.00		4.48	6.63	4.88

Effect of exposing peanut seeds to MR and X-ray on yield components and seed oil percentage:

The effect of exposing peanut seeds to MR and X-ray on yield components and seed oil content was studied under a naturally infested field with *S. rolfsii*. Results in Table (5 a and b) show that yield components (*i.e.*, number of peanut pods and seed per plant and weight of 100-pods and 100-seeds and seed oil content were significantly increased when seeds were exposed to MR and X-ray compared to the control (not exposed seeds). All yield components and seed oil percent of peanut plants significantly varied between MR and X-ray and control seed treatments during 2019 and 2020 seasons and their means over the two growing seasons. Moreover, irradiating peanut seeds by microwave for 30 sec and X-ray at 45 KeV for 5 sec before planting increased the number of-pods and seeds per plant compared to non-irradiated seeds (Table 5a). The exception was in the height of peanut plants generated from seeds exposed to X-ray at 95 KeV for 5 sec, which was increased compared to those generated from not exposed seeds. Data also show that irradiating peanut seeds by microwave for 30 sec and X-ray at 45 KeV for 5 sec before planting significantly increased the weight of 100-pods and 100-seeds and seed oil percent compared to the control of non-irradiated seeds (Table 5b).

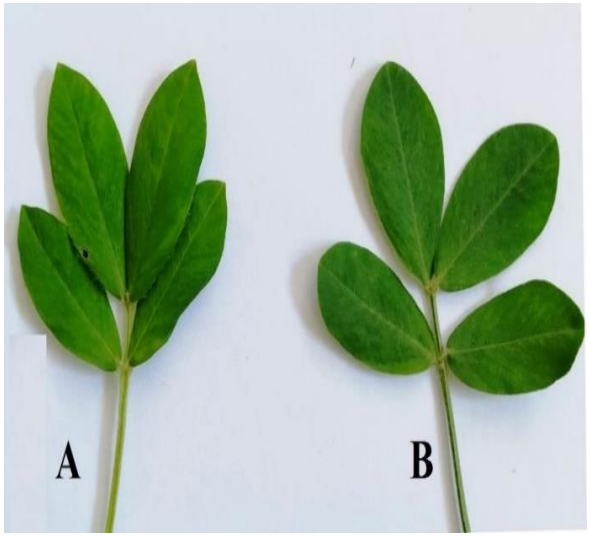

Fig. (3): A novel fused full leaflet observed as a mutant of plant leaf generated from peanut seeds exposed to X-ray radiation at 45 KeV for 5 sec (A) and control leaflet of plants generated from not exposed seeds (B).

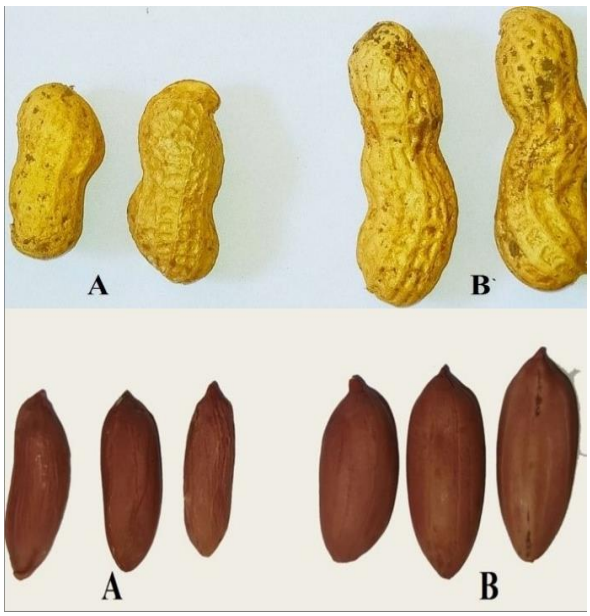

Fig. (4): Control pods (above) and seeds (down) of peanut plants generated from seeds not exposed to X-ray radiation (A). Large pods and seeds observed as mutants of peanut plants generated from seeds exposed to X-ray radiation at 45 KeV for 5 sec (B).

Fig. (5): Control seeds of peanut plants generated from seeds not exposed to X-ray radiation and after harvesting and storing at room conditions, showing *S. rolfsii* rot infection (A). Healthy seeds of peanut plants generated from seeds exposed to X-ray radiation at 45 KeV for 5 sec (B).

Besides observations on plant morphometrics, a novel fused full leaflet (Fig. 3) of leaves, large pods, and seeds (Fig. 4) as mutants have appeared in peanut plants generated from seeds exposed to X-ray at 45 KeV for 5 sec. Also, healthy and uninfected seeds by *S. rolfsii* of peanut plants generated from seeds exposed to X-ray at 45 KeV for 5 sec were noticed compared to those of control plants generated from not exposed seeds, which showed *S. rolfsii* rot infection (Fig. 5) after harvesting and storing at room conditions.

Table (5a): Effect of exposing peanut seeds to microwave radiation on yield/plant.

Time of dose	No. of pods/plant			No. of seeds/plant			Weight of 100-pods (g)			Weight of 100-Seeds (g)			Seed oil%		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
0 sec	25.91	2020	26.37	36.08	35.08	35.58	72.23	73.50	72.86	35.20	35.87	35.53	35.31	34.91	35.11
20 sec	44.41	26.83	44.78	57.33	58.08	57.70	104.50	105.37	104.93	51.93	52.29	52.11	40.58	41.32	40.95
30 sec	55.66	45.16	56.66	72.00	73.02	72.51	126.33	129.23	127.78	62.82	63.16	62.99	47.21	47.11	47.16
40 sec	42.66	56.66	43.10	47.25	45.75	46.50	109.14	108.43	108.78	53.70	54.00	53.85	42.77	43.00	42.89
Mean	42.16	43.54	42.60	53.17	52.98	53.07	103.05	104.13	103.58	50.92	51.33	51.12	41.46	41.58	41.52
L.S.D. at 5%	1.23	1.81	1.17	4.36	6.56	1.99	4.75	8.83	0.58	2.29	1.62	3.01	2.53	1.12	1.21

Table (5b): Effect of exposing peanut seeds to X-ray radiation on yield/plant.

Dose	No. of pods/plant			No. of seeds/plant			Weight of 100-pods (g)			Weight of 100-Seeds (g)			Seed oil%		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
0KeV	25.91	26.83	26.37	36.08	35.08	35.58	72.23	73.50	72.86	35.20	35.87	35.53	35.31	34.91	35.11
45 KeV	44,41	45.37	44.89	60.10	60.77	60.43	119.73	121.63	120.68	57.24	57.30	57.27	45.27	45.41	45.34
75KeV	35.58	37.91	36.74	35.91	36.33	36.12	74.17	74.84	74.50	35.61	35.63	35.62	36.66	36.34	36.50
95KeV	40.50	41.21	40.85	42.41	43.16	42.78	82.93	83.55	83.24	40.20	40.66	40.43	37.23	38.11	37.67
Mean	36.60	37.83	37.21	43.63	43.84	43.73	87.26	88.38	87.82	42.06	42.36	42.21	38.62	38.69	38.66
L.S.D.at 5%	4.65	5.33	1.01	4.60	5.21	1.30	3.07	2.62	1.48	1.28	1.33	3.17	1.27	1.05	1.98

RAPD-PCR mutagenic analysis of peanut plants following seed exposure to MR and X-ray:

A- Polymorphism of peanut plants generated from seeds exposed to MR:

The present study evaluated the effects of MR on peanut plants' DNA plants generated from irradiated seeds. The DNA samples from peanut plants generated from seeds exposed to MR for 20, 30, and 40 sec were obtained. Then the changes in the DNA bio-molecules were investigated by employing 8 RAPD-PCR primers to detect any changes in the genetic profile (Tables 6 and Fig. 6). Changes in the number of amplified bands (fragments) by each tested primer in the peanut plants generated from seeds exposed to MR compared to those arising from not exposed seeds of the control could reflect the mutagenic effect of MR affecting the DNA sequence in the annealing sites of each tested primer. In the present study, changes in plant DNA sequences could be detected by new or lost DNA bands compared to the DNA profile of the control. Thus, five new DNA bands at sequence bps of 700_{OPE-5}, 660_{OPA-2}, 1000_{OPC-18}, $350_{OPC-18,}$ and 180_{OPC-18}, and no missing bands were detected in peanut plants generated from seeds exposed to MR for 20 sec. Therefore, the irradiating effect resulted in a mutagenic frequency of 17.24% (5 out of the 29 bands).

On the other hand, six new DNA bands at sequence bps of 450_{OPU-7}, 700_{OPE-5}, 820_{OPA-3}, $350_{OPC-18,}$ 180_{OPC-18}, and 980_{OPY-5}, and no missing bands were also detected in peanut plants generated from seeds exposed to MR for 30 sec. Therefore, the irradiating effect resulted in a mutagenic frequency of 20% (6 out of the 30 bands). In contrast, three new DNA bands were detected at sequence bps of 700_{OPE-5}, 820_{OPA-3}, 420_{OPA-15}, and 2 missing bands at sequence bps of 1000_{OPA-3} and 810_{OPA-15} in peanut plants generated from seeds exposed to MR for 40 sec. Therefore, the irradiating effect resulted in a high mutagenic frequency of 21.73% (5 out of the 23 bands).

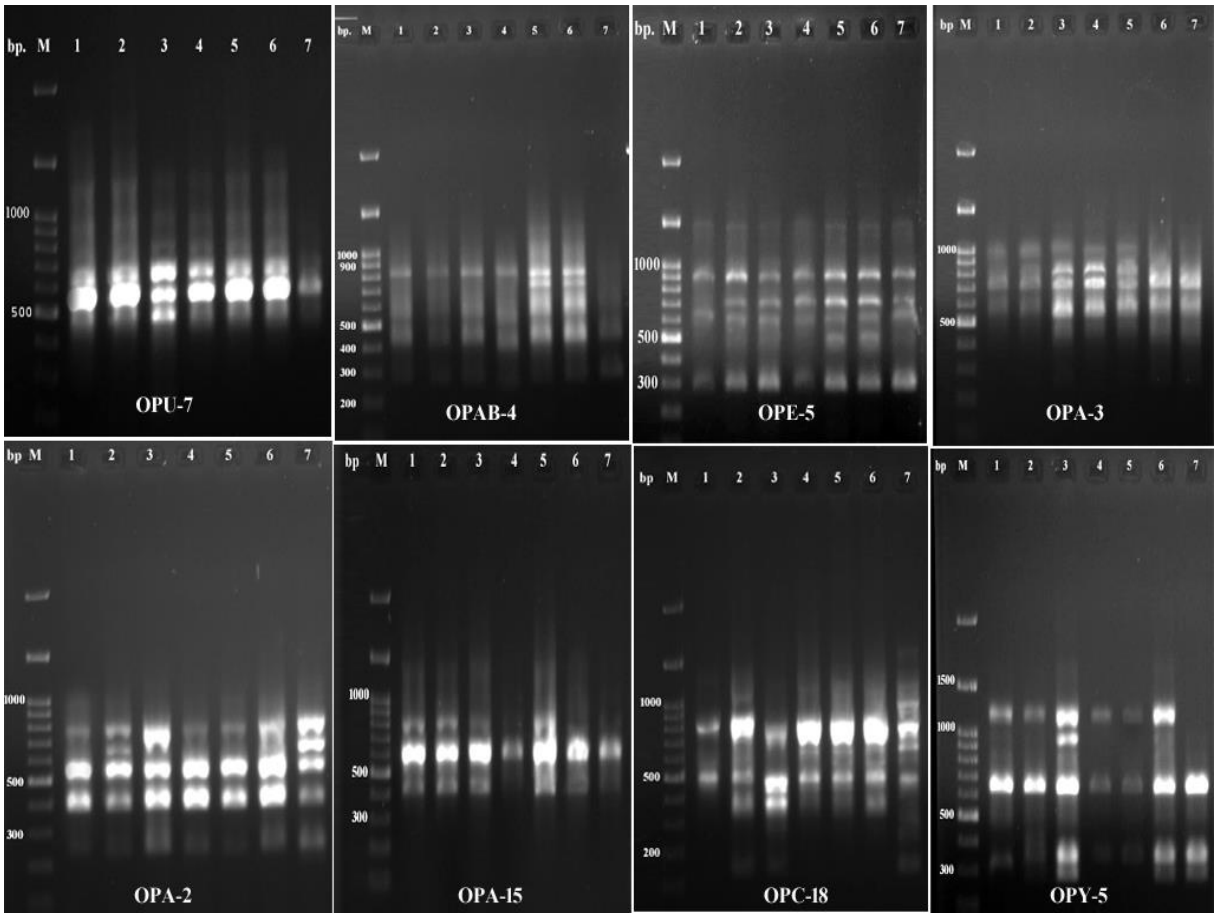

Fig. (6): Agarose-gel electrophoresis of RAPD-PCR profiles with eight primers. "M" denotes a 1000 bp ladder DNA size standard. Lanes 1- 7 show RAPD-PCR results from the peanut DNA of control plants originated from not exposed seeds to radiation (Lane 1), DNA of plants originated from seeds exposed to microwave radiation for 20 sec (Lane 2), 30 sec (Lane 3), and 40 sec (Lane 4), and X-ray radiation at 45 KeV (Lane 5), 75 KeV (Lane 6) and 95 KeV (Lane 7) for 5 sec.

Table (6): DNA bands (fragments) amplified by 8 RAPD-PCR primers of peanut plants generated from exposed seeds to microwave radiation for 0, 20, 30, and 40 seconds.

Primers	Exposure time (Seconds)									
	0	20			30			40		
	Total bands	Sequence bp of new bands	Sequence bp of missing bands	Total bands	Sequence bp of new bands	Sequence bp of missing bands	Total bands	Sequence bp of new bands	Sequence bp of missing bands	Total bands
OPU-7	2	-	-	2	450	-	3 (1)	-	-	2
OPAB-4	4	-	-	4	-	-	4	-	-	4
OPE-5	3	700	-	4 (1) *	700	-	4 (1)	700	-	4 (1)
OPA-3	3	-	-	3	820	-	4 (1)	820	1000	3 (2)
OPA-2	4	660	-	5 (1)	-	-	4	-	-	4
OPA-15	3	-	-	3	-	-	3	420	810	1 (2)
OPC-18	2	1020, 350, 180	-	5 (3)	350, 180	-	4 (2)	-	-	2
OPY-5	3	-	-	3	980	-	4 (1)	-	-	3
Total bands	24	29 (5)			30 (6)			23 (5)		
Mutagenic frequency %	0	17.24			20			21.73		

*Values between brackets are the numbers of new and missing DNA bands (fragments). – means no new or missing bands were detected.

B- Polymorphism following X-ray:

In this study, DNA profiles of peanut plants generated from seeds exposed to X-ray at doses of 45, 75, and 95 KeV for 5 sec compared to those generated from not exposed seeds (0 KeV) were obtained using 8 RAPD-PCR primers (Tables 7 and Fig. 6). Results show that four new DNA bands at sequence bps of 800_{OPAB-4}, 500_{OPE-5}, 1000_{OPC-18}, and $700_{OPE-5,}$ and only one missing band at sequence bp of 420_{OPA-15}were detected in peanut plants generated from seeds exposed to X-ray at 45 KeV for 5 sec. Therefore, the irradiating effect resulted in a mutagenic frequency of 18.5`% (5 out of the 27 bands).On the other hand, four new DNA bands at sequence bps of 800_{OPAB-4}, 500_{OPE-5}, 700_{OPE-5}, 350_{OPC-18}, and 3 missing bands at sequence bps of 1000_{OPA-3}, $810_{OPA-15,}$ and 420_{OPA-15}were also detected in peanut plants raised from seeds exposed to X-ray at a high dose of 75 KeV for 5 sec. Therefore, the irradiating effect resulted in a high % mutagenic frequency of 28% (7 out of the 25 bands). In contrast, six new DNA bands were detected at sequence bps of 700_{OPE-5}, 660_{OPA-2}, 1020_{OPC-18}, 710_{OPC-18}, 220_{OPC-18}, and $180_{OPC-18}$and 5 missing bands at sequence bps of 650_{OPU-7}, 850_{OPAB-4}, 1000_{OPA-3}, 810_{OPA-15}, and 450_{OPA-15}in peanut plants generated from seeds exposed to X-ray at a higher dose of 95 KeV for 5 sec. Therefore, the irradiating effect resulted in a high % mutagenic frequency of 50% (12 out of the 24 bands).

Table (7): DNA bands (fragments) amplified by 8 RAPD-PCR primers of peanut plants generated from exposed seeds to different doses of X-ray radiation for 5 seconds.

Primers	Exposure X-ray dose for 5 seconds									
	0 KeV	45 KeV			75 KeV			95 KeV		
	Total bands	Sequence bps of new bands	Sequence bps of missing bands	Total bands	Sequence bps of new bands	Sequence bps of missing bands	Total bands	Sequence bps of new bands	Sequence bp of missing bands	Total bands
OPU-7	2	-	-	2	-	-	2	-	650	1 (1)
OPAB-4	4	800	-	5 (1) *	800	-	5 (1)	-	850	3 (1)
OPE-5	3	500, 700	-	5 (2)	500, 700	-	5 (2)	700	-	4 (1)
OPA-3	3	820	-	4 (1)	-	1000	2 (1)	-	1000	2 (1)
OPA-2	4	-	-	4	-	-	4	660	-	5 (1)
OPA-15	3	-	420	2 (1)	-	810, 420	1 (2)	-	810 420	1 (2)
OPC-18	2	-	-	2	350	-	3 (1)	1020, 710, 220, 180	-	6 (4)
OPY-5	3	-	-	3	-	-	3	-	1300	2 (1)
Total bands	24	27 (5)			25 (7)			24 (12)		
Mutagenic frequency %	0	18.51			28			50		

*Values between brackets are the numbers of new and missing DNA bands (fragments). – means no new or missing bands were detected.

DISCUSSION

Alternative eco-friendly approaches include seed treatments using physical methods such as radiation exposure, especially by planting seeds exposed to MR and X-ray are rapidly used globally to manage many plant diseases. In this study, under greenhouse conditions planting peanut seeds exposed to MR for 30 sec and X-ray at 45 KeV for 5 sec highly decreased infection by stem rot caused by *S. rolfsii* compared with those not exposed. Additionally, planting seeds exposed to MR for 40 sec and X-ray at 75 and 95 KeV for 5 sec resulted in the least stem rot protection. These findings align with Ikram *et al*. (2010), who demonstrated that mungbean seeds irradiated with gamma rays (60-cobalt) for 4 min and stored for up to 90 days showed a significant decrease in the infection with *R. solani*, *M. phaseolina*, and *Fusarium* spp., causing root-rot diseases. Azzam *et al.*, 2007 have selected groundnut mutants for resistance against *A. flavus* infection using gamma-rays treated seeds of the local variety Giza 5. All mutants showed a very high resistance *in vitro* seed colonization by an aflatoxin-producing strain of *A. flavus*. Khalifa *et al.*, (2006) evaluated 10 mutants of peanut (selected from previously mutant generations using gamma-rays treated seeds) and their parental variety (Giza-5) against damping off and root-rot diseases caused by *R. solani*, *S. rolfsii*, *M. phaseolina*, and *F. solani* under

greenhouse and field conditions and found that mutants RT-10, RT-11 and RT-7 were the most resistant mutants; however, the parental cultivar Giza-5 was the most susceptible to these fungi. Azzam *et al*. (2007) evaluated ten groundnut mutants (RT6- RT15) that were selected from earlier mutant generations using gamma-rays treated seeds of the parental cultivar Giza-5 for their response to pod rot fungal pathogens, attack by aflatoxigenic fungi and aflatoxin contamination in greenhouse and field trails throughout the summer seasons 2004 and 2005. All groundnut mutants showed a significant reduction in the percentages of pod rot diseases, the incidence of aflatoxigenic fungi, and aflatoxin contamination compared to Giza-5 cultivar. Results showed that RT-10, RT-12, and RT-7 mutants were highly resistant against all groups of pod rot diseases. They had the lowest levels of aflatoxin B1 and/or B2 after the soil inoculation with the aflatoxigenic fungi *Aspergillus flavus* and *A. parasiticus*, individually or in a mixture in the greenhouse by which they were free from any contamination with aflatoxin in field trails compared to Giza-5 cultivar and the other tested mutants. In another study, infection with *Penicillium* spp., the cause of common bean seedling and seed death, was reduced when seeds were subjected to MR for 45 and 60sec (Tylkowska*et al.*, 2010). Also, cowpea and mungbean seed treatment with X-ray at 45 and 75 KeV for 5 and 10 sec showed complete control of root-rot fungal pathogens, *Fusarium* spp., *R. solani*, and *M. phaseolina* (Ikram *et al*., 2015). In recent studies, peanut seed treatment with gamma radiation considerably decreased microbial load and improved the protection of seed samples (Al-Bachir, 2016). Azzam and Khalifa (2016) irradiated seeds of eight groundnut varieties with five gamma-ray doses of 0, 50, 100, 200, and 300Gy, and then they evaluated them under the culture filtrate of the aflatoxigenic fungi in the Lab. The resistant R_0 plantlets were transferred to the greenhouse for the acclimatization process. The populations of R1 and R2 were evaluated under artificial infestation with the aflatoxigenic fungi *Aspergillus flavus* and *A. parasiticus*. As a result, three newly developed mutants, CMPM-2, CMPM-6, and CMPM-8, presented a highly resistant to aflatoxin contamination compared to the parental varieties. Also, chickpea, sunflower, and bean seeds were exposed to MRs for 20 and 30 sec suppressed root-rot-causing fungi *M. phaseolina*, *R. solani*, and *Fusarium* spp. (Kanwal *et al.*, 2018).

Under field conditions and natural infestation of the soil with *S. rolfsii* in each growing season of 2019 and 2020 and their mean, all peanut seed treatments with MR and X-ray before planting decreased the incidence of stem rot compared to the control of non-irradiated seeds. Stem rot disease was wholly inhibited after a year of storing peanut seeds generated from seeds treated with radiation while the control seeds were infected. Such results were also reported by Ikram *et al*. (2010). Concerning the effects of exposing peanut seeds to MR and X-ray on plant growth, yield, and seed oil content, all plant growth parameters, yield component, pod yield, and seed oil content during 2019 and 2020 growing seasons significantly increased when seeds were exposed to MR and X-ray before planting under field conditions and natural soil infestation with *S. rolfsii* compared to the control as not exposed seeds. Moreover, irradiating peanut seeds by X-ray at 45 KeV for 5 sec and microwaving for 30 sec before planting increased the pods and seeds. Also, irradiating peanut seeds by microwave for 30 sec and X-ray at 45 KeV for 5 sec significantly increased yield components (weight of 100-pods and 100-seeds) and seed oil percent compared to the control of not irradiated seeds. Therefore, our study demonstrated that seeds exposed to MR for less than a minute enhance the quality of plants. However, other treated seeds by radiation have responded differently. For instance, after being exposed to chickpea seeds for 10 sec, mashed beans for 40 and 20 sec, and sunflower seeds for 30 sec, all exposure times exhibited positive benefits (Kanwal *et al.*, 2018). Gadgil & Mitra (1983) proved that X-rays of slight-seeded (Georgia Brown) and several large-seeded lines with high variability for disease incidence led to the yielding high and disease-resistant, and pod yield, total mature kernels also increased pod weight and seed weight. It was discovered that variations in leaf part size, texture, type, and alterations have predominated over other features. For microwaves with an output power of 730 W, Azzam *et al.* (2007) reported that the mean values of pod yield m^{-2}, 100-pod weight, total sound mature kernels percentage (TSMK %), the fancy pods percentage (FP %) and the oil content of the ten peanut mutants that selected from previously mutant generations using gamma-rays treated seeds of the parent variety (Giza 5) were significantly higher compared to those of parent variety. However, the beneficial effects of microwave stimulation were reduced at greater radiation intensities.

Besides observations on plant morphometrics, a novel fused full leaflet of leaves, large pods, and seeds as mutants have appeared in peanut plants originating from seeds exposed to X-ray at 45 KeV for 5 sec. Also, healthy, and uninfected seeds by *S. rolfsii* of peanut generated from seeds exposed to X-ray at 45 KeV for 5 sec were noticed compared to those of control plants generated from seeds not exposed, which showed *S. rolfsii* rot infection after harvesting and storing at room conditions. In addition to plant mutations, an X-ray was employed to identify fused leaflets in peanut plants, although the expressivity was only 20–50% between the plants (Patil, 1966). Later, Reddy *et al.* (1977) obtained some mutants in peanuts induced by mutagens for radiation traits, including plant stature, growth habit, branching pattern, leaf, flower, sterility, stem, pod, kernel, pod yield, days to maturity, seed dormancy, oil content, and quality, resistance to diseases. The top leaflets of our mutants caused by X-ray at 45 KeV for 5 sec exhibited the same characteristics and produced miniature plants compared to the parents of not irradiated seeds. These findings agree with Mondal *et al.* (2007), who found that two mutants were detected in peanut plants generated from seeds exposed to 200 and 300 Gy of gamma rays, where the M 95 mutant was susceptible to rust but resistant to late leaf spot, whereas M 101 mutant was resistant to both diseases.

The present study also evaluated the effects of MR and X-ray on peanut DNA. The DNA was studied after an exposure time of 0, 20, 30, and 40 sec to MR. The changes in DNA bio-molecules were made by employing 8 RAPD-PCR markers to detect mutagenic changes in the genetic profile. MR affected the DNA sequence in the annealing sites of the tested primer. Similarly, a high mutagenic frequency of 21.74% (5/23 bands) was found in the more extended treatment (the 40s). Also, compared to the control treatment, peanut plants generated from seeds exposed to X-rays at 75 and 95 keV for 5 sec, reflected a high mutagenesis frequency. The most noticeable changes were the increased number of missing bands as the X-ray dose increased, and these results indicated that the effects of X-rays caused severe injuries at high doses of radiation.

In a study applied to moth bean (*Vigna aconitifolia*), Jangid *et al*. (2010) found that the RAPD-PCR analysis detected alteration in DNA sequences due to microwave treatment for 7 sec, and the frequency of mutation was 12.9% (4 bands out of 31). Such a high mutation frequency could be expected with gross chromosomal aberrations, which were produced more frequently after microwave treatments (Pavel *et al.*, 1998). In another study, several molecular markers were associated with pod rot resistance/susceptibility in the ten peanut mutants that were selected from previously mutant generations using gamma-rays treated seeds of the Giza 5 cv obtained by the RAPD primers. Moreover, the ISSRs did not show any marker (positive or negative) associated with pod rot resistance/vulnerability in the mutants and their parental variety. However, the RAPD and ISSR collective data showed that the three most closely related mutants were RT-7, RT-10, and RT-12 (Azzam *et al.*,2007). Azzam and Khalifa (2016) used ten RAPD primers and seven ISSR primers across the eight groundnut varieties and three newly advanced resistant mutants to describe the 11 genotypes genetically.

Similar outcomes were also reported by Arena *et al*. (2017), who irradiated mature tomato plants with different doses of X-ray, and they found that only 4 out of 12 primers showed apparent differences in the RAPD banding forms with 36% polymorphism (mutation rate) compared with the control. Therefore, the RAPD-PCR profiles in the present study showed the occurrence of polymorphic bands in all irradiated peanut plants with X-ray compared to the control plants, confirming the mutagenic effect induced by X-ray (Danylchenko and SoroSchinsky, 2005; Arena *et al*., 2017).

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

REFERENCES

Abdel Haliem, E.; Abdullah, H. and Al-Huqail, A.A. 2013. Oxidative damage and mutagenic potency of fast neutron and UV-B radiation in pollen mother cells and seed yield of *Vicia faba* L. BioMed Res. Int., 2013: 824656

Adu, B.; Otten, L., Afenya, E., and Groenevelt, P. 1995. Thermodynamics of microwave (polarized) heating systems. J. Microw. Power Electromagn. Energy, 30(2): 90-96.

Ahmed, H.A.M. and Abdel-Gayed M.A. 2017. Safe approach to control *Fusarium oxysporum* in sesame crop. Zag. J. Agric. Res., 44(6): 2529-2540.

Alariqi, S.A.; Kumar, A.P.; Rao, B.S.M. and Singh, R.P. 2006. Biodegradation of γ-

sterilised biomedical polyolefins under composting and fungal culture environments. Polym. Degrad. Stab., 91(5): 1105-1116.

Al-Bachir, M. 2016. Evaluation the effect of gamma irradiation on microbial, chemical and sensorial properties of peanut (*Arachis hypogaea* L.) seeds. Acta. Sci. Pol. Technol. Aliment., 15(2): 171-179.

Al-Enezi, N.A, and Al-Khayri, J.M. 2012. Alterations of DNA, ions and photosynthetic pigments content in date palm seedlings induced by X-irradiation. Int. J. Agric. Biol., 14(3) 329-336.

Al-Enezi, N.A.; Al-Bahrany, A.M. and Al-Khayri, J.M. 2012. Effect of X-irradiation on date palm seed germination and seedling growth. Emir. J. Food Agric., 24(5): 415-424

Arena, C.; De Micco,V. and De Maio, A. 2014. Growth alteration and leaf biochemical responses in *Phaseolus vulgaris* exposed to different doses of ionising radiation. Plant Biol., 16: 194-202.

Arena, C.; Turano, M.; Hay Mele, B.; Cataletto, P. R.; Furia, M.; Pugliese, M. and De Micco, V. 2017. Anatomy, photochemical activity, and DNA polymorphism in leaves of dwarf tomato irradiated with X-rays. Biol. Plant., 61(2): 305-314.

Ashri, A. 1976. Plasmon divergence in peanuts (*Arachis hypogaea*): A third plasmon and locus affecting growth habit. Theor. Appl. Genet., 48(1): 17-21.

Atienzar, F.A. and Jha, A.N. 2006. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutat. Res., 613(2-3): 76-102.

Azzam, C.R. and Khalifa, M.M.A. 2016. Peanut mutants resistant to aflatoxin induced through gamma ray and somaclonal variation and its associated genetic molecular markers. Proceedings of the IRES 26th International Conference, Paris, France, 30th January 2016.

Azzam, C.R.; Azer, S. A.; Khalifa, M. M. A. and Abol-Ela, M.F. 2007. Characterization of peanut mutants and molecular markers associated with resistance to pod rot diseases and aflatoxin contamination by RAPD and ISSR. Arab J. of Biotech., 10(2): 301-320.

Cilliers A.J.; Pretorius Z.A. and Van Wyk P.S. 2003. Integrated control of *Sclerotium rolfsii* on Groundnut in South Africa. J. Phytopath., 151(5): 249-258.

Danylchenko, O. and Sorochinsky,B. 2005. Use of RAPD assay for the detection of mutation changes in plant DNA induced by UV-B and γ-rays. BMC Plant Biol., 5(Suppl 1): S9.

De Micco, V.; Arena, C.; Pignalosa, D.and Durante, M. 2011. Effects of sparsely and densely ionizing radiation on plants. Radiat. Environ. Biophys. 50(1): 1-19.

Dhakshanamoorthy, D.; Selvaraj, R. and Chidambaram, A.L.A. 2011. Induced mutagenesis in *Jatropha curcas* L. using gamma rays and detection of DNA polymorphism through RAPD marker. C.R. Biol., 334(1): 24-30.

Domsch K.H.; Gams W. and Traute-Heidi A.W. 2007. Compendium of soil fungi. second edition, taxonomically revised by W. Gams. IHW-Verlag, Eching, 2007. 672 pp.

El-Sherif, F.; Khattab,S.; Ghoname, E.; Salem, N. and Radwan, K. 2011. Effect of gamma irradiation on enhancement of some economic traits and molecular changes in *Hibiscus sabdariffa* L. Life Sci. J., 8(3): 220-229.

Esnault, M.A.; Legue, F. and Chenal, C. 2010. Ionizing radiation: advances in plant response. Environ. Exp. Bot., 68(3):231-237.

Evans, H. J. and Scott, D. 1964. Influence of DNA synthesis on the production of chromatid aberrations by X-rays and maleic hydrazide in *Vicia faba*. Genetics, 49(1):17-38.

FAOSTAT. 2021. C2023 Rome, Italy: Food and Agriculture Organization (FAO); [2023 March 19]. http://www.fao.org/index_en.htm

Gadgil, J.D. and Mitra, R. 1983. Chemical composition of seeds in induced groundnut mutants and their derivatives. Indian J. Agric. Sci., 53(5): 295-298.

Gomez, K. and Gomez, A. 1984. Statistical procedures for agricultural research, 2nd edn. Wiley, New York, p 680.

Grondeau, C.; Samson, R. and Sands, D.C. 1994. A review of thermotherapy to free plant materials from pathogens, especially seeds from bacteria. CRC Crit. Rev. Plant Sci., 13(1): 57-75.

Hamada, E.A.M. 2007. Effects of microwave treatment on growth, photosynthetic pigments and some metabolites of wheat. Biol. Plant., 51(2): 343-345.

Ikram, N.; Dawar, S. and Imtiaz, F.2015. X-rays exposure on leguminous seeds in combination with *Aerva javanica* parts powder for the promotion of growth and management of root rot fungal pathogens. J. Plant Pathol. Microbiol, S3: 003

Ikram, N.; Dawar, S.; Abbas, Z. and Zaki, M.J. 2010. Effect of (60 cobalt) gamma rays on

growth and root rot diseases in mungbean (*Vigna radiata* L.). Pak. J. Bot., 42(3): 2165-2170.

Jangid, R.K.; Sharma, R.; Sudarsan, Y.; Eapen, S.; Singh, G. and Purohit, A.K. 2010. Microwave treatment induced mutations and altered gene expression in *Vigna aconitifolia* Biol. Plant., 54(4): 703-706.

Kalinin, L.G.; Panchenko, G.I.; Boshkova, I.L. and Kolomiichuk, S.G. 2005. Influence of low-frequency and microwave electromagnetic fields on seeds. Biophys., 50(2): 334-337.

Kanwal, S., Tariq, M. and Dawar, S. 2018. Effect of microwave radiation on plants infected with root rot pathogens. Pak. J. Bot., 50(6): 2389-2393.

Khalifa, M.M.A.; Azzam, C.R. and Azer, S.A. 2006. Biochemical markers associated with disease resistance to damping-off and root-rot diseases of peanut mutants and their productivity. Egypt. J. Phytopathol., 34(2): 53-74.

Kovalchuk, I.; Abramov, V.; Pogribny, I. and Kovalchuk, O. 2004. Molecular aspects of plant adaptation to life in the Chernobyl zone. Plant Physiol., 135(1):357-363.

Kovalchuk, I.; Molinier, J.; Yao, Y.; Arkhipov, A. and Kovalchuk, O. 2007. Transcriptome analysis reveals fundamental differences in plant response to acute and chronic exposure to ionizing radiation. Mutat. Res-Fund. Mol. M., 624(1-2): 101-113.

Kranz, A.R.; Gartenbach, K.E. and Zimmermann, M.W. 1994. Initial approach to comparative studies on the evolutionary potentials of space radiation effects in a plant system. Adv. Space Res., 14(10): 383-388.

Kumar, A.; Pushpangadan, P. and Mehrotra, S. 2003. Extraction of high-molecular-weight DNA from dry root tissue of *Berberis lycium* suitable for RAPD. Plant Mol. Biol. Rep., 21: 309a-309d.

Kumari, R. and Singh, Y. 1996. Effect of gamma rays and EMS on seed germination and plant survival of *Pisum sativum* L. and *Lens culinaris* Medic. Neo Botanica, 4(1): 25-29.

Martin, S.L.; Mooney, S.J.; Dickinson, M.J. and West, H.M. 2012. The effects of simultaneous root colonisation by three *Glomus* species on soil pore characteristics. Soil Biol. Biochem., 49: 167-173.

Martínez, E.; Carbonell, M.V. and Flórez, M. 2003. Estimulación de la germinación y el crecimientoporexposición a campos magnéticos. Investig. cienc., 324: 24-28.

Mondal, S.; Badigannavar, A.M.; Kale, D.M. and Murty, G.S.S. 2007. Induction of genetic variability in a disease resistant groundnut breeding line. BARC Newsletter, Founders Day Special issue, 285: 237-246.

Murray, M.G. and Thompson, WF. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res., 8(19): 4321-4326.

Naito, K.; Kusaba, M.; Shikazono, N.; Takano, T.; Tanaka, A.; Tanisaka, T. and Nishimura, M.2005. Transmissible and nontransmissible mutations induced by irradiating *Arabidopsis thaliana* pollen with γ-rays and carbon ions. Genetics**,**169(2): 881-889.

Nunan, N.; Ritz, K.; Rivers, M.; Feeney, D.S., and Young, I.M. 2006. Investigating microbial micro-habitat structure using X-ray computed tomography. Geoderma, 133(3-4): 398-407.

Patil, S.H. 1966. Mutations induced in groundnut by X-rays. Indian J. Genet. Plant Breed., 26A: 334-348.

Pavel, A.; Ungureanu, C.E.; Băra, I.I.; Gassner, P.and Creangă, D.E. 1998. Cytogenetic changes induced by low-intensity microwaves in the species *Triticum aestivum*. Rev. Med. Chir. Soc. Med. Nat. Iasi., 102(3-4): 89-92.

Priya, R.S.; Chinnusamy, C.; Manicaksundaram, P.; Babu, C. 2013. A review on weed management in groundnut (*Arachis hypogaea* L.). Int. J. Agri. Sci. Res., 3(1): 163-172.

Rangaswamy, G. 1972. Diseases of Crop Plants in India. Prentice Hall of India Pvt. Ltd., New Delhi, 520 pp.

Reddy, P.S.; Reddi, M.V.; Raju, B.T. and Ali, S.M. 1977. Creation of genetic variability by recourse to irradiation in groundnut (*Arachis hypogaea* L.). Oleagineux, 32(2): 59-62.

Reddy, P.; Mycock, D.J. and Berjak, P. 2000. The effect of microwave irradiation on the ultrastructure and internal fungi of soybean seed tissues. Seed Sci. Technol., 28(2): 277-289.

Roux D.; Vian, A.; Girard, S.; Bonnet, P.; Paladian, F.; Davies, E. and Ledoigt, GG .2006. Electromagnetic fields (900MHz) evoke consistent molecular responses in tomato plants. Physiol. Plant., 128(2): 283-288.

Roy, R.M.; Donini, B. and Brunori, A. 1972. Biochemical and cytological studies on developing cotyledons of *Pinus pinea* following X-irradiation of dry seeds. Radiat, Bot., 12(4): 249-260.

Rubayet, M.T. and Bhuiyan M.K.A. 2016. Integrated management of stem rot of potato caused by *Sclerotium rolfsii*. Bangladesh J. Plant Pathol., 32 (1&2): 7-14.

Sivaprakasam, K. 1994. Management of Fungal Diseases by Plant Products. In: Sivaprakasam, K. Crop Disease Innovative techniques and Management. New Delhi, Kalyani, p. 107- 111,

Spadaro, D. and Gullino, M.L. 2005. Improving the efficacy of biocontrol agents against soil-borne pathogens. Crop Prot., 24(7): 601-613.

Tracy, S.R.; Black, C.R.; Roberts, J.A.; Sturrock, C.; Mairhofer, S.; Craigon, J.and Mooney, S.J. 2012. Quantifying the impact of soil compaction on root system architecture in tomato (*Solanum lycopersicum*) by X-ray micro-computed tomography. Ann. Bot., 110(2): 511-519.

Tylkowska, K.; Turek, M. and Prieto, R.B. 2010. Health, germination and vigour of common bean seeds in relation to microwave irradiation. Phytopathol., 55: 5-12.

Watanabe, K.; Pacher, M.; Dukowic, S.; Schubert, V.; Puchta, H. and Schubert, I. 2009. The structural maintenance of chromosomes 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in *Arabidopsis thaliana*. Plant Cell, 21(9): 2688-2699.

Williams, J.G.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18(22): 6531-6535.

Yokota, Y.; Yamada, S.; Hase, Y.; Shikazono, N.; Narumi, I.; Tanaka, A. and Inoue, M. 2007. Initial yields of DNA double-strand breaks, and DNA fragmentation patterns depend on linear energy transfer in tobacco BY-2 protoplasts irradiated with helium, carbon and neon ions. Radiat. Res., 167(1): 94-101.

Yoshida, H. and Kajmoto, G. 1988. Effects of microwave treatment on the trypsin inhibitor and molecular species of triglycerides in soybeans. J. Food Sci., 53(6): 1756-1760.