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Physical and Chemical Mutagens Induced Mutations in *Codiaeum Variegatum var.* Mollucanum under Saline Conditions

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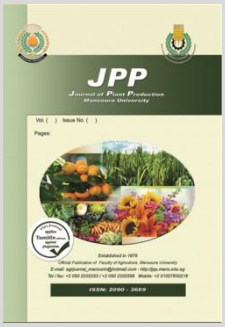

ABSTRACTS

The present study was conducted at the Experimental Farm of the Horticulture Department, Faculty of Agriculture, Moshtohor, Benha University, Egypt through the two successive seasons of 2020/2021 and 2021/2022. This investigation aimed to examine different types of mutagens on the induction of variability *Codiaeum Variegatum var* mollucanum Physical mutagen,(gamma rays at 0.0, 100, 200, and 300 grays), and two chemical mutagens [Ethyl methane sulphonate (EMS) and Dethylmethane sulphonate (DES) at 0.0, 0.01, 0.02 and 0.03%] were applied on the plant materials. The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replicates. The obtained data cleared that the physical and chemical mutagens (0.02 DEMS and EMS, and 100 or 200 Gy gamma rays) increased the plant growth compared with the higher radiation of gamma rays (300 Gy). All treatments of gamma rays at 100 or 200 Gy increased growth parameters, i.e. number of leaves/plant, plant height, and dry and fresh weight of leaves (g)/plant as compared with the control. The best treatments were obtained by gamma rays at 100 Gray.

Keywords: *Codiaeum variegatum*, Radiation, Gamma, EMS, DES

INTRODUCTION

Many breeding methods are applied to enhance plants, including selective breeding, cross, polyploid, and monadic breeding. There are some disadvantages, such as a heavy workload and a long breeding time (Li et al, 2021). Mutations are an important tool to increase genetic variability in plant breeding, which could effectively solve these drawbacks (Kishi-Kaboshi, Aida & Sasaki, 2018). Gamma rays induce damage to plants by changing DNA, promoting the production of new varieties of plants (Yamaguchi, 2018). Irradiation also causes genetic changes, cytological, and physiological in tissues, and cells which alter the plant morphology (Fan et al., 2014). *Codiaeum Variegatum var. mollucanum.* is a species of the genus *Codiaeum* which belongs to the family Euphorbiaceae. At a macroscopic level, the saline aerosol provokes visible injuries (e.g., leaf necrosis and burns), alters buds and stems structure, and reduces the whole plant growth, Although the detrimental effects of the saline aerosol on vegetation are well documented (as previously reported), there are still few experimental studies that have evaluated the tolerance/ resistance of different ornamental species to this stressor (Toscano et al., 2021).

Therefore, studying the genetic and physiological effects of gamma-ray irradiation on croton *(Codiaeum Variegatum L.)* has great importance for breeding new varieties and improving the quality of growth. Thus, this study aimed to indicate the biological effects of irradiation with gamma rays on *Codiaeum Variegatum* by determining the optimal dosage for mutation breeding and the potential fertile mutants on the morphological parameters of the plant. In addition to evaluate Diethyl sulfate (DES) and Dimethyl sulfate on the same plant.

MATERIALS AND METHODS

The present study was conducted during the two successive seasons of 2020/2021 and 2021/2022 at the Experimental Farm of the Horticulture Department, Faculty of Agriculture, Moshtohor, Benha University, Egypt.

Plant preparation:

Cuttings of *Codiaeum Variegatum var. mollucanum* plant were collected from the Experimental Farm of Horticulture department.

Analyzed at Lab. of Soil and Water, Deptartment., Moshtohor Faculty of Agriculture, Benha University according to Rainwater and Thatcher (1960).

The layout of the experiment:

The experiment was in a randomized RCPD with three replicates each replicate containing four plants.

Treatments:

Plantlets of the *Codiaeum Variegatum var. mollucanum* were irradiated before planting in farms using Indian Gamma cell 40/Date (April-77) –curies (3032)-(cesium-137) source from a unit gamma chamber at dose rate of 0 .843 rad/second (100 Gy = 7 minutes at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt . Irradiation doses were 0,0, 100, 200, 300 Gray or Ethyl sulfate and Dimethyl sulfate treatments application one percent stock solution of EMS was prepared and was used for preparing working DMS solutions at 0.1, 0.2, and 0.3% then the Plantlets were planted on 1/4/2020 and 2021 and 2021 and 2022, under the influence of four concentrations of salts 0.00, 1500, 3000 and 6000 ppm and the water used is diluted seawater. for the two summer seasons (khlifa *et al.*, 2016). The achieved experiments could be summarized as follows:

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Sampling for vegetative and chemical analysis.

Studied character:

The vegetative growth: Plant height (cm), number of leaves/plant, leaves fresh and dry weight (g), number of branches, roots length, roots fresh and dry weight.

PCR METHODS:

1. Leaf protein:

SDS-polyacrylamide gel electrophoresis was performed in 12 % acrylamide slab gels following the system of Laemmli (1970) to identify their protein profiles.

Gel preparation

The following stock solutions were prepared:

Acrylamide stock solution (30 %): prepared by dissolving 30 g (acrylamide and 0.8 g N, N, methylene bis–acrylamide) in about 70 ml distilled water

Resolving gel buffer (1.5 M Tris-HCl, pH 8.8):

The buffer was prepared by dissolving 18.15 g Tris in 50 ml distilled water and kept at 4°C.

Stacking gel buffer (0.5 M Tris-HCl, pH 6.8):

The buffer was prepared by dissolving 6.05 g Tris in 50 ml distilled water.

Sodium dodecyl sulfate (SDS 10 %, W/V):

A stock solution was prepared by dissolving 10 g SDS in 70 ml distilled water.

Ammonium persulfate solution (APS 10 % W/V):

The solution was prepared by dissolving 1.0 g ammonium persulfate in 10 ml distilled water.

Table A. Composition of separating and stacking gels:

Stock Solutions	12% separating gel	4% Stacking gel
Acrylamide	40 ml	2.6ml
Separating gel buffer	25 ml	-
Stacking gel buffer	-	5.0 ml
Distilled water	33.5 ml	12.2 ml
10 % SDS	1.0 ml	0.2 ml
10 % APS	0. 5 ml	0.1 ml
TEMED	60 µl	25 µl

Sample buffer:

This buffer was prepared by mixing the following components:

2.5 ml of 0.5 M Tris buffer (pH 6.8), 4 ml of 10 % SDS., 1 ml of 2 mercaptoethanols, 1 g of Sucrose, 1 ml Bromophenol blue (0.4 %)

Up to 10 ml of distilled water.

Extraction of leaf total proteins

Protein extraction was conducted by mixing 0.2 g of Insect tissue with an equal weight of pure, clean, sterile fine sand.

The Insect tissue were then ground to fine powder using a mortar and pestle and homogenized with 1 M Tris-HCl buffer, pH 6.8 in a clean Eppendorf tube and left in the refrigerator overnight.

Application of samples:

Control wells were loaded with standard protein marker Medium ranging from 14.20 KDa to 66.00 KDa (Fermentas.Com).

Gel running and staining:

Gels were agitated gently overnight. The composition of the staining and destaining solutions was as follows

Reagents	staining	destaining
Commassie Brilliant blue R-250	1 gm	-
Methanol	455 ml	455 ml
Glacial acetic acid	90 ml	90 ml
Distilled water	455 ml	455 ml

Gel Analysis:

Gels were photographed scanned and analyzed using the Gel Doc VILBER LOURMAT system. According to Laemmli (1970) to identify their protein profiles.

The experimental pots in the open field recommended a dose of (NPK) inorganic fertilizer according to the Egyptian Ministry of Agriculture and Land Reclamation (Reclaim). The normal agricultural practices (irrigation, ……e t c) were carried out for the experiment.

Chemical analysis:

Plant samples were used for chemical analysis as follows:

Chlorophyll A, B content in fresh leaves.

Nitrogen N, p, K, Ca, and Na content in leaves.

Determination of chlorophyll content:

Total chlorophyll content was determined in fresh leaves of plants according to Wintermans and De Mots (1965)

1)Determination of N, P, K, content:

The dry matter of leaves was ground, and 0.2 g of each sample was digested with sulphuric acid to determine the element's content (Guzman and Romero,1988)

1) Nitrogen according to the micro Kjeldahl method (A.O.A.C.,1980) using nitrogen distillation instrument model Buchi323.
2) Phosphorus was colorimetrically determined by the vanadate–molybdate-y method (Chapman and Pratt, 1961) using spectrophotometer model (spectronic 21), Potassium, calcium, and sodium were determined by using the flame photometer model (corning 410)

Statistical analysis:

The study was subjected to analysis of variance as factorial experiments in a complete randomized block design. L.S.D. a 5 % method was used to differentiate between means according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Effect of Gamma rays, EMS, and DMS on vegetative growth of *Codiaeum variegatum var. mollucanum* during two seasons 2020/2021 and 2021/2022.

1. Plant height (cm):

Ethyl methane sulphonate at 0.1% significantly increased the plant height of *Codiaeum variegatum* plant in Table (1) in both seasons, respectively. While, gamma rays at dose 100 gray gave next in this concern in both seasons, respectively. On the other hand, Ethyl methane sulphonate t 0.1% or 0.2% produced the best value in the connection in both seasons. These results were reported by Karki and Srivastava (2010). This may be because Ethyl methane sulphonate at 0.1% or 0.2% is more suitable for a growth-promoting hormone that enhances the growth of plant height Also, Sudha, (2022) found that the stimulative effect on growth may be due to the increase of cell length or cell number and size shifting in metabolism which promoted the stimulating effect of phytohormones on the biosynthesis of nucleic acids.

2. The number of leaves /p:

Data shown in Table (2) indicated that the number of leaves per plant in the first season (2020 and 2021/2021 and 2022) revealed that the gamma at 200 Gy gave the maximum number of leaves per plant as (15.81) compared to other treatments. gamma at 100 Gy or Ethyl methane sulphonate at 0.1% gave the next value in this concern. In this respect, the number of leaves per plant was 12.54 and 11.58 leaves per

plant. but the D'Ethyl methane sulphonate at 0.3% gave the minimum number of leaves per plant.

Data in the same Table (3) appear similar to those obtained in the first one. In this connection, Patil *et al.*, (2018) on *(Gladiolus hybrid)* It was found that low doses of gamma rays stimulate vegetative growth, while high doses of gamma rays inhibition of vegetative growth.

Table 1. Effect of Gamma rays, EMS, and DMS on Plant height (cm) of *Codiaeum variegatum var. mollucanum* under saline conditions during two seasons 2020/2021 and 2021/2022.

Character	Water concentration (ppm)	Tap water		1500		3000		6000	
	Seasons	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control saline water	0.00	25.0	27.0	26.0	29.0	25.0	27.0	20.0	24.0
Control without saline water	0.00	30.0	33.0	35.0	38.0	30.0	32.0	32.0	31.0
Gamma rays	100Gy	35.0	40.0	32.0	30.0	45.0	50.0	36.0	45.0
	200Gy	53.0	40.0	42.0	45.0	40.0	35.0	33.0	31.0
	300Gy	50.0	55.0	45.0	37.0	45.0	43.0	35.0	37.0
	mean	46.0	45.0	39.7	37.3	43.3	42.7	34.7	37.7
Ethyl Methane Sulphonate	0.1%	50.0	47.0	43.0	39.0	33.0	30.0	35.0	36.0
	0.2%	45.0	50.0	31.0	35.0	45.0	44.0	30.0	33.0
	0.3%	38.0	60.0	41.0	50.0	30.0	28.0	29.0	30.0
	mean	44.3	52.3	38.3	41.3	36.0	34.0	31.3	33.0
DiEthyl Methane Sulphonate	0.1%	35.0	40.0	60.0	55.0	32.0	36.0	30.0	31.0
	0.2%	33.0	34.0	42.0	41.0	47.0	42.0	33.0	31.0
	0.3%	30.0	57.0	44.0	45.0	30.0	37.0	20.0	25.0
	mean	32.7	43.7	48.7	47.0	36.3	38.3	27.7	29.0
L.S.D. at 0.5%		12.11	12.47	10.41	8.98	8.24	7.18	4.16	8.05

Table 2. Effect of gamma rays, ethyl methane sulphonate, and diethyle methane sulphonate on the number of leaves per plant of *Codiaeum variegatum var. mollucanum* under saline conditions during two seasons 2020/2021 and 2021/2022.

Character	Water concentration (ppm)	Tap water		1500		3000		6000	
	Seasons	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control saline water	0.00	10.25	11.07	9.83	11.80	8.59	9.45	7.89	8.60
Control without saline water	0.00	11.02	11.90	11.25	13.50	10.22	11.24	10.71	11.67
Ethyl Methane Sulphonate	0.10%	12.54	13.54	4.50	5.40	20.58	22.64	3.80	4.14
	0.20%	15.81	17.07	9.52	11.42	2.88	3.17	3.94	4.29
	0.30%	13.74	14.84	5.79	6.95	3.51	3.86	2.88	3.14
	mean	14.03	15.15	6.60	7.92	8.99	9.89	3.54	3.86
DiEthyl Methane Sulphonate	0.10%	11.58	12.51	6.18	7.42	6.88	7.57	3.58	3.90
	0.20%	11.28	12.18	8.99	10.79	3.99	4.39	2.58	2.81
	0.30%	10.51	11.35	6.28	7.54	5.51	6.06	3.58	3.90
	mean	11.12	12.01	7.15	8.58	5.46	6.01	3.25	3.54
Gamma rays	100Gy	9.82	10.61	7.53	9.04	4.50	4.95	4.00	4.36
	200Gy	7.85	8.48	6.47	7.76	7.22	7.94	7.58	8.26
	300Gy	5.53	5.97	5.89	7.07	6.58	7.24	2.98	3.25
	mean	7.73	8.35	6.63	7.96	6.10	6.71	4.85	5.29
L.S.D.at 0.05%		3.64	3.89	2.17	3.70	3.40	3.99	2.59	2.82

2. Phosphorus percentage

The obtained results of P% of *Codiaeum variegatum* plants are tabulated in Table (3). These data revealed that in both seasons, which was significantly increased by all tested treatments, Ethyl methane sulphonate at 0.2% treatment gave the most promising effect in increasing the percentage of P% in the first season. Diethyle methane sulphonate at 0.1% and gamma rays at 100 gray showed increasing p% in leaves and ranked second in this direction in the two seasons results agree with Hussein *et al*.,(1995) on *Datura metel.*

Table 3. Effect of Gamma rays, EMS, and DMS on Phosphorus % of *Codiaeum variegatum var. mollucanum under saline conditions* during two seasons 2020/2021 and 2021/2022.

Character	Water concentration (ppm)	Tap water		1500		3000		6000	
	Seasons	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control saline water	0.00	0.23	0.25	0.22	0.26	0.25	0.27	0.22	0.25
Control without saline water	0.00	0.21	0.24	0.21	0.25	0.22	0.26	0.20	0.23
Ethyl Methane Sulphonate	0.10%	0.33	0.36	0.31	0.33	0.29	0.32	0.21	0.24
	0.20%	0.32	0.35	0.20	0.23	0.23	0.28	0.25	0.28
	0.30%	0.31	0.35	0.28	0.31	0.32	0.35	0.32	0.35
	mean	0.32	0.35	0.26	0.29	0.28	0.32	0.26	0.29
DiEthyl Methane Sulphonate	0.10%	0.29	0.32	0.23	0.28	0.30	0.33	0.24	0.28
	0.20%	0.29	0.33	0.26	0.29	0.23	0.25	0.24	0.28
	0.30%	0.27	0.29	0.22	0.28	0.25	0.29	0.27	0.31
	mean	0.28	0.31	0.24	0.28	0.26	0.29	0.25	0.29
Gamma rays	100Gy	0.24	0.27	0.24	0.27	0.27	0.31	0.18	0.21
	200Gy	0.23	0.25	0.22	0.26	0.25	0.27	0.22	0.25
	300Gy	0.21	0.24	0.21	0.25	0.22	0.26	0.20	0.23
	mean	0.23	0.25	0.22	0.26	0.25	0.28	0.20	0.23
L.S.D.at 0.05%		0.525	0.505	0.502	0.505	0.508	0.499	0.502	0.502

3. Potassium percentage

Results of potassium percentage presented in Table (4) cleared that, Ethyl methane sulphonate at 0.2% gave the maximum percentage of K and Diethyle methane sulphonate at 1 0.1% gave the next result in leaf potassium percentage.

Table 4. Effect of Gamma rays, EMS, and DMS on Potassium% of *Codiaeum variegatum var. mollucanum under saline conditions* during two seasons 2020/2021 and 2021/2022.

Character	Water concentration (ppm)	Tap water		1500		3000		6000	
	Seasons	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control saline water	0.00	1.38	1.41	1.30	1.35	1.72	1.73	1.23	1.26
Control without saline water	0.00	1.23	1.28	1.61	1.54	1.65	1.68	1.11	1.14
Ethyl Methane Sulphonate	0.10%	1.95	1.98	1.38	1.43	1.61	1.65	1.50	1.53
	0.20%	1.84	1.88	1.61	1.65	1.42	1.49	1.34	1.37
	0.30%	1.80	1.83	1.42	1.46	1.53	1.52	1.30	1.33
	mean	1.86	1.90	1.47	1.51	1.52	1.55	1.38	1.41
DiEthyl Methane Sulphonate	0.10%	1.65	1.68	1.69	1.73	1.27	1.33	1.27	1.31
	0.20%	1.61	1.64	1.19	1.21	1.46	1.51	1.19	1.22
	0.30%	1.57	1.61	1.57	1.61	1.19	1.23	1.50	1.34
	mean	1.61	1.64	1.48	1.52	1.31	1.36	1.32	1.29
Gamma rays	100Gy	1.45	1.48	1.27	1.32	1.34	1.38	1.42	1.45
	200Gy	1.38	1.41	1.30	1.35	1.72	1.73	1.23	1.26
	300Gy	1.23	1.28	1.61	1.54	1.65	1.68	1.11	1.14
	mean	1.35	1.39	1.39	1.40	1.57	1.60	1.25	1.28
L.S.D.at 0.05%		0.798	0.798	0.752	0.759	0.793	0.800	0.793	0.791

4. Calcium%

Data shown in Table (5) indicated that, the content of Fe (mg/g D.W.) in the dry leaves of *Codiaeum variegatum* was greatly affected by gamma rays at 100 gray and Diethyle methane sulphonate at 1 0.1% treatments as compared to control in two seasons. While control plants gave the least level in this concern. Gamma rays at 200 Gy and Ethyl methane sulphonate at 0.1% gave the third level of Fe (mg/g D.W.) in the two seasons. Statistical analysis showed a significant difference between these treatments during the two seasons. These results agree with obtained by EL-Esawy (1995)

Table 5. Effect of Gamma rays, EMS, and DMS on Calsium% of *Codiaeum variegatum var. mollucanum under saline conditions* during two seasons 2020/2021 and 2021/2022.

Character	Water concentration (ppm)	Tap water		1500		3000		6000	
	Seasons	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control saline water	0.00	0.91	0.95	1.16	1.19	1.12	1.15	1.07	1.11
Control without saline water	0.00	0.79	0.82	1.12	1.15	0.87	0.92	0.95	0.99
Ethyl Methane Sulphonate	0.10%	1.36	1.40	0.87	0.91	0.95	0.98	1.08	1..11
	0.20%	1.44	1.48	1.20	1.60	1.03	1.04	1.28	1.31
	0.30%	1.12	1.16	1.28	1.31	1.32	1.35	0.99	1.02
	mean	1.31	1.35	1.12	1.27	1.10	1.12	1.12	1.17
DiEthyl Methane Sulphonate	0.10%	1.07	1.11	1.24	1.25	1.28	1.31	0.91	0.95
	0.20%	1.07	1.11	1.28	1.31	1.07	1.12	0.87	0.90
	0.30%	1.03	1.05	1.08	1.11	1.07	1.11	1.03	1.06
	mean	1.06	1.09	1.20	1.22	1.14	1.18	0.94	0.97
Gamma rays	100Gy	0.95	0.98	1.12	1.16	0.95	0.96	0.99	1.03
	200Gy	0.91	0.95	1.16	1.19	1.12	1.15	1.07	1.11
	300Gy	0.79	0.82	1.12	1.15	0.87	0.92	0.95	0.99
	mean	0.88	0.92	1.13	1.17	0.98	1.01	1.00	1.04
L.S.D.at 0.05%		0.717	0.582	0.758	0.752	0.783	0.662	0.664	0.684

5. Sodium %

The obtained results of Sodium % of *Codiaeum variegatum* plants in response to different treatments are tabulated in Table (6). These data revealed that in both seasons, gamma rays at 100 gray and Diethyle methane sulphonate at 1 0.1% gave the most promising effect in increasing sodium %in the first and second seasons.

Gamma rays at 200Gy increase Sodium % in leaves and ranked the second in this concern in the two seasons. Ethyl methane sulphonate at 0.2% gave the third value in this respect in both seasons. These results of nutrients agree with those obtained by Hussein *et al*. (1995) on *Datura metel.*

6. Chlorophyll "a" (mg/g. f w) of leaves

Data shown in Table (7) indicated that, the content of chlorophyll "a" in the fresh leaves of *Codiaeum variegatum* was greatly affected by Diethyle methane sulphonate at 1 0.1% treatment as compared to other all treatments and two control in the two seasons. while control plants gave the least level in this concern. Diethyle methane sulphonate at 2 0.1% produced the second highest level of chlorophyll "a" in both seasons. However, in both seasons treating *Codiaeum variegatum* with Diethyle methane sulphonate appeared to be the most effective treatment for increasing chlorophyll "a " when compared with all treatments and control.

7. V.B. 8. Chlorophyll "b" (mg/g. f w) of leaves

Data obtained in Table (8), it could be mentioned that the content of chlorophyll "b" Although was more effective by using Diethyle methane sulphonate at 0.1% gave the maximum level in the two seasons, while control plants gave the least level in this concern. Generally, the results of ch/ B

were similar in harmony with those obtained of ch/ A. The results agreed with Youssef (2003) proved that the percentage and content of N, as well as (chl a, b, and carotenoids) contents in leaves of fennel plants was considerably augmented as a result of Ethyl methane sulphonate treatment.

Table 6. Effect of Gamma rays, EMS, and DMS on Sodium % of *Codiaeum variegatum var. mollucanum* under saline conditions during two seasons 2020/2021 and 2021/2022.

Character	Water concentration (ppm)	Tap water		1500		3000		6000	
	Seasons	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control saline water	0.00	0.89	0.91	0.67	0.68	0.86	0.91	0.78	0.96
Control without saline water	0.00	0.82	0.85	0.82	0.84	1.04	1.07	0.93	0.74
Ethyl Methane Sulphonate	0.10%	1.16	1.19	0.78	0.83	0.74	0.77	0.97	1.02
	0.20%	1.12	1.14	1.01	1.04	0.86	0.88	0.82	0.89
	0.30%	1.12	1.14	0.89	0.93	1.01	1.05	0.93	0.85
	mean	1.13	1.16	0.89	0.93	0.87	0.90	0.91	0.92
DiEthyl Methane Sulphonate	0.10%	1.11	1.15	0.97	0.99	0.86	0.90	0.86	1.02
	0.20%	1.04	1.07	0.78	0.81	0.89	0.93	0.82	0.82
	0.30%	1.01	1.04	1.11	1.13	0.97	1.01	0.71	0.85
	mean	1.05	1.09	0.95	0.98	0.91	0.95	0.80	0.90
Gamma rays	100Gy	0.93	0.96	0.71	0.75	1.11	1.02	0.78	0.94
	200Gy	0.89	0.91	0.67	0.68	0.86	0.91	0.78	0.96
	300Gy	0.82	0.85	0.82	0.84	1.04	1.07	0.93	0.74
	mean	0.88	0.91	0.73	0.76	1.00	1.00	0.83	0.88
L.S.D.at 0.05%		0.702	0.617	0.612	0.640	0.642	0.686	0.610	0.613

Table 7. Effect of Gamma rays, EMS, and DMS on Chlorophyll "a " (mg/g. f w) of *Codiaeum variegatum var. mollucanum* under saline conditions during two seasons 2020/2021 and 2021

Character	Water concentration (ppm)	Tap water		1500		3000		6000	
	Seasons	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control saline water	0.00	0.310	0.340	0.170	0.210	0.090	0.120	0.130	0.160
Control without saline water	0.00	0.330	0.360	0.340	0.380	0.040	0.080	0.190	0.220
Ethyl Methane Sulphonate	0.10%	0.040	0.070	0.080	0.110	0.070	0.090	0.200	0.240
	0.20%	0.600	0.090	0.170	0.210	0.250	0.280	0.200	0.230
	0.30%	0.500	0.070	0.240	0.270	0.120	0.150	0.160	0.190
	mean	0.380	0.077	0.163	0.197	0.147	0.173	0.187	0.220
DiEthyl Methane Sulphonate	0.10%	0.260	0.300	0.320	0.350	0.140	0.170	0.150	0.180
	0.20%	0.110	0.140	0.250	0.290	0.090	0.130	0.260	0.290
	0.30%	0.070	0.090	0.230	0.260	0.020	0.060	0.190	0.210
	mean	0.147	0.177	0.267	0.300	0.083	0.120	0.200	0.227
Gamma rays	100Gy	0.060	0.080	0.040	0.070	0.060	0.080	0.150	0.180
	200Gy	0.060	0.090	0.170	0.210	0.090	0.120	0.130	0.160
	300Gy	0.030	0.050	0.340	0.380	0.040	0.080	0.190	0.220
	mean	0.050	0.073	0.183	0.220	0.063	0.093	0.157	0.187
L.S.D.at 0.05%		0.114	0.122	0.201	0.204	0.120	0.119	0.188	0.190

Table 8. Diethyle methane sulphonate on Carotenoids (mg/g. f w) of leaves of *Codiaeum variegatum var. mollucanum under saline conditions* during two seasons 2020/2021 and 2021/2022.

Character	Water concentration (ppm)	Tap water		1500		3000		6000	
	Seasons	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control saline water	0.00	3.44	3.22	3.45	3.03	3.26	3.28	3.19	3.22
Control without saline water	0.00	3.78	3.81	2.60	2.64	3.06	3.08	3.73	3.78
Ethyl Methane Sulphonate	0.10%	1.57	1.61	3.65	3.68	3.07	3.11	3.65	3.68
	0.20%	2.15	2.18	3.45	3.46	3.28	3.31	3.36	3.41
	0.30%	2.11	2.15	2.28	2.30	2.04	2.08	3.68	3.69
	mean	1.94	1.98	3.13	3.15	2.80	2.83	3.56	3.59
DiEthyl Methane Sulphonate	0.10%	2.00	2.05	2.07	2.11	2.30	2.33	3.74	3.81
	0.20%	1.95	1.96	2.45	2.48	3.36	3.40	2.86	2.90
	0.30%	1.85	1.88	1.47	1.51	3.48	3.50	3.49	3.52
	mean	1.93	1.96	2.00	2.03	3.05	3.08	3.36	3.41
Gamma rays	100Gy	1.29	1.31	2.35	2.37	3.41	3.44	2.67	2.70
	200Gy	1.29	1.30	3.00	3.03	3.26	3.28	3.19	3.22
	300Gy	1.15	1.18	2.60	2.64	3.06	3.08	3.73	3.78
	mean	1.24	1.26	2.65	2.68	3.24	3.27	3.20	3.23
L.S.D.at 0.05%		0.195	0.199	0.087	0.089	0.211	0.214	0.115	0.118

8. Carotenoids (mg/g. f w) of leaves:

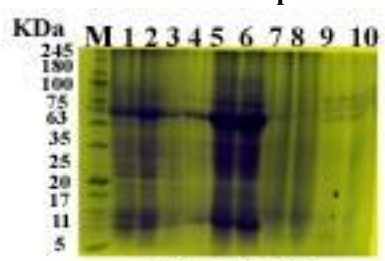
Data obtained in Table (9), showed that, the content of carotenoids although was more effective by using physical treatments and Ethyl methane sulphonate as compared with control in both seasons Diethyle methane sulphonate at 1 0.1% gave the maximum level in the two seasons, while gamma rays 100 gray gave the second level in this concern. while Ethyl methane sulphonate at 0.2 concentration gave

the third value in level of carotenoids in the two seasons. Several investigators also found similar trends with a positive correlation (Zaharia *et al.*,1991). was found between gamma doses and pigment accumulation in seedlings of *Tagetes erecta, Zinnia elegans* and *Callistephus chinensis.*

Table 9. Effect of Gamma rays, EMS and DMS on Carotenoids (mg/g. f w) of leaves of *Codiaeum variegatum var. mollucanum under saline conditions* during two seasons 2020/2021 and 2021/2022.

Character	Water concentration (ppm)	Tap water		1500		3000		6000	
	Seasons	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control saline water	0.00	4.55	4.57	5.24	5.48	4.68	4.70	5.45	5.49
Control without saline water	0.00	4.04	4.08	5.51	5.54	5.49	5.52	6.02	6.05
Gamma rays	100Gy	5.59	5.62	5.57	5.59	5.42	5.45	6.04	6.07
	200Gy	6.04	6.08	6.04	6.07	5.99	6.05	5.41	5.48
	300Gy	5.45	5.47	5.53	5.56	6.22	6.24	5.84	5.87
	mean	5.69	5.72	5.71	5.74	5.88	5.91	5.76	5.81
Ethyl Methane Sulphonate	0.10%	5.08	5.10	4.27	4.31	5.98	6.01	5.35	5.33
	0.20%	4.87	4.09	5.74	5.77	6.29	6.33	5.75	5.78
	0.30%	4.79	4.83	5.45	5.47	4.54	4.57	5.41	5.44
	mean	4.91	4.67	5.15	5.18	5.60	5.64	5.50	5.52
DiEthyl Methane Sulphonate	0.10%	4.76	4.01	5.80	5.83	6.27	6.31	5.80	5.83
	0.20%	4.55	4.57	5.24	5.48	4.68	4.70	5.45	5.49
	0.30%	4.04	4.08	5.51	5.54	5.49	5.52	6.02	6.05
	mean	4.45	4.22	5.52	5.62	5.48	5.51	5.76	5.79
L.S.D at 5%		1.25	1.40	1.85	1.90	2.01	2.06	2.07	2.04

SDS-Protein electrophoresis



SDS-PAGE

Band No	M.W Bp	1	2	3	4	5	6	7	8	9	10
1	86	0	0	0	0	1	1	0	0	0	0
2	75	0	0	0	0	1	1	0	0	0	0
3	68	1	1	1	1	1	1	1	1	1	1
4	65	1	1	1	1	1	1	1	1	1	1
5	59	1	1	1	1	1	1	1	1	1	1
6	57	1	1	1	1	1	1	1	1	1	1
7	49	1	1	1	1	1	1	1	1	1	1
8	47	1	1	1	1	1	1	1	1	1	1
9	33	1	1	1	1	1	1	1	1	1	1
10	31	1	1	1	1	1	1	1	1	1	1
11	28	1	1	1	1	1	1	1	1	1	1
12	19	1	1	1	1	1	1	1	1	1	1
13	16	1	1	1	1	1	1	1	1	1	1
14	14	1	1	1	1	1	1	1	1	1	1
Total		12	12	12	12	14	14	12	12	12	12

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المطفرات الفزيائية والكيماوية لإستحداث الطفرات علي نبات الكروتن صنف موليكانم تحت الظروف الملحية

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الملخص

أجريت التجربة بالمزرعة التجريبية التابعة لقسم البساتين بكلية الزراعة، بمشتهر، جامعة بنها، مصر خلال موسمي 2020 - 2021 و 2021 - 2022 بغرض إستحداث بعض الطفرات علي نبات الكروتن صنف موليكانم حيث تم تطبيق أشعة جاما (0.00 ، 100 ، 200 ، 300 جراي)، وكذلك طافرين كيميائيين هما إيثيل ميثان سلفونات (EMS) وداي إيثيل ميثان سلفونات (DMES) بتركيزات (0.00 ، 0.01 ، 0.02 و 0.03٪) ، وتحت تأثيرأربعة تركيزات من الأملاح 0.00 , 1500, 3000 و 6000 جزء في المليون والماء المستخدم ماء بحر مخفف للتركيزات السابق ذكرها في ثلاثة مكررات بنظم القطاعات الكاملة العشوائية. وكانت النتائج المتحصل عليها كالتالي: أشعة جاما عند الجرعة 100 و200 جراي وكذلك المطفرات الكميائية EMS و DEMS أعطت تأثيراً إيجابياً على النمو مقارنة باستخدام الجرعات العالية 300 جراي . أظهرت جميع معاملات أشعة جاما عند (100 ، 200 غرام) زيادة في صفات النمو المدروسة ، أي عدد الأوراق / نبات ، ارتفاع النبات ، والوزن الرطب والجاف للأوراق (جم) / نبات مقارنة مع الكنترول. تم الحصول على أفضل النتائج بواسطة أشعة جاما عند 100 جراي.