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Marker-assisted Selection of New Compounds in *Moringa oleifera* Lam. Induced by Gamma Irradiation

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THE DRY seeds of the medicinal plant *Moringa oleifera* were exposed to four gamma rays doses (0, 20, 40 and 60Gy) using ^{60}Co gamma unit (Indian gamma cell), that delivered 2.25kGy per hour. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to identify the chemical profile of the ethanol extracts of non-irradiated and gamma irradiated (20, 40 and 60Gy) leaf samples of *Moringa oleifera*. New chemical compounds such as 5-hydroxymethylfurfural (5-HMF) and 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) were detected in the irradiated samples. Ten start codon-targeted (SCoT) primers were used to detect markers correlated with the appearance of 5-HMF and DDMP. Twenty four markers have been used for assessing the new compounds at the three radiation doses used. Nine of them represented positive markers that appeared in the plants treated with each of the three doses under study (20, 40 and 60Gy), but they were absent in the corresponding control. On the other hand, fifteen bands represented negative markers that appeared in non-irradiated samples and were absent in the irradiated ones. The primer SCoT 5 showed the highest percentage of polymorphism (83.3%).

Keywords : *Moringa oleifera*, Gamma rays, GC-MS, SCOT-marker.

Introduction

Since earliest times, herbs have been prized for their pain-relieving and healing abilities and even at present, people still rely largely on medicinal plants for their curative properties. According to the World Health Organization, 80% of people living in rural areas depend on medicinal herbs as primary health care systems (Sundaram et al., 2011).

Moringa species are well documented as herbs for their extraordinary nutritional and medicinal properties. *Moringa oleifera* Lam. is the most widely cultivated species of the monogenic family, Moringaceae (Fahey, 2005). These species are known as anti-helminthics antibiotics, detoxifiers, immune builders and have been used to treat malaria (Thilza et al., 2010). There are about 13 species of *Moringa* trees in the family Moringaceae. They are native to India, the Red Sea area, and/or parts of Africa, including Madagascar. Ancient Egyptians used *Moringa oleifera* oil for its cosmetic value and skin preparations (Mahmood et al., 2010). Although *Moringa oleifera* was not popular among the Greeks and Romans, they were aware of its medical properties (Fahey, 2005). *Moringa oleifera* is an interesting plant for its contribution to bioactive compounds. In particular, the leaves are the most used part of the plant (Leone et al., 2015). Many investigators have reported that the leaves are rich in phytochemical compounds; vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins, in addition to several antioxidant enzymes. However, the leaves showed a high variation in the amounts of bioactive compounds because of the plants genetic characteristics (Mukunzi et al., 2011).

Gamma radiation (high frequency electromagnetic radiation) is an important ionizing ray, as it comprises of high-energy

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photons. The high penetration property of photons causes ionization of matter in plants by indirect interaction (Vandenhove et al., 2010).

Esnault et al. (2010) showed that gamma rays modified plant growth and development, caused DNA damage, and interrupted metabolic pathways. Gamma radiation induced changes in the gas chromatography- mass spectrometry (GC-MS) profile of essential oils in plants (Shim et al., 2009 and Silva et al., 2013).

Collard & Mackill (2009) developed a novel method for generating plant DNA markers based on the short conserved region flanking the ATG start codon in plant genes. Using single 18-mer primers in a single primer polymerase chain reaction (PCR), the authors reported that start codon targeted (SCoT) markers were generally reproducible, but it was suggested to be used in conjunction with markers for applications such as genetic analysis, bulked segregant analysis, and quantitative trait loci mapping, especially in laboratories with a preference for agarose gel electrophoresis.

Gorji et al. (2011) compared three molecular marker techniques: Start codon targeted (SCT), inter-simple sequence repeats (ISSRs) and random amplified polymorphic DNA (RAPD) markers for fingerprinting of 24 varieties and a segregating population of tetraploid potato. SCoT markers were more informative, followed by ISSRs marker, as compared to other markers, for assessing varieties based on polymorphism information content. DNA markers are produced by PCR using a single primer that is designed from the short conserved region flanking the ATG start codon. Interestingly, out of 2857 scientific publications on *M. oleifera* in the primary database (Web of Science), only 12 included genetic characterization based on molecular markers. Furthermore, data of only 77 fragments of DNA and RNA sequences are available in the National Center for Biotechnology Information (NCBI) nucleotide database (Leone et al., 2015).

The objectiveof the present study is to identify the possible effects of different doses of gamma irradiation on the phytochemical constituents in the ethanol extracts of *Moringa oleifera* leaves, using GC-MS. It has been also intended to detect molecular markers associated with active ingredients.

Materials and Methods

A field experiment was conducted in a sunny greenhouse at the Natural Products Research Department, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA), Cairo, Egypt. The laboratory experiments were distributed between NCRRT, the Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt and the Central Laboratory of Pesticides, Ministry of Agriculture, Giza, Egypt.

Plant materials

The seeds of *M. oleifera* were obtained from the Desert Research Center, Cairo, Egypt. The dry seeds were exposed to four gamma rays doses (0, 20, 40 and 60Gy). Gamma irradiation was performed at the NCRRT, Cairo, Egypt. Irradiation treatments were conducted using a ^{60}Co gamma unit (Indian gamma cell), which delivered 2.25kGy per hour.

Methods

Some seeds were not irradiated and used as control. The irradiated and non-irradiated seeds were soaked in warm water for 30min to activate germination and then were sown in plastic bags (10 × 20cm) each filled with 1kg of soil. The soil was a mixture of compost and sand at a rate of 1:3 to 2:3, respectively, with three replicates for each treatment containing 25 plastic bags with one seed per bag. After 2 months, the plantlets were transferred to the field. The experiment was carried out in a completely randomized experimental design.

GC-MS analysis

Sample preparation: Five individual plants were taken from every replicate for each treatment and the control to determine the effect of gamma rays on the phytochemical profile of *M. oleifera* leaves.

Fresh leaves (10g) were macerated in 20-30ml 80% ethanol for at least 10 days at 37°C. The alcohol was clarified and the remained tissue was re-extracted with 20-30ml 80% ethanol for three times. Finally, clarified extracts were collected together and evaporated

Sample analysis: The samples were analyzed on a Hewlett-Packard model 6890 Series GC system equipped with a HP 5973 MS detector

(EI mode, 70eV). A capillary column type, HP-5 (5% phenyl dimethyl-siloxane), 30m length, inner diameter 0.25mm and a film thickness of 0.25µm was used. The column temperature was programmed to be increased after 5min from 70 to 150°C at the rate of 2°C/min and then after 5min from 150 to 250°C at the rate of 1°C/min. Helium was used as a carrier gas at a flow rate of 1ml/min. The injector and detector temperatures were 250°C and 280°C, respectively. The components in the sample extract were identified by comparing gas chromatographic retention indices (Adams 1), and mass spectra from Wiley MS Chemstation Libraries (6th ed., G 1034, Rev.C.00.00, Hewlett-Packard, Palo Alto, CA, USA).

SCoT-marker analysis

Genomic DNA extraction: DNA extraction from leaves of the first season plants was performed according to the method outlined by Graham & Henry (1997).

SCoT-marker method: Start codon targeted (SCoT) polymorphism is a simple DNA marker technique for generating gene-targeted markers in plants. Ten primers with 18-mer, ATG sequence and with GC% content between 50% and 72% were used Table 1.

TABLE 1. The sequences of the ten used SCoT primers.

SCoT primer	Sequence (5′-3′)	%GC
S5	CAACAA**ATG**GCTACCACGA	50
S7	CAACAA**ATG**GCTACCACGG	56
S10	CAACAA**ATG**GCTACCAGCC	56
S18	ACC**ATG**GCTACCACCGCC	67
S22	AACC**ATG**GCTACCACCAC	56
S26	ACC**ATG**GCTACCACCGTC	61
S31	CC**ATG**GCTACCACCGCCT	67
S32	CC**ATG**GCTACCACCGCAC	67
S34	ACC**ATG**GCTACCACCGCA	61
S35	C**ATG**GCTACCACCGGCCC	72

SCoT protocol: The amplification reactions were used in a final volume of 25µl containing the following:

Buffer 10X (2.5µl), $MgCl_2$ 25 mM (2.5µl), dNTPs 40mM (0.5µl), Taq DNA polymerase 1unit/1µL (1.0µl), Template DNA 50ng/µl (2.0µl), Primer 0.4µM (2.0µl), H_2O dd (up to 25.0µl). Reactions were performed in a DNA thermal cycler– PCR (Progene 30, Techno, Cambridge Ltd., Duxford, Cambridge, UK). The thermal cycler was programmed for three main steps as follows: At 94ºC for 3min, 35 cycles, {94ºC for 30sec, 50ºC for 1min, 72ºC for 90sec} then one cycle at 72ºC for 7min and at 4ºC (infinite).

Results

The results of ethanol extract of *M. oleifera* leaves showed a wide range of differences among radiation treatments, or within the same treatment, as compared with the control. In general, 84 chemical compounds were recognized as shown in Table 2. The compounds were classified into different groups and arranged according to their retention time (RT).

In the control sample, 29 chemical compounds were found as shown in Table 2. The compounds included alcohols, carbonyl compounds, lipids, steroids compounds, phenols, fatty acids and benzenoids. Most of the recognized compounds were classified as fatty acids (Table 2d). The compound 9, 12, 15-octadecatrienoic acid, ethyl ester (linoleic acid, ethyl ester) scored the highest ratio in *Moringa* leaf ethanol extract (29.77%). Many important compounds were recognized in the extract with a peak percentage mentioned in parentheses. These compounds represented phytol (7.14%), acetic acid (2.85%), alpha-tocopherol "Vitamin E" (2.16%) (Table 2a), beta-sitosterol (2.84%) fucosterol (2.24%) (Table 2b). Table 2d shows the occurrence of hexadecanoic acid "palmitic acid" (5.62%), decanoic acid "n-capric acid" (1.52%) and octanoic acid "caprylic acid" (2.15%) Benzenoids compounds; 1 2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (0.98%), 1, 2-benzenedicarboxylic acid, diethyl ester "Diethyl phthalate (DEP)" (2.49%) and 1, 2-benzenedicarboxylic acid, and bis (2-methylpropyl) ester "Diisobutyl phthalate (DiBP)" (2.24%) are also shown in Table 2e.

Gamma irradiation showed a difference between the irradiated and non-irradiated leaf sample extracts. As shown in Table 2c, polyphenols showed an increase in the level of the examined samples under the three used doses (20, 40, 60Gy), compared with the control. New polyphenol compounds such as 1, 2 benzenediol, benzaldehyde, 4-hydroxy (vanillin), phenol, 2-ethyl and phosphoric acid were found in some irradiated samples, but not in those of corresponding control.

TABLE 2. Phyto-components (as percentages) identified in the ethanol extracts of *Moringa oleifera* leaves non irradiated and irradiated with different doses of gamma rays, using GC-MS analysis.

a- Aliphatic alcohols and carbonyl compounds.

Compounds	Retention time	Control sample	Gamma doses								
			20 Gy			40Gy			60Gy		
			R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Aliphatic alcohols											
2-furanmethanol	4.45	----	2.36	3.68	3.83	4.94	4.55	0.20	4.32	1.98	0.47
2-propanol,1-(1-methylpropoxy)	5.54	0.45	0.53	----	----	-----	----	----	----	0.58	0.22
2,5-Dimethyl-4-hydroxy-3(2H)-furanone (Furaneol)	8.68	----	----	2.08	----	1.43	0.42	0.79	----	----	----
Diglycerol	10.19	----	----	1.36	----	0.78	----	----	----	----	----
2-aminobenzyl alcohol	13.88	----	----	0.98	----	----	----	----	----	----	1.43
Phytol	23.69	7.14	----	1.29	0.46	1.04	----	2.75	----	----	----
Neo-Inositol /allo- Inositol	24.44	----	----	------	----	------	1.06	0.14	-----	0.22	3.31
(2-Amino-cyclohexyl)-phenyl-Methanol	26.40	1.50	0.53	1.43	----	------	---	----	1.07	----	----
alpha- Tocopherol= Vitamin E	34.50	2.16	2.01	1.32	----	2.01	1.69	1.54	0.99	0.78	1.78
Total		11.25	5.43	12.14	4.29	10.2	7.72	5.42	6.38	3.56	7.21
Carbonyl compounds											
3-pyridinecarboxamide = Niacinamide or vitamin B3	16.77	2.86	2.71	1.12	1.35	----	----	1.17	0.43	0.34	1.46
Cyclopentanecarboxylic acid, hexyl ester	18.81	0.28	----	----	----	----	----	----	----	----	----
4-(3,4-Dimethoxybenzylidene)-1-(4-nitrophenyl)-3-phenyl-2-pyrazolin-5-one	28.30	5.84	0.68	----	----	----	----	----	----	3.56	----
Acetic acid	29.01	2.85	2.18	1.05	----	----	1.80	----	2.08	2.24	----
3-(4-N,N-Dimethylaminophenyl) propenoic acid, 2-(diethoxyphosphinyl)-ethyl ester	30.82	2.52	0.86	-----	----	----	----	----	----	----	-----
Total		14.35	6.43	2.17	1.35	0	1.80	1.17	2.5	6.14	1.46

Values refer to the peak area (%), R= Replications for each treatment.

b- Lipids, steroids, sulphur and nitrogen compounds.

Compounds	Retention Time	Control Sample	Gamma doses								
			20Gy			40Gy			60Gy		
			R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Lipids & Steroids compounds											
4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3- en-2-one	15.66	0.12	0.49	1.14	1.69	0.39	1.24	1.40	0.65	1.83	1.42
Trans-carveol	18.07	----	----	----	0.41	----	----	----	1.38	----	----
beta-sitosterol	37.61	2.84	0.99	1.76	1.16	----	----	1.99	2.12	----	----
Fucosterol	37.99	2.24	----	2.09	1.17	----	----	2.15	1.98	----	----
Beta.-Amyrin [[lipeds-triterpenoid]]	38.30	1.55	----	1.06	1.22	----	----	1.42	1.23	----	----
Benzene,1,2,3,4-tetramethyl1-4-(1-methylethenyl) [[lipeds-triterpenoid]]	13.34	----	0.18	0.41	0.34	----	----	0.35	----	----	0.41
Total		6.75	1.66	6.46	5.99	0.39	1.24	7.31	7.36	1.83	1.83
Sulphur & nitrogen compounds											
1,2,4-Triazin-3-amino	4.83	----	----	-----	0.64	----	----	----	----	----	---
1,2-benzene dicarbonitrile	8.72	----	----	-----	-----	----	0.93	-----	0.96	----	-----
5-amino-3-phenylpyrazole	13.48	----	----	----	----	----	----	----	0.19	----	----
2-Methylfuro (2,3)pyridine	16.58	----	----	----	----	----	----	----	0.48	1.15	2.15
2,4,5-Trimethylthiazole (azole)	17.25	----	----	----	----	4.06	----	----	----	5.92	----
Acridine/ 1H-indole,2-phenyl/Acridine, 9-methyl	18.81	----	----	0.63	0.64	0.51	----	----	0.51	1.00	----
1-benzyl-4,6-dimethoxy-2,3-diphenylindole	31.25	1.11	----	----	----	----	----	----	1.04	----	----
2-Ethylacridine	38.01	----	----	----	----	0.55	----	----	----	----	----
Total		1.11	0	0.63	1.28	5.12	0.93	0	2.91	8.07	2.15

Values refer to the peak area (%), R= Replications for each treatment.

c- Alkaloid, alkanes, ketons and phenols compounds.

Compounds	Retention time	Control sample	Gamma doses								
			20Gy			40Gy			60Gy		
			R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Alkaloid											
2-Methyl-6-propylpiperidine	5.56	----	----	----	----	----	----	----	0.94	----	----
Phenaleno[2,3-g]quinolin-7-one	29.74	0.24	0.23	----	----	----	----	----	----	0.48	----
Total		0.24	0.23	0	0	0	0	0	0.94	0.48	0
Alkanes											
Hexadecen acid	23.68	----	0.17	----	----	----	----	----	----	----	----
1-octadecene / 1- Heptadecene	19.75	0.37	----	----	----	----	----	----	----	----	----
Butane	9.74	----	----	----	----	----	----	0.15	----	----	----
Total		0.37	0.17	0	0	0	0	0.15	0	0	0
Ketons											
Ethanone, 1-(2-furanyl) =(2-Acetylfuran)	5.16	----	----	----	----	----	0.43	0.36	----	----	----
Cyclohexanone	13.9	----	----	----	----	----	----	0.81	----	----	----
3-buten-2-one,1-(2,3,6-trimethylphenyl)	17.65	----	0.75	----	----	----	1.78	1.97	0.77	2.21	----
Megastigmatrienone	17.75	----	0.22	0.49	0.44	----	----	0.61	----	----	----
Total		0	0.97	0.49	0.44	0	2.21	3.75	0.77	2.21	0
Phenols											
Phenol alchohol	6.85	1.76	----	1.15	----	----	----	0.23	0.25	2.15	----
1,2-Benzenediol	11.62	----	2.59	----	0.31	0.34	0.47	0.06	0.82		----
2-Butyn-1-one, 1-phenyl	13.76	----	----	----	----	----	----	----	3.29	3.21	----
Phenyl-1-thio-alpha-d-glucopyranoside	13.68	----	----	----	----	----	----	0.46	----	----	----
Benzaldehyde, 4-hydroxy= (vanillin)	14.73	----	----	1.87	2.09	1.20	1.69	1.55	----	1.56	1.99
1-(2,4,6-Trimethylphenyl)buta-1,3-dien	15.28	----	0.83	----	----	1.18	1.23	1.34	1.37	----	----
Phenol,3,5-bis(1,1-dimethylethyl)	16.06	0.54	----	0.46	0.38	----	----	0.38	----	----	1.11
Guaifenesin = guaiacola glycerol ether	18.62	----	3.00	----	----	----	----	----	----	----	----
Phenol, 2-ethyl	23.38	----	1.40	3.26	5.68	1.65	0.88	----	----	----	1.92
2-(6,7-Dimethoxy-1-isquinolinyl) phenol	27.87	----	0.42	----	----	----	----	----	----	----	----
Phosphoric acid…../ phenol	31.16	----	2.48	----	----	----	1.42	----	4.12	3.02	----
Total		2.40	10.72	6.74	9.08	4.37	5.69	4.02	9.85	9.94	5.02

Values refer to the peak area (%), R= Replications for each treatment.

d- Fatty acids and esters compounds.

Compounds	Retention time	Control sample	Gamma doses								
			20Gy			40Gy			60Gy		
			R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Fatty acids and esters											
2-Butenedioic acid, 2-methyl	7.15	----	0.18	----	----	0.36	0.54	----	----	----	----
2-Hexenoic acid, 2-methyl	7.86	----		----	----	0.33	1.92	1.04	0.49	----	----
Butanoic acid,3-hydroxy-ethyl ester	9.79	----	0.91	----	1.78	----	----	----	0.36	1.64	----
propanoic acid	11.51	----	----	0.26	3.64	----	----	----	----	----	----
Malic acid	14.04	----	1.36	1.57	----	----	----	----	----	----	----
9-octadecenoic acid (oleic acid)	14.73	----	0.26	0.98	0.79	----	----	----	----	0.74	----
Heptanoic acid,2-acetyl-ethyl ester	16.88	----		0.33	0.39	----	----	----	----	----	----
Decanoic acid/ n-capric acid	17.37	1.52	0.56	1.35	0.84	----	----	----	9.95	12.39	2.38
Dodecanoic	18.37	----	2.72	-----	-----		2.55		----	----	1.51
Octanoic acid / caprylic acid	19.41	2.51	0.12	----	1.40	12.66	2.87	1.01	0.90	----	12.60
Heptanoic acid	20.09	----	0.59	----	----	----	0.26	1.73	----	----	10.00
Hexadecanoic acid / palmitic acid	21.97	5.62	1.31	2.52	0.19	1.83	----	0.11	----	----	----
Hexadecanoic acid, ethyl ester	22.38	3.57	----	1.47	0.43	----	----	----	----	----	----
9,12,15-octadecatrien-1-ol	24.12	11.45	----	----	----	----	----	----	----	----	----
Tetradecanoic acid	24.21	2.5	----	----	------	------	-----	0.33	----	----	2.43
9,12,15-octadecatrienoic acid, ethyl ester =linoleic	24.47	29.77	8.64	12.86	9.75	12.0	----	1.32	5.57	7.27	----
9,12-octadecadienoic acid, methyl ester /linoleic acid	24.40	----	----	0.43	----	2.06	----	----	----	----	----
Octadecanoicacid,ethyl ester	24.56	1.58	----	0.18	----	----	----	----	----	----	----
Total		58.52	16.65	21.95	19.21	29.24	8.14	5.54	17.27	22.03	29.00

Values refer to the peak area (%), R= Replications for each treatment.

e- Benzenoids, flavonoids and furan compounds.

Compounds	Retention time	Control sample	Gamma doses								
			20Gy			40Gy			60Gy		
			R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Benzenoids											
Difluorobenzene, 1-methoxy	6.38	----	----	----	----	0.83	----	0.93	0.92	1.22	----
Benzeneacetaldehyde	7.83	----	----	0.48	0.56	----	----	----	----	----	----
2-Naphthalenol	13.78	----	----	----	----	3.02	2.04	0.46	----	----	----
Benzeneacetonitrile, 4-hydroxy	16.39	----	0.68	0.92	0.91	0.15	2.15	1.74	----	1.85	----
1,2-Benzenedicarboxylic acid, diethyl ester =Diethylphthalate (DEP)	17.25	2.49	0.17	----	----	----			----	1.53	----
Benzaldehyde, 3,4-dimethoxy (Vanillin methyl ether)	20.57	----	5.37	----	----	----	12.95	19.24	1.85	1.16	----
1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester =Diisobutyl phthalate(DiBP)	21.86	2.24	----	----	4.09	----	----	----	----	----	----
1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	28.38	0.98	----	2.17	2.25	----	----	----	----	3.30	2.42
Total		5.71	6.22	3.57	7.81	4.00	17.14	22.37	2.77	9.06	2.42
Flavonoids											
4H-pyran-4-one,2,3-Dihydro-3-5-dihydroxy-6-methy	10.47	----	20.45	22.85	14.74	13.01	11.75	11.82	10.04	20.66	13.08
Furan/aldehydes											
2-furancarboxaldehyde	3.71	----	3.92	3.17	5.00	4.97	4.94	4.96	4.35	----	----
2-furancarboxaldehyde, 5-methyl	6.12	----	1.79	----	----	1.92	2.04	2.12	0.35	----	0.91
2-furancarboxylic acid	9.68	----	-----	----	----	1.17	----	0.65	0.42	----	----
2-Furancarboxaldehyde, 5-(hydroxymethyl)/5-(Hydroxymethyl) furfural	12.33	----	25.36	19.83	31.01	25.62	36.40	30.72	34.08	17.18	37.01
Total		0	31.07	23.00	36.01	33.68	43.38	38.45	39.2	17.18	37.92

Values refers to the peak area (%), R= Replications for each treatment.

As shown in Table 2b, radiation did not show similar effects, regarding the levels of total lipids, steroid compounds, as well as sulphur and nitrogen compounds. Some samples showed increases in total ratios, while others exhibited decreases in these ratios, compared with their control. Radiation caused the appearance of some new compounds that were not detected in non-irradiated leaf extracts. However, the induced new compounds showed variable levels according to the applied dose of irradiation; e.g. trans-carveol, 2,4,5-trimethyl thiazole (azole), acridine, 1,2,4 triazin-3-amino, 1,2 benzene dicarbonitrile, 5 amino-3-phenylpyrazole and 2 -methylfuro (2,3) pyridine.

At the level of total benzenoids (Table 2e**)** radiation did not show a similar effect on the irradiated leaf extracts regarding the antimicrobial chemical compound 1, 2-benzene dicarboxylic acid, bis (2-ethylhexyl) ester.

All irradiated samples showed decreases in the total ratio of fatty acids compounds, compared with the control. The results showed asymmetrical distribution of various fatty acids between the samples. Compared with the control, many saturated and non-saturated fatty acids were found in some irradiated samples for example, 2-butenedioic acid; 2-methyl; 2-hexenoic acid; 2-methyl, butanoic acid; 3-hydroxy-ethyl ester; propanoic acid malic acid; 9-octadecenoic acid (oleic acid); heptanoic acid and heptanoic acid 2-acetyl-ethyl ester. Contrariwise, the compound 9, 12, 15-octadecatrien-1-ol that was detected in the control sample did not appear in any of the irradiated leaf samples (Table 2d).

In general, several phytochemicals (four compounds) called furan compounds appeared in response to application of the different doses of gamma irradiation, but they were not found in the control samples. A similar result was also observed for 4H-pyran-4-one, 2,3-dihydro-3-5-dihydroxy-6-methyl (DDMP) that are classified as flavonoid compounds. It is important to mention that for furans compounds, three of them showed unequal distribution among the irradiated samples, whereas 2-furancarboxaldehyde, 5-(hydroxymethyl) compound was detected in all irradiated samples (Table 2e).

Both 4H-pyran-4-one, 2,3-dihydro-3-5-dihydroxy-6-methyl and Furan carboxaldehyde, 5-(hydroxymethyl) compounds were recorded as new ones that appeared in all irradiated samples, but not present in the control. These compounds were found in high ratios, compared with other detected compounds (Table 2e).

Molecular genetic studies

SCoT markers

DNA was isolated from each of the irradiated and non-irradiated leaf samples. The DNA samples obtained from every gamma-treated leaf samples were mixed together for SCoT markers analysis to obtain a marker band that might be related to the appearance or disappearance of DNA fragment in the irradiated samples and linked strongly to the change in phytochemical compounds of the tested samples. The results of the 10 SCoT primers are shown in Fig.1 and Table 3.

The total number of bands was 103 which ranged between 6 to 13 bands using the 10 primers under investigation. Three SCoT primers; S7, S18 and S22 generated the highest number of bands (13), while S34 generated the lowest number (6). Primer S5 corresponded to the highest number of polymorphic bands (10 bands, 83.3%), while primer S34 resulted in the lowest number (1 band, 16.0%). Primers S5, S18 and S26 showed 4 markers, both of primers S22 and S35 exhibited 3 markers, both of primers S7 and S32 showed two markers, primers S31 and S34 produced only one marker, while primer S10 did not represent any marker. This means that 24 markers were present as a total, nine of them were positive markers distinctive to the three doses of 20, 40 and 60GY, while being absent in control sample. Fifteen bands were negative markers that were distinctive to the non-irradiated samples while they were absent in the irradiated samples.

According to the results shown in Table 2e, all irradiated samples were distinctive for appearance of the chemical compounds; 4H-pyran-4-one,2,3-dihydro-3-5-dihydroxy-6-methyl and Furancarboxaldehyde, 5-(hydroxymethyl), which were not present in the control sample. Therefore, the appearance and absence of the SCoT bands in the irradiated samples can be used as positive and negative markers that linked to these compounds. According to that, SCoT can be applied to differentiate between different gamma treatments and obtain markers associated with new compound production.

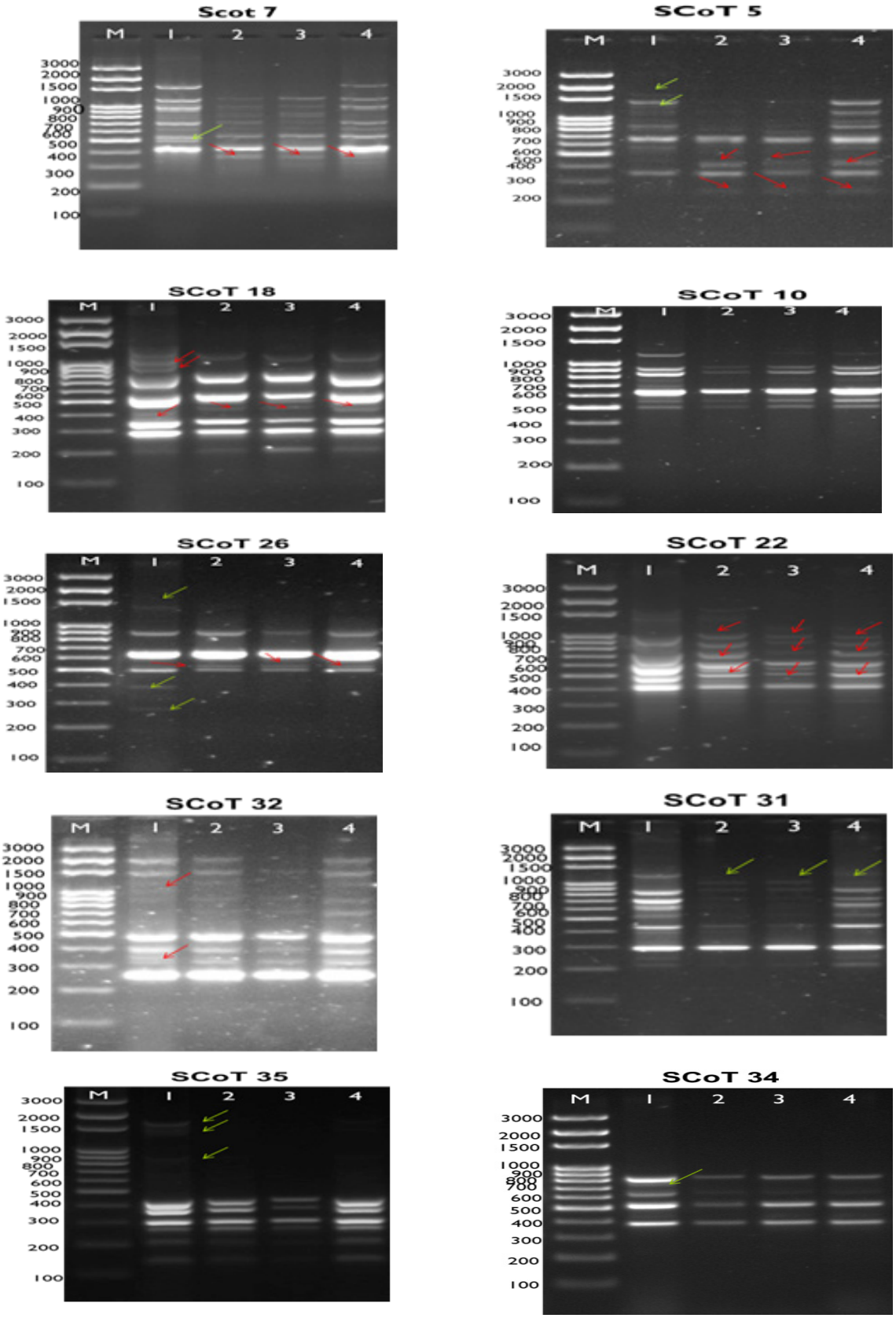

Fig. 1. Bands pattern of ScoT primer with *Moringa* leaves under four treatments; M=marker, 1=control, 2=Gy, 3=40Gy and 4=60Gy; Yellow arrow = Band present, Red arrow = Band absent.

TABLE 3. Total number of bands, polymorphic, monomorphic bands and positive and negative markers with each primer.

Primers	Total band no.	Polymorphic bands	Polymorphism (%)	Monomorphic bands	Positive markers	Negative markers	Total markers no.
S 5	12	10	83.3	2	2	2	4
S 7	13	7	53.8	6	1	1	2
S 10	8	3	37.5	5	0	0	0
S 18	13	8	61.5	5	1	3	4
S 22	13	8	61.5	5	3	0	3
S 26	7	4	57.1	3	1	3	4
S 31	11	5	45.5	6	1	0	1
S 32	11	4	36.4	7	0	2	2
S 34	6	1	16.0	5	0	1	1
S 35	9	3	33.3	6	0	3	3
Total	103	53	51.5	50	9	15	24

Discussion

The results of ethanol extraction of *Moringa oleifera* leaves showed a wide range of differences among radiation treatments, or within the same treatment as compared with the control. This was in partial agreement with the results of Bhattacharya et al. (2014) who identified hexadecanoic acid (palmitic acid), alpha-tocopherol and beta-sitosterol phytochemical compounds in ethanol extract of *Moringa* leaves of plants cultivated in India. The authors found that hexadecanoic acid was the major compound in the studied leaf extract (28.84%). Similarly, Karthika et al. (2013) identified both 1, 2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester and diethyl phthalate (DEP) in *Moringa* leaf extract, and found that the phytochemical compound identified with the maximum peak percentage area was methyl lactate (21.07%). Furthermore, the authors found that at the level of total benzenoids, the antimicrobial chemical compound (1, 2-benzene dicarboxylic acid, bis (2-ethylhexyl) ester) showed a twofold increase in four irradiated samples (two at each 20 and 60Gy), compared with the control.

The compounds 4H-pyran-4-one, 2, 3-dihydro-3-5-dihydroxy-6-methyl and Furan carboxaldehyde, 5-(hydroxymethyl) were concomitant with all irradiated samples and have been recorded in many plants. A five-carbon-ring aromatic aldehyde; 5-hydroxymethylfurfural (5-HMF) was present in coffee, dried fruits and some flavoring agents (Xu et al., 2007). In the last decades, researchers considered the 5-HMF to be hazardous to human health. Several studies have suggested that 5-HMF damages striated mucous membranes and irritates the skin. However, recent studies have shown the pharmacological effects of 5-HMF. These include antioxidant effects (Zhao et al., 2013), anti-ischemic, and anti-tyrosine enzyme effects, and positive hemorheology effects (Li et al., 2011). In addition, hepatoprotective effects were examined by Li et al. (2015) who suggested that 5-HMF hepatoprotective effects on alcohol-induced liver oxidative injury might be due to its potent antioxidant properties.

Mohamed & Khan (2013) examined the antioxidant activity of chewing stick (miswak), by using the methanol extract of miswak and GC-MS analysis, where the results detected three compounds of furan group; one of them was 2-Furancarboxaldehyde,5-(hydroxymethyl). They suggested that such furan derivatives that contained hydroxyl groups could possess antioxidant activities.

Moreover, according to Gopalakrishnan & Kalaiarasi (2013), the major phytoconstituents that were found in ethanolic extract of the fruits of *Cucumis sativus* (using GC-MS analysis) were 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (10.86 %), 2-furancarboxaldehyde, 5-(Hydroxyl methyl) (64.69 %).

The authors suggested that those active constituents might be responsible for many biological activities of cucumber such as antimicrobial, anti-inflammatory and antimicrobial preservative.

Similar effects of gamma radiation was shown on the phytochemical compounds in other medicinal plants. Thus, Seo et al. (2007) found an increase in the amount of essential

oils in the irradiated samples in *Angelica gigas* plant, compared with the non-irradiated samples. Similarly, Fanaro et al. (2012) found that gamma irradiation had a substantial influence on the profile of odor chemical compounds in oolong tea and that the dose of 10kGy caused informed more new compounds.

SCoT markers have recently become the marker of choice in case of genetic diversity studies. The diversity information generated by RAPD, SSRs or ISSRs is based on the non-coding regions of DNA, so the gained information is not directly useful. However, it is useful only when it is linked strongly to some traits, whereas, SCoT markers reveal the genetic diversity at the level of genes. Thus, these markers can help in finding new alleles in a given germplasm collection.

The appearance and absence of the SCoT bands in the irradiated samples can be, respectively used as positive and negative markers linked to these compounds. According to that, SCoT can be applied to differentiate between different gamma treatments and for obtaining markers associated with new compound production.

The simplicity and reproducibility of using SCoT markers led to their being successfully applied for the assessment of genetic diversity and taxonomic study of citrus plants (Han et al., 2011), *Dimocarpus longan* (Chen et al., 2010) and date palm cultivars (AL-Qurainy et al., 2015) and 53 *Elymus sibiricus* from distribution area in China (Zhang et al., 2015). However, Sankhla et al. (2015) reported that SCoT markers have emerged as a superior system, compared to RAPD, and Shahlaei et al. (2014) found that SCoT markers were more informative than the ISSR molecular system in the genetic diversity analysis of ten tomato varieties. In contrast, polymorphism detected by SCoT markers in the genetic diversity study of date palm cultivars (AL-Qurainy et al., 2015) was low as compared with other markers such as RAPD (Bahraminejad & Nejad, 2015) and SSR (Racchi et al., 2014).

The results of this study are in agreement with those reported by Hussein (2012) who stated that gamma irradiation resulted in appearance or disappearance of bands that can be considered as molecular markers for radiation process for *Ambrosia maritima* plant. Jyoti et al. (2009) explained the effect of gamma irradiation on DNA that gamma rays induce ionization of molecules to create free radicals that attack the DNA molecule which causes breaks in one or the two strands of DNA during sterilization.

Conclusion

Gamma radiation can be used as a mutagenic agent for forming and producing new compounds in the medicinal plants *Moringa*. The SCoT technique can be applied to confirm the effect of gamma rays treatments and obtain markers associated with new compound production.

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References

AL-Qurainy, F., Khan, S., Nadeem, M. and Tarroum, M. (2015) SCoT marker for the assessment of genetic diversity in Saudi Arabia date palm cultivars. *Pak. J. Bot.* **47**(2), 637-643.

Bahraminejad, A. and Nejad, G.M. (2015) Genetic diversity analysis of date palm (*Phoenix dactylifera* L.) genotypes using RAPD markers. *Annual Rese. Rev. Biol.* **5**(1), 41-47.

Bhattacharya, A., Ghosh, G., Agrawal, D., Sahu, P.K., Kumar, S. and Mishra, S.S. (2014) GC-MS profiling of ethanolic extract of *Moringa oleifera* leaf. *Inter. J. Pharm. Biol. Sci.* **5**(4), 263-275.

Chen, H., He, X.H., Luo, C., Zhu, J.H. and Li, F. (2010) Analysis on the genetic diversity of 24 longan (*Dimocarpus longan*) accessions by SCoT markers. *Acta Hort. Sin*. **37**(10), 1651-1654.

Collard, B.C.Y. and Mackill, D.J. (2009) Start codon targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol. Biol. Rep.* **27**, 86-93.

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

Fahey, J.W. (2005) *Moringa oleifera*: A review of the

medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees Life J.* **1**, 1-15.

Fanaro, G.B., Duarte, R.C., Santillo, A.G., Pinto e' Silva, M.E.M., Purgatto, E. and Villavicencio, A.L.C.H. (2012) Evaluation of γ-radiation on oolong tea odor volatiles. *Rad. Phys. Chem*. **81**, 1152-1156.

Gopalakrishnan, S. and Kalaiarasi, T. (2013) Determination of biologically active constituents of the fruits of *Cucumis sativus* Linn. using GC-MS analysis. *Inter. J. Biol. Pharm Res.* **4**(7), 523-527.

Gorji, A.M., Poczai, P. and Polgar, Z. (2011) Efficiency of arbitrarily amplified dominant markers (SCOT, ISSRs and RAPD) for diagnostic finger printing in tetraploidpotato. *Am. J. Pot. Res.* **88**, 226-237.

Graham, C.S. and Henry, C. (1997) Inheritance of fertility restoration of cytoplasmic male sterility in Indica rice. Rice Genetics. *Proceedings of the Int. Rice Genetics Symposium,* 27-31, pp. 649-654.

Han, G.H., Su, Q., Wang, W.S., Jia, Z.G., Hong, Q.B. and Liang, G. (2011) Establishment and application of SCoT molecular marker system for Citrus. *Acta Hort. Sin.* **38**(7), 1243-1250.

Hussein, O.S. (2012) Protein electrophoresis and DNA in herbs produced from irradiated *Ambrosia maritima* seeds grown under soil salinity and their resistance to insect. *Am. J. Plant Physiol*. **7**(6), 261-268.

Jyoti, P.M., Sukalyan, C.S.K., Subrata, P., Jiin-Shuh, J., Alok, C., Anindita, C. and Subhas, C.S. (2009) Effects of gamma irradiation on edible seed protein, amino acids and genomic DNA during sterilization. *Food Chem*. **114**, 1237-1244.

Karthika, S., Ravishankar, M., Mariajancyrani, J. and Chandramohan, G. (2013) Study on phytoconstituents from *Moringa oleifera* leaves. *Asian J. Plant Sci. Res.* **3**(4), 63-69.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristiland, J. and Bertoli, S. (2015) Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An Overview. *Int. J. Mol. Sci.* **16**, 12791-12835.

Li, M.M., Wu, L.Y., Zhao, T., Xiong, L., Huang, X., Liu, Z.H., Fan, X.L., Xiao, C.R., Gao, Y., Ma, Y.B., Chen, J.J., Zhu L.L. and Fan, M. (2011) The protective role of 5-HMF against hypoxic injury. *Cell Stress Chaperones,* **16**, 267-273.

Li, W., Qu, X., Han, Y., Zheng, S., Wang, J. and Wang, Y. (2015) Ameliorative effects of 5-hydroxymethyl-2-furfural (5-HMF) from *Schisandra chinensis* on alcoholic liver oxidative injury in mice. *Int. J. Mol. Sci.* **16**, 2446-2457.

Mahmood, K., Mugal, T. and Haq, I.U. (2010) *Moringa oleifera*: A natural gift- A review. *J. Pharm. Sci. Res.* **2**, 775-781.

Mohamed, S.A. and Khan, J.A. (2013) Antioxidant capacity of chewing stick miswak *Salvadora persica*. BMC Complement. *Alternat. Med*. **13**(40), 1-6.

Mukunzi, D., Nsor-Atindana, J., Xiaoming, Z., Gahungu, A., Karangwa, E. and Mukamurezi, G. (2011) Comparison of volatile profile of *Moringa oleifera* leaves from Rwanda and China using HS-SPME. *Pak. J. Nutr.* **10**(7), 602-608.

Racchi, M.L., Bove, A., Turchi, A., Bashir, G., Battaglia, M. and Camussi, A. (2014) Genetic characterization of Libyan date palm resources by microsatellite markers. *Biotech*. **4**, 21-32.

Sankhla, A.K., Malik, C.P. and Parashar, M. (2015) A review on start codon targeted (SCoT) marker. *J. Plant Sci. Res.* **31**(2), 153-160.

Seo, H.Y., Kim, J.H., Song, H.P., Kim, D.H., Byun, M.W., Kwon, J.H. and Kim, K.S. (2007) Effects of gamma irradiation on the yields of volatile extracts of *Angelica gigas* Nakai. *Rad. Phys. Chem.* **76,** 1869-1874.

Shahlaei, A., Torabi, S. and Khosroshahli, M. (2014) Efficiacy of SCoT and ISSR marekers in assesment of tomato (*Lycopersicum esculentum Mill*.) genetic diversity. *Int. J. Biosci*. **5**(2), 14-22.

Shim, S.L., Hwang, I.M., Ryu, K.Y., Jung, M.S., Seo, H.Y., Kim, H.Y., Song, H.P., Kim, J.H., Lee, J.W., Byun, M.W., Kwon, J.H. and Kim, K.S. (2009) Effects of γ- irradiation on the volatile compounds of medicinal herbs, *Paeoniae radix*. *Rad. Phys. Chem.* **78**, 665-669.

Silva, T.M., Miranda, R.R.S., Ferraz, V.P., Pereira, M.T., de Seiqueira, E.P. and Alcantara, A.F.C. (2013) Changes in the essential oil composition of

leaves of *Echinodorus macrophyllus* exposed to γ-radiation. *Brazil. J. Pharmacognosy*, **23**(4), 600-607.

Sundaram, S., Verma, S.K. and Dwivedi, P. (2011) *In vitro* cytotoxic activity of Indian medicinal plants used traditionally to treat cancer. *Asian J. Pharm. Clinic. Res.* **4**(1), 27-29.

Thilza, I., Sanni, S., Zakari, A., Muhammed, T. and Musa, B. (2010) *In vitro* antimicrobial activity of water extract of *Moringa oleifera* leaf stalk on bacteria normally implicated in eye disease. *Academia Arena,* **2**, 80-83.

Vandenhove, H., Vanhoudt, N., Cuypers, A., Van, H.M., Wannijn, J. and Horemans, N. (2010) Life-cycle chronic gamma exposure of *Arabidopsis thaliana* induces growth effects but no discernible effects on oxidative stress pathways. *Plant Physiol. Biochem*. **48,** 778-786.

Xu, Q., Li, Y.H. and Lv, X.Y. (2007) Investigation on influencing factors of 5-HMF content in *Schisandra*. *J. Zhejiang Univ. Sci. B*. **8**, 439-445.

Zhang, J., Xie, W., Wang, Y. and Zhao, X. (2015) Potential of start codon targeted (SCoT) markers to estimate genetic diversity and relationships among Chinese *Elymus sibiricus* accessions. *Molecules,* **20,** 5987-6001.

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z. and Chen, T. (2013) *In vitro* antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. *J. Agric. Food Chem.* **61** (44), 10604-10611.

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كاشفات إنتخابية لمركبات جديدة فى المورينجا مستحثة بالتشعيع الجامى

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تم تعريض البذور الجافة لنبات المورينجا الطبى لأربع جرعات من اشعة جاما (0، 20، 40، 60 جراى) وذلك بواسطة الوحدة Co^{60} التى تنتج 2.25 كيلو جراى/ساعه. وقد أمكن التعرف على أهم المركبات الموجوده فى مستخلص اوراق المورينجا المنماة إثر معاملة البذور باستخدام جهاز كروماتوجرافيا الغاز/ مطياف الكتلة GC-MSوذلك لكل من العينات المعامله بالإشعاع (20، 40، 60 جراى) أو غير المعاملة، وقد تبين من التحليل ظهور مركبات جديده مع جميع الجرعات المستخدمة والتى لم تكن موجوده فى عينات المقارنة (الكنترول) مثل مركب 5-ميثيل فيرفيورال (5-HMF)، ومركب 2،3 ثنائى هيدرو- 5،3- ثنائى هيدروكسى – 6- ميثيل-4H-بيوران -4-واحد (DDMP). وقد تم استخدام عشرة بادئات خاصة بتقنيه SCoT لإيجاد كاشفات مرتبطة بظهور المركبات الجديدة فى العينات المشعة وقد تم الحصول على 24 حزمة منفردة يمكن أن تستخدم ككاشفات مميزه للجرعات المستخدمة والمركبات الناتجة، تسع منها موجبه ظهرت فى النباتات المعاملة بالجرعات الثلاث المستخدمة من أشعاع جاما (20، 40، 60 جراى) ولم تظهر فى عينات المقارنة وخمس عشرة حزمة سالبه ظهرت فى العينات غير المشعة ولم تظهر فى المشعه. وظهرت أعلى نسبة من تعدد الأشكالpolymorphism مع البادئ SCoT 5 بنسبة 83.3%.