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Morphological, Phytochemical, and Molecular Studies on Gamma-irradiated *Coriandrum Sativum*

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Abstract

The aromatic herb coriander (*Coriandrum sativum* L.) has a lot of uses as a spice and has potential medical benefits. In this study, the impact of Gamma rays was evaluated on coriander plants. The results showed that 20, 40 and 60 Gy doses activated most of growth and yield characters significantly (plant height, dry weight of plant, number of branches/plants, number of umbels, weight of umbels/plant, weight of seeds/plant and weight of 100 seed/plant), with 60 Gy and 20 Gy being the best doses each one of them in some characters. Also, GC-MS analysis was performed to determine the changes in the phytochemical compounds in ethanolic leaves and seeds extract. Linalool fatty acid was the most main compound in the ethanol extract, the dose 40 Gy was the highest linalool percentage in leave extract (59.48%), while the dose 60 Gy was the greatest content of linalool in seed extract (62.41%). Moreover, some minerals (N, Na, Mg, P, K, Ca, Mn, and Fze) were determined in irradiated and non-irradiated samples for both leaves and seeds fine powder. On the other hand, the effect of radiation on genetic materials was studied using ten ISSR primers as well as nine SCoT primers which determined the changes in the genome and calculate the polymorphism in leaves samples which were exhibited (55.5%) with ISSR and (84.3%) with SCoT primers.

Key words: *Coriandrum sativum* L, gamma rays, GC-MS, EDEX, ISSR, SCoT.

Introduction

Coriander (*Coriandrum sativum* L.) is an annual plant that belonging to the *Apiaceae* family, commonly used as a spice, as well as in folk medicine, especially in Egypt **(Önder, 2018),** also used in the pharmaceutical and food industries due to medicinal properties **(Prachayasittikul *et al*., 2018).**

Coriander has a significant antioxidant activity and anticancer activity (**Nithya and Sumalatha, 2014)**. Previous studies have proven the medicinal effects of coriander plant as an anticonvulsant **(Hosseini *et al*., 2021),** it has been recommended for stimulate digestion, treatment of rheumatis **(Szemplinskiet *et al*., 2018)** as well as a valuable functional food against obesity, metabolic syndrome and diabetes **(Scandar *et al*., 2023).**

Gamma-rays are one tool used to enhance crop growth and quality, particularly when used in small doses **(El-Beltagi *et al*., 2022),** also it is a good inducer of mutation and can improve the number of bioactive compounds at a level of medicinal plants. Moreover, gamma irradiation can enhance the antimicrobial activities, antioxidant activity as well as total phenolic content of plants **(Khawory *et al*., 2020).** Hence, the possibility of utilized of gamma irradiation to create high-grade raw materials that satisfy the demands of the food and pharmaceutical industries **(Radomir, 2021).**

The mutagenic sensitivity of *Coriandrum sativum* L. seeds exposed to varying doses of gamma radiation had been studied pollen fertility, seed yield was examined, field germination rate and survivability, mitotic and meiotic anomalies, and seedling growth. Also, physiological and cytological disruptions were brought about by gamma irradiations **(Pramanik *et al*., 2018).**

DNA marker protocols mediated by PCR applications have become commonly used in plant genomic analysis that DNA markers have been very valuable in revealing the extent and distribution of variation in a diversity of crop species **(Hailu and Asfere, 2020).** Molecular markers may be dominant like RAPD, ISSR, AFLP, and SCoT-DNA or co-dominant like SSR **(Yadav and Malik, 2018).**

Start codon targeted (SCoT) polymorphism and inter simple sequence repeats (ISSR) molecular marker systems were proven to be highly reproducible, persistent, yet simple approaches for assessing the genetic fidelity of the in vitro-regenerated plantlets **(Collard and Mackill 2009; Bhattacharyya *et al*., 2023; Rai, 2023)**

ISSR markers technique is a quick, simple, and normally show excessive polymorphism, without

necessary to prior information about genomic sequence is required **(Idrees and Irshad, 2014),** high annealing temperature and longer sequence products, made it very reliable **(Yuan *et al*., 2019).**

The SCoT markers have been effectively employed to study the genetic variation and population structure of different species within the *Apiaceae* family, including *Ferula asafoetida* **(Tajbakht *et al*., 2018)**, *Foeniculum vulgare* Mill **(Ramadan *et al*., 2019),** and *Anethum graveolens* L **(Kadoglidou *et al*., 2023).**

Materials and Methods

Plant Material: In this study, the seeds of coriander (*Coriandrum sativum* L) were obtained from Research Section, Horticulture Research Institute (HRI), Agricultural Research Center (ARC, Giza, Egypt).

Gamma irradiation: The dry seeds of coriander were subjected to three different doses of gamma radiation (0, 20, 40, and 60 Gy). The National Center for Radiation Research and Technology in Nasr City (NCRRT), Cairo, Egypt, is where gamma irradiation was carried out by 60 Cobalt with dose rate 0,806 kg/h.

Field Methodology: The cultivar's seeds which were exposed to gamma irradiation, were sown along with the control seeds in sandy-loam soil. The three replications of the randomized complete block design (RCBD) were employed on the 2nd half of November 2022.

At the start of the experiment, the soil was fertilized using the recommendations provided by the Egyptian Ministry of Agriculture and Land Reclamation.

Growth and Yield Parameters: Ten plants were chosen and length of shoot (cm), dry weight of the plant (gm), number of branches per plant (branches/plant), Number of umbels per plants (umbels/plant), Weight of umbel clusters/plant (gm), Seed yield /plant (gm), and 100-seed weight (gm) were recorded.

MStat statistical analysis software: MStat version software was used to analyze the data on vegetative traits. **Gomez and Gomez (1984)** The Duncan's test, or Duncan's multiple range as per **Duncan (1955)**, was employed to confirm the variations in means

Phytochemical parameters:

GC-Mass analysis

Preparing the samples : Weight of 5 g of each fresh leaves and finely ground dry seed samples were macerated for 5 days at room temperature in 25 ml of 80% ethanol. After filtering the alcohol, the remaining tissues were extracted three more times using 25 milliliters of 80% ethanol. Finally, filtered extracts were gathered and allowed to evaporate.

Analysis of samples: The samples were processed using a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness) and a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA).

Mineral Composition: Coriander leaves and seeds fine powder were used to measure the changes of some minerals between the control and irradiated plants. The National Center for Radiation Research and Technology used the Zeiss SmartEDX energy dispersive X-ray analysis model to measure a few minerals in the samples. Iron, manganese, calcium, phosphorus, potassium, sodium, and nitrogen were among the elements that were estimated.

Molecular genetic studies

DNA extraction: The DNA was extracted from young leaves of coriander of each treatment using Cetyle Trimethyl Ammonium Bromide (CTAB) method **(Doyle and Doyle, 1990)** modified 5, which includes 2.8% CTAB; 2.5 M NaCl; 0.1 M Tris-HCl; 0,02 M EDTA; 3% mercaptoethanol; 2.5% PVP (**Sari *et al.*, 2015)**. About 0.2 g of fresh leave samples of each treatment was homogenized with 1.5 ml of prewarmed extraction buffer.

Polymerase chain reaction (PCR) and gel analysis: A set of both ISSR and SCot primers were chosen for PCR amplification. Quantity of DNA was diluted to final concentration of 10 ng/µl using nuclease free H_2O. The amplification was implemented in 25 µl of reaction mixture, using PCR Master mix (Promega, M7502, USA), 1 µl DNA 10 ng/µl, 2 µl of a primer (10 pmol/ µl), 12.5 µl of PCR master mix, and 8.4 µl nuclease free H_2O. The Reactions of this study were carried out in a thermocycler - PCR (Himedia, Prima-96 plus, Type LA1015) using the cycling parameters for ISSR and SCot primers which their sequences are represented at Table (1) and (2). PCR amplification was programmed for ISSR primers to fulfill 40 cycles after an initial denaturation cycle for 3 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 30 s, an annealing step at 41.5°C for 30 s and an elongation step at 72°C for 2 min. The primer extension segment was done for 5 min at 72°C in the final cycle. PCR amplification was programmed for SCoT primers to fulfill 36 cycles after an initial denaturation cycle for 3 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1min, an annealing step at 50.5°C for 1 min and an elongation step at 72°C for 2 min. The primer extension segment was done for 7 min at 72°C in the final cycle. The amplified products were then separated by gel electrophoresis on 1.2% agarose gel. Further, the gel was viewed under UV trans-illuminator and photographed the bands on the gel.

Molecular data analysis: Using a UV transilluminator, the ISSR and SCoT technique bands were seen, and a photo camera were taking the images. The differences in band intensity between the profiles of the different samples were not considered. The binary data generated was used to estimate the polymorphism levels by dividing the total number of scored bands by the number of polymorphic bands. Band size was estimated using the gel analyzer Ver. 23.1.1 program by comparing it to a 1 kb ladder (Cleaver Scientific Ltd.).

Table 1. Primer names, their sequences and GC% used for ISSR analysis.

Primer Name	Sequences (5'→3')	GC%
ISSR 6	CGCGATAGATAGATAGAT	38.9%
ISSR 7	GACGATAGATAGATAGATA	31.6%
ISSR 8	AGACAGACAGACAGACGC	55.6%
ISSR 10	GACAGACAGACAGACAAT	44.4%
UBC 807	AGAGAGAGAGAGAGT	47.1%
UBC 808	GAGAGAGAGAGAGAGC	52.9%
UBC 809	AGAGAGAGAGAGAGAGG	52.9%
UBC 825	ACACACACACACACACT	47.1%
UBC 826	ACACACACACACACACC	52.9%
UBC 827	ACACACACACACACACG	52.9%

Table 2. Primer names, their sequences and GC% used for SCoT analysis.

SCoT Primer	Sequence (5`-3`)	GC%
S1	CAACA**ATG**GCTACCACCA	50
S4	CAACA**ATG**GCTACCACGG	56
S15	CAACA**ATG**GCTACCAGCC	56
S20	ACC**ATG**GCTACCACCGCC	67
S25	AACC**ATG**GCTACCACCAC	56
S30	AGACAGCTAA**ATG**GGGTG	61
S33	CC**ATG**GCTACCACCGCCT	67
S40	CC**ATG**GCTACCACCGCAC	67
S40	ACC**ATG**GCTACCACCGCA	61

The results and discussion

The yield-related traits experiment

Plants for each treatment (0, 20, 40, 60 Gy doses) were taken to measure seven vegetative yield-related traits; Height/plant (cm), Dry weight/plant (gm), No of branches/plant, No of umbels/plant, Weight of umbels/plant (gm), Weight of seeds/plant, Weight of 100 seed (gm) table (3).

Vegetative yield-related traits of *Coriandrum sativum*

The irradiated plants with dose 20 Gy showed significant decrease in plant height, but the differences between other doses were insignificant compared with the control. The highest value for stem length was recorded at 60 Gy dose (99.60 cm).

Both 20 Gy and 40 Gy showed insignificant decrease in the number of branches per plant compared with the control (4.6 branches/ plant for both), while 60 Gy appeared significant increase compared with the control and other doses, the 60 Gy recorded 6.2 branches/plant.

About the number of umbels/plants, the results viewed decrease significantly in dose 40 Gy (43.80 umbels/plant) compared with other doses which have insignificant result between them. The non-irradiated plants were having the highest number of umbels (99.40 umbels/plant).

For weight of umbels/plant, the test showed that there were no remarkable different between the control and dose 60 Gy, while a significant decrease with the dose 20 Gy were recorded the best result of the weight of umbels/plant is in dose 40 Gy (11 gm).

The weight of seeds/plant showed a significant variance between all doses and the control, the best result was with 20 Gy dose (7.76 gm) while the worst result was obtained with 40 Gy dose (1.86 gm).

The weight of 100 seed recorded that there is no significant change in the results between all doses except dose 40 Gy which recorded the lowest value (0.600 gm) and the best result was obtained from the dose 20 Gy which recorded (0.782 gm).

For the dry weight of plants, the results showed that there is a significant decrease in the (40 Gy) treated plants compared with the other treatments, and significant increase in the dose 60 Gy compared with control, the compared value between the control and dose 20 Gy had insignificant increase, the highest value for this trait was showed in dose 60 Gy (22.8 gm). In general, the highest values for the seven studied traits were mostly observed at 60 Gy for (three traits), 20 Gy

Table 3. Means of the ten vegetative yield-related traits for the non-irradiated treatment (control) and three gamma irradiated treatments (20, 40, 60 Gy) of the *Coriandrum sativum***.** Different letters indicate significant variation.

	Yield parameters						
Doses	**Height**	**No of branches/ plant**	**No of umbels/plant**	**Weight of umbels/plant**	**Weight of seeds/plant**	**Weight of 100 seed**	**Dry weight**
Control	97.60^{a}	5.600^{ab}	99.40^{a}	8.600^{b}	6.120^{b}	0.7720^{a}	17.80^{bc}
20 Gy	88.00^{b}	4.600^{b}	99.20^{a}	11.00^{a}	7.760^{a}	0.7820^{a}	20.20^{ab}
40 Gy	96.40^{a}	4.600^{b}	43.80^{b}	4.740^{c}	1.860^{d}	0.6000^{b}	15.60^{c}
60 Gy	99.60^{a}	6.200^{a}	91.20^{a}	7.820^{b}	4.560^{c}	0.7540^{a}	22.00^{a}
LSD	5.174	1.076	11.54	2.355	0.8930	0.1571	5.046

Phytochemical analysis

The leaves and seeds of three selected plants were collected from each treatment addition to the control to determine the change in the phytochemical content profile individually by GC-MS technique. The findings revealed a variety of variations between the radiation treatments which applied to each cultivar or within each treatment as compared to the control. In general, the phytochemicals which recognized were displayed in Table (4) and (5).

Gamma irradiation effects on phytochemical contents of *Coriandrum sativum*

A. leaves extraction

The irradiated plants showed different changes between different doses and the control in extracted leaves sample. These changes are displayed in table (4). The hexadecanoic acid (Palmitic Acid) showed increase in three used doses compared with the control, Oleic Acid increased in two used doses 20 and 60 Gy and disappeared in 40 Gy.

Table 4. Phytochemical components (as percentage) of *Coriandrum sativum* leaves for non-irradiated treatment (control) and three gamma irradiated treatments (20, 40, 60 Gy) of the plants using GC-MS**.**

R.T	**Compound name**		**Area**			
			Control	**20 Gy**	**40 Gy**	**60Gy**
23.88	1, 2-Benzenedicarboxylic acid, butyl octyl ester **[Phthalic acid dihexyl ester]**	**lipophilic chemicals**	**2.36**	-	-	-
24.17	Z,Z-3,15-Octadecadien-1-ol acetate	**Fatty alcohol ester**	**1.79**	-	-	-
24.17	Ethanol, 2-(9-octadecenyloxy)-, (Z) **[Icosanoic acid]**	**saturated fatty acid**	-	**3.84**	-	-
24.87	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z) **[Monolinolenin]**	**monoglyceride , saturated fatty acid**	**2.18**	-	-	-
25.63	Hexadecanoic acid [**Palmitic Acid]**	**saturated fatty acid**	**20.24**	**20.98**	**25.86**	**24.03**
28.81	**Oleic Acid**	**monounsaturated omega-9 fatty acid**	**4.44**	**10.59**	-	**32.39**
29.14	**Phytol**	**Terpene**	**6.58**	-	-	-
35.81	1,2-Benzenedicarboxylic acid	**Saturated fatty acid**	**4.75**	-	-	**12.80**
42.52	9,12-Octadecadienoic acid (z,z)-, 2,3-bis[(trimethylsilyl)oxy]propyl ester **[Monolinolein TMS]**	**Unsaturated fatty acid ester**	**52.72**	**46.36**	**59.48**	**28.51**
44.73	Dasycarpidan-1-methanol, acetate (ester)	**Alkaloid**	**4.96**	**18.06**	**3.96**	**2.26**
44.72	Arabinitol, Pentaacetate	**pentose sugar alcohol**	-	-	**10.69**	-

The 9,12-octadecadienoic acid (z,z)-, 2,3- bis [(trimethylsilyl)oxy]propyl ester (monolinolein TMS) showed different level of changes when be compared the four doses with each other, the compound reached high level at 40 Gy and lowest level at 60 Gy. The alkaloid Dasycarpidan-1-methanol, acetate (ester) was recorded the highest level at 20 Gy and lowest at 60 Gy. Radiation caused disappearing of some compounds (Phthalic acid dihexyl ester; Z,Z-3,15-Octadecadien-1-ol acetate; 9,12,15-Octadecatrienoic acid; 2,3-dihydroxypropyl ester, (Z,Z,Z)-; Phytol), and appearing of some compound (Icosanoic acid appears in treatment with dose 20 Gy only. The Arabinitol, Pentaacetate which considered as pentose sugar alcohol had been recorded at dose 40 Gy only.

B. Seeds extraction

The linalool which is considered as the main compound in coriander detected at retention time 6.66 and gamma irradiation induced unsymmetrically change, linalool content increased in treatments with dose 60 Gy and decreased in the other treatments (20 and 40 Gy) compared with control. The compound named (camphor) gave the same behavior with gamma irradiation comparing with the control.

The saturated fatty acid (palmitic acid) showed different changes in used treatments and the highest level detected at dose 40 Gy, while the lowest level detected at dose 60 Gy.

The Oleic Acid which classifies as unsaturated fatty acid showed different trend, decreased in 20 Gy, increased in 40 Gy, and disappeared in 60 Gy compared with the control.

Saturated fatty acid (phthalic acid) showed decrease in the total ratio in all doses and disappeared in 20 Gy compared with the control. The lipophilic chemicals like Butyl Octyl Phthalate were disappeared in 40 Gy, and some Monosugar (Mannose) was appeared in all irradiated treatments which didn't appear in the control, The Antioxidant (Farnesol) showed in control and disappeared in irradiated treatment doses.

Terpenoid (Camphor) and Monoterpenoid (Geraniol) appeared in all doses and the control, The Anticancer (Desulphosinigrin) and Coumarin as phenol were detected at 40 Gy only.

Vardhan and Shukla (2017) suggested that increasing in the yield of secondary metabolites by ionizing radiation is due to enhancing the activity of certain key biosynthetic enzymes.

This suggestion is consistent with Moghaddam *et al*., (2011) who reported that regarding the response to gamma irradiation, PAL activity affects flavonoid synthesis in the phenylpropanoid pathway. Hence, our results can be interpreted according to **Mansour *et al.* (2018)** who found significant increase in phenols content in irradiated Moringa plants leaves that was accompanied with increasing in PAL enzyme activity in both pre and post-flowering stage.

The results obtained in our study are in good agreement with observations in earlier studies by **Mansour *et al.* (2018).**

Table 5. Phytochemical components (as percentage) of *Coriandrum sativum* seeds for for non-irradiated treatment (control) and three gamma irradiated treatments (20, 40, 60 Gy) of the plants using GC-MS

R.T	Compound name	Compound ID	Area			
			control	20 Gy	40 Gy	60 Gy
6.66	1,6-OctadieN-3-ol, 3,7-dimethyl**[Linalool]**	Unsaturated fatty acid	57.17	54.82	47.55	62.41
7.37	**Camphor**	Terpenoid	1.61	1.48	1.46	1.85
8.88	Benzene, 1-methoxy-4-(1-propenyl) **[Anethole]**	phenylpropanoid	-	3.38	4.98	3.68
13.49	2,6-Octadien-1-ol, 3,7-dimethyl **[Geraniol]**	Monoterpenoid	2.27	2.52	1.62	2.17
14.06	**Coumarin**	Phenol	-	-	7.28	-
18.81	(2Z,6E)-Farnesol **[V K]**	Antioxidant	5.91	-	-	-
18.96	**Mannose**	Monosugar	-	6.11	1.64	5.02
19.01	**Desulphosinigrin**	Glucosinolate Anticancer	-	-	3.15	-
23.87	1,2-Benzenedicarboxylic acid, butyl octyl ester **[Butyl Octyl Phthalate]**	lipophilic chemicals	4.59	4.12	-	4.85
25.73	Hexadecanoic acid **[Palmitic Acid]**	saturated fatty acid	10.08	8.61	12.64	7.70
29.61	**Oleic Acid**	Unsaturated fatty acid	6.15	5.51	7.97	-
35.80	**1,2**-Benzenedicarboxylic acid **[phthalic acid]**	Saturated fatty acid	6.86	-	5.95	5.35

Minerals content

According to the results recorded in table (6) most of the mineral percentages increased differently among the irradiated plans with different doses (0, 20, 40, 60 Gy). The most mineral percentages in coriander leaves and seeds were potassium, calcium, and nitrogen in leaves and seed's fine powder. However, the N % in the leaves decreased in the

irradiated samples comparing with the control, while increased to 20.72 in the 20 Gy seed's sample. Comparison to the control, the K % appeared increased significantly 58.19 in the 20 Gy leave's sample, but insignificantly improved to 30.05 in 60 Gy seed's sample. On the other hand, a large variation in the Ca content has been reported, it was noticed that the dose 40 Gy recorded the highest value comparing with the control in both leaves and seeds powder. The other minerals (Fe, Mn, P, Mg, and Na) were found in little amounts and followed different which were increased in most irradiated samples.

Table 6. Minerals components (as percentage) of *Coriandrum sativum leaves and* seeds for non-irradiated treatment (control) and three gamma irradiated treatments (20, 40, 60 Gy) of the plants using EDEX.

	N%	Na%	Mg%	P%	K%	Ca%	Mn%	Fe%
Coriander leaves EDEX								
Coriander control	30.34	4.25	5.70	2.91	37.99	17.05	0.81	0.96
Coriander (20Gy)	12.38	3.46	3.33	3.91	58.19	15.17	1.08	2.48
Coriander (40Gy)	22.55	4.99	6.78	3.84	34.39	24.39	1.43	1.62
Coriander (60Gy)	29.18	5.00	6.10	2.91	36.36	16.36	2.10	1.99
Coriander seeds EDEX								
Coriander control	15.49	5.34	7.40	4.79	29.88	32.20	1.21	3.69
Coriander (20Gy)	20.72	7.99	8.86	5.53	27.18	27.40	0.63	1.69
Coriander (40Gy)	0.03	9.90	13.93	10.28	28.68	34.29	1.16	1.73
Coriander (60Gy)	14.42	6.97	7.78	6.91	30.05	31.04	0.69	2.14

Molecular analysis

ISSR Primers: Ten ISSR primers were applied, photos of the produced banding patterns are shown in Figure (1). According to the mentioned results shown in Table (7). The total number of bands was 53 bands, which are arranged between 3 to 8 bands. The primer UBC 825 created the highest number of bands (8 bands), while the primer UBC 807 generated the lowest number of bands (3 bands). The highest number of polymorphic bands appeared with primer ISSR 6, ISSR 8, and UBC 825 (5 bands), While the primers ISSR 10 and UBC 807 exhibited the lowest number (1 band). The total markers were 17 markers, thirteen of them were positive markers distinctive to the gamma doses 20, 40, and 60 Gy, while the four were negative markers which are distinctive to the non-irradiated samples (control). The most positive markers obtained from ISSR8, and didn't appear with UBC 807, UBC 808, UBC 809, UBC 825, and ISSR 7. The negative markers obtained with primers ISSR 7, UBC808, UBC 809, and UBC 825. The negative markers showed with primers ISSR7, UBC 808, UBC 809, and UBC 825. The highest markers were appeared with primer ISSR 5 and didn't create with primer UBC 807.

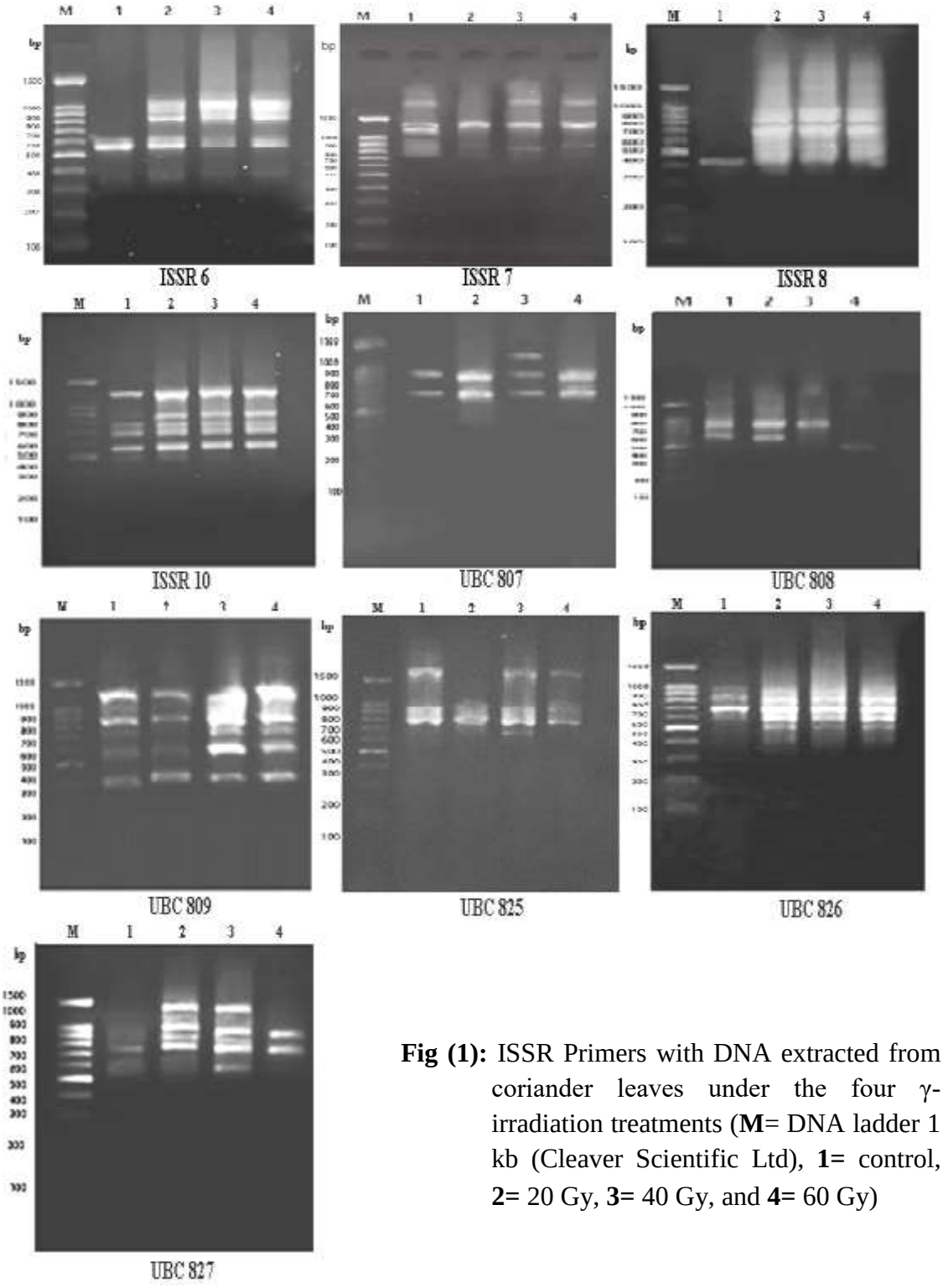

Fig (1): ISSR Primers with DNA extracted from coriander leaves under the four γ-irradiation treatments (**M**= DNA ladder 1 kb (Cleaver Scientific Ltd), **1=** control, **2=** 20 Gy, **3=** 40 Gy, and **4=** 60 Gy)

Table 7. Presence (1) and absence (0) of different bands in Coriander leaves with 10 ISSR primers under four γ-irradiation treatments. Ms=Molecular size, 1=control, 2=20 Gy, 3=40 Gy, and 4=60 Gy

Primer name	Band no.	Ms (bp)	1(Control)	2(20Gy)	3(40Gy)	4(60Gy)
	1	1054	0	1	1	1
	2	970	0	1	1	1
ISSR 6	3	896	0	0	1	1
	4	841	0	1	1	1
	5	636	0	1	0	0
	6	570	1	1	1	1
ISSR 7	1	1587	1	0	0	0
	2	910	1	1	1	0
	3	650	1	1	0	0
	4	503	0	0	0	1
ISSR 8	1	1400	0	1	1	1
	2	885	0	1	1	1
	3	690	0	1	1	1
	4	605	0	1	1	1
	5	540	0	1	1	1
	6	465	1	1	1	1
ISSR 10	1	1186	1	1	1	1
	2	855	0	1	1	1
	3	763	1	1	1	1
	4	695	1	1	1	1
UBC 807	1	1301	0	0	1	0
	2	930	1	1	1	1
	3	690	1	1	1	1
UBC 808	1	1587	1	0	0	0
	2	910	1	1	1	0
	3	650	1	1	0	0
	4	503	0	0	0	1
UBC 809	1	1220	1	1	1	1
	2	1041	1	0	0	0
	3	944	0	0	1	0
	4	830	1	1	1	1
	5	715	1	1	1	1
	6	550	1	1	1	1
	7	395	1	1	1	1
UBC 825	1	1730	1	0	1	1
	2	1041	1	0	0	0
	3	1530	1	0	1	0
	4	1000	0	1	0	1
	5	910	1	1	1	1
	6	830	1	1	1	1
	7	763	1	1	1	1
	8	666	1	0	1	0
UBC 826	1	965	1	1	1	1
	2	844	1	1	1	1
	3	685	1	1	1	1
	4	590	1	1	1	1
	5	505	0	1	1	1
	6	425	0	1	1	1
UBC 827	1	1330	0	1	1	0
	2	1023	0	1	1	1
	3	743	1	1	1	1
	4	561	1	0	1	0

Table 8. Total numbers of total bands, monomorphic, unique bands, polymorphic bands, the percentage of polymorphism and the positive and the negative markers for each ISSR primer with Coriander leave samples under four treatments (0, 20, 40, and 60 Gy).

Primers	Size range (approx. in bp)	Total bands no.	Monomorphic bands	Unique band	Polymorphic bands	Polymorphic %	Positive markers	Negative markers	Total markers
ISSR 6	1054-570	6	1	1	5	83.3%	3	0	3
ISSR 7	2100-880	4	1	0	3	75%	1	1	2
ISSR 8	1400-465	6	1	0	5	83.3%	5	0	5
ISSR 10	1186-600	5	4	0	1	20%	1	0	1
UBC 807	1301-690	3	2	1	1	33.3%	0	0	0
UBC 808	1587-503	4	0	1	4	100%	0	1	1
UBC 809	1220-395	7	5	1	2	28.5%	0	1	1
UBC 825	1730-666	8	3	1	5	62.5%	0	1	1
UBC 826	965-425	6	4	0	2	33.3%	2	0	2
UBC 827	1330-561	4	1	0	3	75%	1	0	1
Total	-	53	22	5	31	59.42%	13	4	17

SCoT Primers: Nine SCoT primers were applied, Photos of the produced banding patterns are shown in Figure (2) According to the mentioned results shown in Table (9) that represents the results of nine SCoT-DNA primers under the four treatments of Coriander leave samples (the control, 20 Gy, 40 Gy, and 60 Gy). The total number of bands was 51 bands, which are arranged between 3 to 10 bands. The primer SCoT 40 viewed the highest number of bands (10 bands), while the primers SCot 1, and SCoT 4 generated the lowest number of bands (3 bands). The highest number of polymorphic bands appeared with primer SCoT 40, while the primers SCoT 4 exhibited no polymorphic bands, the total markers were 24 markers, twelve of them was positive markers distinctive to the gamma doses 20, 40, and 60 Gy, while eleven were negative markers which are distinctive to the non-irradiated samples (the control). Primer SCoT 44 produced the highest positive markers while primer SCoT 4 didn't form any positive marker, the SCoT25, and SCoT 40 primers produced three negative markers which is distinctive to non-irradiated samples. The primer SCoT 30 produced two positive and negative markers. The primers SCoT 44 and SCoT 15 formed one negative marker, while primer SCoT 1, and SCoT 20 didn't produce any negative marker.

This work studied the effect of gamma rays on plant characteristics, the vegetative parameters have recorded an increase in some characteristics similar to **(Moussa, 2018).** The irradiation enhanced the growth characteristics of a number of economically significant plants like coriander plants specially in low doses **(Kim *et al.*, 2004), (Marcu *et al.*, 2013)** and (**Ilyas and Naz, 2014)**. Most of vegetative parameters have been significantly increased in dose 60 Gy in this study which agreed with **(Hong *et al.*, 2018)** that reported that common wheat (*Triticum aestivum* L.) growth traits were positively influenced by low gamma radiation doses. Radiation also can improve the number of bioactive compounds at a level of medicinal plants, also, gamma irradiation can enhance the antimicrobial activities, antioxidant activity as well as total phenolic content **(Khawory *et al.*, 2020).** Depending on the species and the radiation dose, the effects of gamma radiation on the phenolic profile were diverse, also the content of other phytochemical compound where change with a rise in some phytochemicals and a decrease in others **(Calucci *et al.*, 2003).**

In the exposed nine aromatic herb plants by gamma radiation. The phytochemicals percentages in leaves and seeds, noticed increased in the level of Omega 9 fatty acid, Linalool, and Monolinolein (unsaturated fatty acid) which have the same opinion of **(Hussein and Hamideldin, 2016)** and **(Ali *et al.*, 2018)**. A new compound was showed (Anethole) in irradiated samples (Anethole) compared with the control, while the antioxidant [Vitamin K] disappeared in the irradiated samples which disagreed with the results of **(Ahmed *et al.*, 2023).**

The N, K, and Ca are the most percentages of minerals found in plants samples; the dose 60 Gy showed increase in the Ca percentage. Dose 40 Gy raised up the percentage of K agreed with **(Hussein and Hamideldin, 2016),** While N percentage reduced by radiation comparing with non-irradiated plant sample.

In addition, irradiation causes high changes in the molecular level study, ISSR technique brings high levels of polymorphism, which are reproducible and beneficial for the identification of intra-genotype and inter-genotype version in many plants **(Labajová, 2013)** In this study the percentage of the polymorphism between samples using ISSR marker was 59.42% with new 5 unique bands appeared and 17 markers (10 positive markers +7 negative markers) with total bands 55 bands, these results aligned with **(Taheri *et al.*, 2013).**

Using nine SCoT markers with irradiated and non-irradiated samples, the genomic variations were observed in a polymorphism percentage about 84.3% with 7 unique bands, and 24 markers (12 positive + 11 negative) with total number of bands 51, the results of this marker were compatible with **(Essa *et al.*, 2024).**

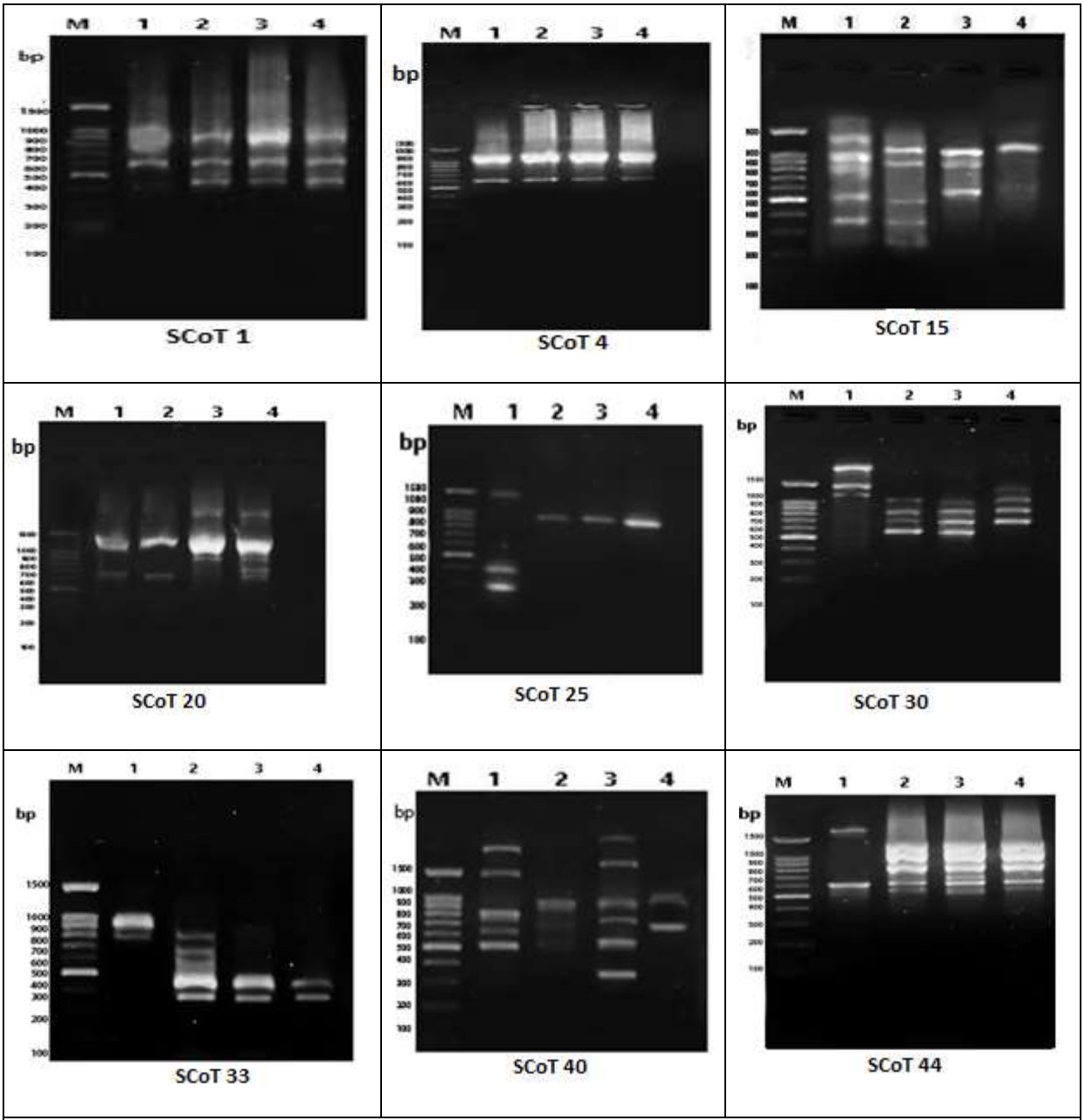

Fig (2): SCoT Primers with DNA extracted from Coriander leaves under the four γ- irradiation treatments (**M=** DNA ladder 1 kb (Cleaver Scientific Ltd), **1=** control, **2=** 20 Gy, **3=** 40 Gy, and **4=** 60 Gy)

Table 9. Presence (1) and absence (0) of different bands in Coriander leaves with 9 SCoT primers under four γ-irradiation treatments. Ms=Molecular size, 1=control, 2=20 Gy, 3=40 Gy, and 4=60 Gy

Primer name	Band no.	Ms (bp)	1(Control)	2(20Gy)	3(40Gy)	4(60Gy)
	1	915	1	1	1	1
SCoT 1	2	613	1	1	1	1
	3	440	0	1	1	1
SCoT 4	1	2275	1	1	1	1
	2	1158	1	1	1	1
	3	630	1	1	1	1
SCoT 15	1	1343	1	0	0	0
	2	1050	1	1	1	1
	3	858	1	1	1	0
	4	741	0	0	1	0
	5	510	1	1	0	0
	6	360	1	1	0	0
	7	255	1	1	0	0
SCoT 20	1	2327	0	0	1	1
	2	1178	1	1	1	1
	3	880	0	0	1	1
	4	725	0	0	0	1
	5	665	1	1	0	1
SCoT 25	1	1418	1	0	0	0
	2	967	0	1	1	1
	3	890	1	0	0	0
	4	399	1	0	0	0
SCoT 30	1	2338	1	0	0	0
	2	1439	1	0	0	0
	3	1230	1	0	0	1
	4	1025	0	1	1	1
	5	833	0	1	1	1
	6	694	0	0	1	1
	7	590	0	1	1	0
SCoT 33	1	965	1	0	0	0
	2	900	1	1	0	0
	3	857	0	1	0	0
	4	832	0	1	1	1
	5	828	0	1	1	1
SCoT 40	1	2811	0	0	1	0
	2	2367	1	0	0	0
	3	1754	0	0	1	0
	4	1074	1	0	0	0
	5	935	0	1	1	1
	6	811	1	0	0	0
	7	733	0	0	1	1
	8	687	1	1	0	1
	9	575	1	1	1	0
	10	459	0	0	1	0
SCoT 44	1	2059	1	0	0	0
	2	1195	0	1	1	1
	3	1020	0	1	1	1
	4	829	0	1	1	1
	5	725	0	0	1	0
	6	661	1	1	1	1
	7	610	0	1	1	1

Table 10. Total number of total bands, monomorphic bands, unique bands, polymorphic bands, the percentage of polymorphism and the positive and the negative markers for each SCoT primer with Coriander leave samples under four treatments (0, 20, 40, and 60 Gy).

Primers	Size range (approx. in bp)	Total bands no.	Monomorphic bands	Unique band	Polymorphic bands	Polymorphic %	Positive markers	Negative markers	Total markers
SCot 1	440-915	3	2	0	1	33.3%	1	0	1
SCoT 4	630-2275	3	3	0	0	0%	0	0	0
SCoT 15	255-1343	7	1	1	6	85.7%	1	1	2
SCoT 20	665-2327	5	1	1	4	80%	1	0	1
SCoT 25	890-1418	4	0	0	4	100%	1	3	4
SCoT 30	590-2203	7	0	0	7	100%	2	2	4
SCoT 33	828-965	5	0	1	5	100%	2	1	3
SCoT 40	459-2811	10	0	3	10	100%	1	3	4
SCoT 44	575-2059	7	1	1	6	85.7%	4	1	5
Total		51	8	7	43	84.3%	12	11	24

Conclusion

Radiation was affected on the coriander on the different ways. These differences appeared in the vegetative characters, phytochemical components, mineral contents, and molecular analysis either leaves or seeds. All doses cause changes on the yield and vegetative parameters and mineral percentages, dose 20 Gy and 60 Gy showed the best values. About the phytochemical components, it is clearly the radiation causes increase in the percentages of linalool which was recorded the highest value at 60 Gy dose. Molecular analyses in this study revealed a high degree of genetic differentiation with high polymorphism percentages among the samples under investigation Using ISSR and SCoT primers as molecular markers.

References

Ahmed, S. M. and A. B. Hassan (2023). Validation of γ-radiation and their effect on phenolic compounds, antioxidant activity, and microbial load of fennel (*Foeniculum vulgare*) seeds and cinnamon *(Cinnamomum verum*) sticks. *Food Science & Nutrition*, 11(4):1994-2001.

Ali, S.I.; G.A. El-Emary and A.A. Mohamed (2018). Effects of gamma irradiation on FT-IR fingerprint, phenolic contents and antioxidant activity of *Foeniculum vulgare* and *Carum carvi* seeds. *Research J. of Pharmacy and Technology*, 11(8): 3323-3329.

Bhattacharyya, P.; S. Kumar; L. Lalthafamkimi; R. Sharma; D. Kumar; D. Singh and S. Kumar (2023). Molecular and phytomedicinal stability of long term micro propagated Malaxis acuminata: An endangered terrestrial orchid of biopharmaceutical importance. *South African J. of Botany*, 155:372-382.

Calucci, L.; C. Pinzino; M. Zandomeneghi; A. Capocchi.; S. Ghiringhelli.; F. Saviozzi and L. Galleschi (2003). Effects of γ-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *J. of Agricultural and Food Chemistry*, 51(4): 927-934.

Collard B. C. Y. and D. J. Mackill (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

Doyle, J. (1991). DNA protocols for plants. In Molecular techniques in taxonomy. Berlin, Heidelberg: *Springer Berlin Heidelberg*, 57:283-293

El-Beltagi, H. S.; R.W. Maraei; T.A. Shalaby; A.A. Aly (2022). Metabolites, Nutritional Quality, and Antioxidant Activity of Red Radish Roots Affected by Gamma Rays. *Agronomy*, (12): 1916. 2022.

Essa, N.M.; A.A. Ibrahim and M.I. Soliman (2024). Association study between some cultivated species and their wild relatives from *Apiaceae*, *Asteraceae* and *Brassicaceae* families based on molecular and DNA barcoding in Egypt. *Genetic Resources and Crop Evolution*, 71(3):1125-1143.

Hailu, G. and Y. Asfere (2020). The role of molecular markers in crop improvement and plant breeding programs: A Review. *Agriculture J.*, 15(6):171-175.

Hong, M.J.; D.Y. Kim; J.W. Ahn; S.Y. Kang; Y.W.B. Seo and J.B. Kim (2018). Comparison of radiosensitivity response to acute and chronic gamma irradiation in colored wheat. *Genetics and molecular biology*, 41: 611- 623.

Hosseini, M.; M.H. Boskabady and M.R. Khazdair (2021). Neuroprotective effects of Coriandrum sativum and its constituent, linalool: *A review. Avicenna J. of Phytomedicine*, 11(5):436-450.

Hussein, O.S. and N. Hamideldin (2016). Influence of pre-sowing treatments by gamma rays on growth, yield and some chemical constituents of *Sesamum indicum* L. *Grasas y Aceites*, 67(1): e111-e111.

Idrees, M. and M. Irshad (2014). Molecular Markers in Plants for Analysis of Genetic Diversity. a review. *European academic research*, *2*(1):1513-1540.

Ilyas, S. and S. Naz (2014). Effect of gamma irradiation on morphological characteristics and isolation of curcuminoids and oleoresins of Curcuma longa L. JAPS: *J. of Animal & Plant Sciences*, 24(5).

Kadoglidou, K.; C. Cook; A. Boutsika; E. Sarrou; I. Mellidou; C. Aidonidou and A. Xanthopoulou (2023). Evaluation of a dill (*Anethum graveolens* L.) gene bank germplasm collection using multivariate analysis of morphological traits, molecular genotyping and chemical composition to identify novel genotypes for plant breeding. *PeerJ*, 11: e15043.

Kim, J.H.; M.H. Baek; B.Y. Chung; S.G. Wi and J.S. Kim. (2004). Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. *J. Plant Biol*., 47(4): 314-321.

Khawory, M.H.; A.A. Sain; M. A.A. Rosli; M.S. Ishak; M.I. Noordin and H.A. Wahab (2020). Effects of gamma radiation treatment on three different medicinal plants: Microbial limit test, total phenolic content, *in vitro* cytotoxicity effect and antioxidant assay. *Applied Radiation and Isotopes*, 157: 109-113.

Labajová, M. (2013). Using of inter microsatellite polymorphism to evaluate gamma-irradiated Amaranth mutants. *Emirates J. of Food and Agriculture*, 2013: 673-681.

Mansour, H.; N. Hamideldin; F. Abdel-Tawab and E. Fahmy (2018). Marker-assisted selection of new compounds in Moringa oleifera Lam. induced by gamma irradiation. *Egyptian Journal of Radiation Sciences and Applications*, 31(1):49-62.

Marcu, D.; G. Damian; C. Cosma and V. Cristea (2013). Gamma radiation effects on seed germination, growth and pigment content, and ESR study of induced free radicals in maize (*Zea mays*). *J. Biol. Phys*., 39(4): 625-634.

Moghaddam, S. S.; H. Jaafar; R. Ibrahim; A. Rahmat; M. A. Aziz and E. Philip (2011). Effects of acute gamma irradiation on physiological traits and flavonoid accumulation of *Centella asiatica*. *Molecules*, 16(6): 4994-5007.

Moussa, M.M. (2018). The effect of gamma irradiation and phosphatic fertilization on growth, yield and chemical composition of caraway plants. *Menoufia j. of plant production*, 3(6): 407-424.

Nithya, T. G. and D. Sumalatha (2014). Evaluation of in vitro anti-oxidant and anticancer activity of Coriandrum sativum against human colon cancer HT-29 cell lines. *International J. of Pharmacy and Pharmaceutical Sciences*, 6(2):421- 424.

Önder, A. (2018). Coriander and its phytoconstituents for the beneficial effects. *Potential of essential oils*, ebook, 2018 (9): 165-185

Prachayasittikul, V.; S. Prachayasittikul; S. Ruchirawat and V. Prachayasittikul (2018). Coriander (*Coriandrum sativum*): A promising functional food toward the well-being. *Food Research International*, *105*: 305-323.

Pramanik, A.; A.K. Datta; S. Gupta; B. Ghosh; D. Das; D.V. Kumbhakar and M. Hore (2018). Gamma irradiation sensitivity in *Coriandrum sativum* L.(coriander). *Cytologia*, 83(4):381-385.

Radomir, A. M. (2021). Effect of Gamma Irradiation on Bioactive Compounds of Medicinal Plants. *Scientific Papers. Series B. Horticulture*, 65(2): 292-299

Ramadan, W. A.; R.M. Shoaib; R.T. Ali and N.S. Abdel-Samea (2019). Assessment of genetic diversity among some fennel cultivars (*Foeniculum vulgare* Mill.) by ISSR and SCoT Markers. *African J. of Biological Sciences*, 15(1): 219-234.

Rai, M. K. (2023). Start codon targeted (SCoT) polymorphism marker in plant genome analysis: current status and prospects. *Planta*, 257(2): 34.

Sari, V. K. and R.H. Murti (2015). An effective method for DNA extraction of mature leaf of sapodilla (*Manilkara zapota* L.) van Royen). AGRIVITA, *J. of Agricultural Science*, 37(1): 18-23.

Scandar, S.; C. Zadra and M.C. Marcotullio (2023). Coriander (*Coriandrum sativum*) Polyphenols and Their Nutraceutical Value

against Obesity and Metabolic Syndrome. *Molecules*, 28(10): 4187.

Szemplinski, W.; J. Nowak and K.J. Jankowski (2018). Coriander (*Coriandrum sativum* L.) response to different levels of agronomic factors in Poland. *Industrial crops and products*, 122:456-464.

Taheri, S.; T.L. Abdullah; N.A.P. Abdullah and Z. Ahmad (2013). Use of intersimple sequence repeat assay for detection of DNA polymorphism induced by gamma rays in Curcuma alismatifolia. *HortScience*, 48(11): 1346-1351.

Tajbakht, P.; M. Talebi and M. Rahimmalek (2018). Genetic diversity among and within *Ferula asafoetida* H. Karst. Populations using molecular and phytochemical markers. *Phytochemistry*, 155: 19-29.

Vardhan, P. V. and L. I. Shukla (2017). Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production. *International J. of radiation biology*, 93(9): 967-979.

Yadav, C. and C. P. Malik (2018). Genetic Variation among Fennel (*Foeniculum vulgare* Mill.) Varieties on the basis of Essential Oil Composition and Molecular Markers (ISSR, SCoT, CDDP and CBDP). Indian *J.*, 7(3):126-131

Yuan, B.; L. Ke; Y. Huang; Z. Zhang; Y. Lian and D. Zhang (2019). Identification of Grifola frondosa by antagonistic test and ISSR. *Fujian J. of Agricultural Sciences*, 34(4):393-399.

دراسات مورفولوجية وفيتوكميائية وجزيئية على الكزبرة المشععة بأشعة جاما

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يعتبر نبات الكزبرة (*Coriandrum sativum* L.) عشبة عطرية لها العديد من الاستخدامات حيث تستخدم كنوع من أنواع التوابل بالإضافة الى استخداماتها في المجال الطبي. في هذه الدراسة : تم تقييم تأثير أشعة جاما على النبات وقد أظهرت النتائج أن الجرعات 20 جراي ، 40 جراي ، و 60 جراي عملت على تنشيط معظم صفات النمو وصفات المحصول بصورة كبيرة ، وهذه الصفات تضمنت (طول النبات ، الوزن الجاف ، عدد الأفرع / نبات ، عدد النورات / نبات ، وزن النورات/ نبات ، وزن البذور/ نبات ، وزن 100 بذرة) ، كما أن كلا من الجرعتين 20 و 60 جراي كانوا أفضل الجرعات حيث أظهر كلا منهما تفوقا في صفات معينة ، كما أنه تم اجراء تحليل GC–MS لتقدير التغيرات في المركبات الفيتوكيميائية الموجودة في المستخلص الايثانولي في الورقة والبذرة ، حيث تواجد الحمض الدهني اللينالول كأعلى مركب رئيسي في المستخلص الايثانولي ، كما أظهرت الجرعة 40 جراي أعلى نسبة مئوية من اللينالول في المستخلص الورقى(59.48 %) ، حينما أظهرت الجرعة 60 جراي أعلى محتوى من اللينالول في مستخلص البذور (62.48 %) . علاوة على ذلك، تم تقدير العناصر (N, Na, Mg, P, K, Ca, Mn, and Fe) في النباتات المعاملة وغير المعاملة بالاشعاع في كلا من مسحوق البذرة والورقة ، كما أنه تم دراسة تأثير الاشعاع على المادة الوراثية وذلك باستخدام بعض المعلمات الوراثية (ISSR and SCot markers) حيث تم دراسة التغييرات على المحتوى الوراثي وحساب نسبة التعدد الظاهري باستخدام عينات الأوراق حيث وصلت (55.5 %) مع معلمات ISSR ، و (84.3%) مع معلمات SCoT.