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Molecular Evaluation of *Capparis spinos*a and its Antibacterial Effects on the Activity of *Bacillus subtilis*

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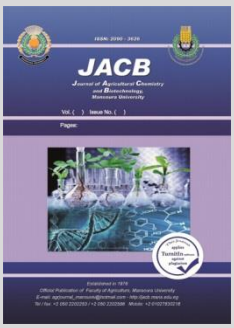

ABSTRACT

Capparis spinosa, belonging to the Capparaceae, was studied to understand the effects of different types of mutagenesis. The study explored gamma rays, ultraviolet rays, colchicine, and caffeine. Various aspects, including appearance, chemical composition, and genetic variation, were evaluated. Radiation mutagenesis of Capparis spinosa using gamma rays (0.5, 1, and 1.5 kGy) and ultraviolet rays (2, 4, and 6 hours) resulted in a decrease in shoot number and length but an increase in the number of leaves. Chemical mutagenesis using colchicine (50, 100, and 150 mg/l) and caffeine (50, 100, and 150 mg/l) led to an increase in shoot and leaf numbers. Genetic variation was assessed using ten primers and the SRAP-PCR technique. The samples treated with colchicine at a concentration of 50 mg/l showed the highest similarity to the control group (89%), while the lowest similarity (81%) was observed in the samples treated with gamma rays at a dose of 0.5 kGy. The highest rutin amount (4.483 mg/ml) was produced when the plant was exposed to ultraviolet rays for 4 hours and gave high inhibition zone (31mm) against *Bacillus subtilis* bacteria while the lowest amount (1.213 mg/ml) was obtained from plants treated with caffeine at a concentration of 150 mg/l. Overall, the best treatment for *Capparis spinosa* was radiation using ultraviolet rays for 4 hours. This treatment resulted in a high number of shoots (2.83 shoots per plant), shoot length (0.66 cm), leaf number (6 leaves per shoot), callus growth (1.849 g), and rutin content (4.483 mg/ml).

***Keywords*:** *Capparis Spinosa*, SRAP-PCR, HPLC, *bacillus subtilis*

INTRODUCTION

The genus *Capparis* is one of some 250 species that belong to the family Capparaceae and had chromosome number 2n = 24, 38 (*Schumann. K .1888*), the European caper bush *Capparis spinosa* is known for its flower buds, *Capparis spinosa* can be found from the Canary Islands' Atlantic coast, Morocco, the Black Sea, Crimea, Armenia, the east side of the Caspian Sea, and Iran (*Inocencio, C. et al., 2002*), the genome of *Capparis spinosa* contained 157728 bp in length and 136 genes, and there were 4 rRNA genes, 31 tRNA genes, and 80 protein-coding genes in the plastome (*Alzahrani, K.M. et al., 2022*). Numerous bioactive substances, including spermidine, rutin, quercetin, kaempferol, stigmasterol, tocopherols, and carotenoids, have been found in *Capparis spinosa* L. These substances are thought to have antioxidant, anti-inflammatory, anti-diabetic, immunomodulatory, antiviral and antibacterial activities like *Bacillus subtilis* is a Gram-positive bacterium, had a rod shape for heat-resistant, its genome contained 4 214 630 base pairs. It was used as a probiotic preparation in the treatment of intestinal disorders and also produce antibiotics, as a fungicide, and in alternative medicine. *Errington, J. and Aart, L.T. ,2020.*

In addition to roots of *Capparis spinosa* is used as a diuretic and against gastrointestinal problems. Bark root has a strong flavor and has been used as an aperitif, astringent, tonic, antidiarrheic, and to cure hemorrhoids and spleen illnesses. It has also been used to treat fever, rheumatism, paralysis, toothache, and destroy earworms (*Zhang. H and Ma. Z.F. 2018*).

Different types of mutagenesis were employed to induce mutation and genetic variation in *Capparis spinosa*, gamma rays are highly energetic electromagnetic ions that are released when an atomic nucleus is excited, Gamma rays, administered at various doses with different doses (0.5,1,1.5 K Gray) changed the cytology, biochemistry, physiology, and morphology of cells and tissues, which had an impact on the growth and development of plants. This effect is attributed producing free radicals in cells, (*Ernest et al, 2020; Hanafy, R.S. and Akladious, S.A. 2018; Kusmiyati, F. et al, 2017; Elkhateed, M.A. et al, 2016; Fulzela, P.D. et al, 2015* and *Kartini, E.M. et al, 2015).*

Ultraviolet radiation, which extends from the visible light spectrum's violet end to the X-ray zone, was also utilized. Ultraviolet rays were administered at different durations (2, 4, and 6 hours). These rays have been found to affect genetic variation and as results of induced mutations in *Capparis spinosa* (*Dwivedi,K.et al,2021; Castronuovo,D.et al,2017; Sztatelman, O. et al,2016; Neelamegam, R. and Sutha, T., 2015*).

Colchicine, an important mutagen, exerts its effects by avoiding the formation of microtubules and doubling chromosome number. It has been applied in various concentrations (50, 100, and 150 mg/l) in various studies. (*Eng,W.H. et al. ,2021; Gupta,G. et al, 2021; Rosmaina et al, 2021; Hailu,M.J. et al ,2021; Cimen ,B.,2020; Dai,J. et al, 2020; Cuong Le,k. et al ,2020)*

Regarding caffeine, many studies have examined the effect of different concentrations and revealed that it acts by

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(50, 100, and 150 mg/l). (*Shahwar,D. et al, 2020; Ansari,S.M. 2020; Hanafi,N.N.M. et al ,2020)*

Tissue culture and molecular markers are crucial fields for studying the effects of different mutagenesis on medicinal plants. Several studies have utilized these approaches to investigate the impact of mutagenesis on genetic material. For instance, have highlighted the significance of tissue culture and molecular markers in studying mutagenesis in medicinal plants. Among the molecular markers, SRAP markers have emerged as important tools for assessing the effects of mutagenesis on genetic variation. SRAP markers target coding regions of DNA and are amplified using specific primers. Researchers such as (*Gianguzzi,V. et al,2020; Dhutmal R.R. et al 2018; Robarts, D.W. H. and Wolfe, A. D. ,2014; Yarar,G. et al,2022 ; El-Nashar,Y.I. and Ammar,M.H., 2016)* found that.

Rutin, also known as rutoside or quercetin-3-O-rutinoside, is a citrus flavonoid glycoside found in various plants, including citrus fruits. It has antioxidant and anti-inflammatory properties and protects against cancer and other diseases. Rutin is commonly used for various purposes, such as autism, aging skin, and airway infections caused by exercise. The leaf tissue of *Capparis spinosa* contains 2.8% rutin. The biosynthesis of rutin involves several key genes, including phenylalanine ammonium lyase (PAL), cinnamate-4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone-3-hydroxylase (F3H), flavonoid-3'-hydroxylase (F3'H), flavonol synthase (FLS), flavonoid-3-O-glucosyltransferase (UFGT), and flavonol-3-O-glucoside L-rhamnosyl transferase (RT). (*Liu, Z. et al, 2021; Kianersi F. et al, 2020.*

This study's objective is to assess the effect of different types of mutagenesis (Gamma-ray, ultraviolet ray, colchicine, and caffeine) on the morphological traits, biochemical composition, and genetic variation of *Capparis spinosa* and to study the effect of *capparis spinosa* extracts on the activity of bacillus subtilis bacteria

MATERIALS AND METHODS

Lab experiments were conducted in the tissue culture laboratory at the Horticulture Research Institute, Agricultural Genetic Engineering Research Institute; ARC, Giza, Egypt. And Lab Research Park, Faculty of Agriculture, Banha University, and Egyptian Atomic Energy Authority. During two subculture mutations of 2021/2022, to study the response of caper (*Capparis Spinosa* L. plants) by radiation (Gamma-ray and ultraviolet ray) and by chemical mutagens (colchicine and caffeine), to evaluate their effects on survival, shoot length, number of shoots, number of leaves, callusing, rooting, weight, total protein and genetic variation (SRAP analyses).

Plant Materials

The caper stem segments (*C. spinosa* L.) were gathered from various locations in Sina's southwest. The collection locations included Dahab, Safaga, and Hurghada.

Media composition for different treatments:

Disinfecting stage

The micronodes stem segments that were obtained from the field were first washed for one hour under running water, followed by a 30-minute soak in water and soap with constant stirring. Then, for 15 minutes, they were immersed in water with 0.5% systemic fungicide benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate, 50% active components). The cuttings were moved into 1% Clorox (sodium hypochlorite 5.5%) for 3 minutes with constant agitation after being moved into 70% alcohol for 1 minute under sterile circumstances. The cuttings were then washed three times for five minutes each with sterile distilled water.

Media preparation:

For shootlet production, Murashige and Skooge media (MS) was used supplemented with kinetin (6-furfural-amino purine) at 3 mg/l and BAP (6-Benzyl amino purine) hormone 3 mg/l. The PH of the media was adjusted to 5.7+ 0.2 by adding agar at 7 g/l and sucrose at 25 g/l.

The callus induction from the leaves disc applying MS media plus 2,4-D (2,4-Dichlorophenoxyacetic acid) at 3 mg/l.Treatments by radiation of Gamma and ultraviolet rays:

Three doses of gamma radiation were irradiated to the explants and callus (0.5, 1, 1.5 K Gray) of 60^Co-γ using India gamma cell at a dose rate (0.782 K Gy/h) and also, UV-C rays exposure was used for three times (2,4,6 hour), and it was type C. Employing model G15T8 ultraviolet light UV-C lamp: philp-TuV-15W-54 V- 0.34 A, and long at 45 cm, diameter at 2.8 cm, containing 2.0 mg of mercury (Hg), and disinfects water air. UV-C light is a short–wavelength linear tube (254 nm). The distance of 10 cm from the UV-C lamp in October 2021 at the National Center for Research and Technology, Nasr City, Cairo, Egypt. *Dwivedi,K.et al,* 2021*; Babina,D.et al,*2020*; Bahmani, M.et al* 2016 and *Sztatelman, O. et al, 2016.*

Treatments of Colchicine and Caffeine:

The explants and callus were treated with three doses of colchicine (50, 100, 150 mg/l) by using colchicine tablets 500 mg source on growth media and also, three doses of caffeine (50, 100, 150 mg/l) by using caffeinospire (caffeine 10 mg/ml) source on growth media at the Horticulture Research Institute – ARC, Giza.

Morphological characteristics shootlets for the multiplication stage

The culture in this stage was incubated for one month then survival percentage, number of shoots, shoot length (cm), number of leaves per each shoot, callusing %, the fresh and dry weight of both new shootlets and callus (gm) and the callus %, callus color manually were determined.

Molecular marker techniques

In this study, two molecular marker techniques, namely (Sequence Related Amplified Polymorphism) and SDS-protein electrophoresis, were used to assess the extent to which the genetic material of *Capparis spinosa* was affected by various treatments. These techniques were used to investigate the genetic changes and variations resulting from the different mutagenesis treatments.

Genomic DNA extraction and purification

Extraction of DNA from *Capparis spinosa* by DNeasy Mini Kit (Qiagen Santa Clarita, CA), the primers showed in table (1) from Macrogen company according to *Ibrahim, S.D. et al,* (2019).

SRAP-PCR Reactions

The polymorphism was identified using a set of ten combination primers. The 25 l reaction volume used for the amplification reaction contained 12.5 l of the Sigma Master Mix, 1.5 l of forward primer, 1.5 l of reverse primer (10 pcmol), 2.5 l of template DNA (10 ng), and 7 l of dH2O.

The PCR Thermocyling Profile:

The Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) was used to carry out the PCR amplification. This system is programmed to denaturation cycle for 5 minutes at 94°C, and 40 cycles for a denaturation step at 94°C for 50 sec, an annealing step at 40°C for 50 sec, and an elongation step at 72°C for 1 min. In the last cycle, the primer extension phase was prolonged to 7 minutes at 72 degrees.

Table 1. Sequence of 10 primers of SRAP:

Primer	Forward Primer	Reverse Primer
SRAP-01	me1- 5′-TGAGTCCAAACCGGATA-3′	em1- 5′-GACTGCGTACGAATTAAT-3
SRAP-02	me1- 5′-TGAGTCCAAACCGGATA-3′	em3- 5′-GACTGCGTACGAATTGAC-3′
SRAP-03	me1- 5′-TGAGTCCAAACCGGATA-3′	em4- 5′-GACTGCGTACGAATTTGA-3′
SRAP-04	me2- 5′-TGAGTCCAAACCGGAGC-3′	em1- 5′-GACTGCGTACGAATTAAT-3
SRAP-05	me2- 5′-TGAGTCCAAACCGGAGC-3′	em2- 5′-GACTGCGTACGAATTTGC-3′
SRAP-06	me2- 5′-TGAGTCCAAACCGGAGC-3′	em3- 5′-GACTGCGTACGAATTGAC-3′
SRAP-07	me3- 5′-TGAGTCCAAACCGGAAT-3′	em1- 5′-GACTGCGTACGAATTAAT-3
SRAP-08	me3- 5′-TGAGTCCAAACCGGAAT-3′	em3- 5′-GACTGCGTACGAATTGAC-3′
SRAP-09	me4- 5′-TGAGTCCAAACCGGACC-3′	em1- 5′-GACTGCGTACGAATTAAT-3
SRAP-10	me4- 5′-TGAGTCCAAACCGGACC-3′	em2- 5′-GACTGCGTACGAATTTGC-3′

The PCR Products' Detection:

Electrophoresis was used to separate the amplification products in a 1.5% agarose gel with ethidium bromide (0.5 ug/ml) in 1X TBE buffer at 95 volts. A Gel Documentation System (BIO-RAD 2000) was used to photograph and visualize PCR results under UV light, Ibrahim, S.D. et al, (2019).

In the SRAP analysis, only distinct and unmistakable bands were visually rated as either present (1) or absent (0) for all samples, and the final data sets comprised both polymorphic and monomorphic bands. A binary statistic matrix was subsequently built. The unweighted pair group technique with arithmetic averages (UPGMA) was then used to calculate the genotype-to-genotype similarity matrix coefficients. Using the PAST software Version 1.91, this matrix was used to create a phylogenetic tree (dendrogram) based on the Euclidean similarity index (Hammer, A.T. et al., 2001).

SDS-protein electrophoresis

According to the procedure (*Matsumoto, H. et al. (2018)*), SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) was performed.

High–performance liquid chromatography (HPLC)

The separation was accomplished using a reversed-phase Zorbax SB-C18 column (3.5 m particle size, i.d. 4.6 mm 250 mm) and HPLC Agilent Technologies 1260 Infinity (USA and Canada) with solvent delivery system quaternary pumps (61311B), including a diode array detector (DAD 61315D with 10-mm flow cell. The volume of injection stayed at 5 l. With a 25-minute total analysis period for each sample, a photodiode array UV detector was set to detect HPLC chromatograms at 210 nm in accordance with the absorption maxima of the studied chemicals. Performed method according to Mutuli, G.P. *et al.* (2022).

Antibacterial assay

The explants of *Capparis spinosa* belonging to the Family, Capparidaceae were collected from successive explants obtained from elicitation treatments (gamma ray 1.5 k gray, ultraviolet ray 4 hour, colchicine 50 mg/l, caffeine 50 mg/l) contained high levels of rutin to cold mortar. Two grams of the explants were macerated in cold methanol 70 % over night in the laboratory for further processing. The cold extraction procedure was used for extracting explants with solvents as per the procedure given below (Prakash, M. and Karmegam, N. 2012; Vigneshwari, *C.* et al., 2014).

Disc diffusion method of antibacterial assay was used to test the sensitivity of selected test organism to the methanolic extracts adopting the method of *Bauer* et al. (1966). Each extract (10 µl) was applied to filter paper discs (Whatman No. 1) measuring 2 mm diameter and allowed to dry before being placed on the agar plate.

The Petri plates of 100mm diameter with nutrient agar media were swabbed with broth culture of the test bacteria in separate plates by using sterile swab. Over this, prepared antimicrobial discs were placed under aseptic conditions. Three discs of original extract, 1:1 and 1:2 (extract: methanol 70%) were placed in triangle. Also, the discs without treated explant extract were also maintained as control. The plates were then incubated at 37ºC for 24 hrs and the zone of inhibition (ZI) was measured in diameter (cm) around the discs and recorded. The assays were performed with three replicates as per the procedure adopted by *Prakash,M. and Karmegam,N.* (2016).

Statistical analysis

The experimental design used in this study was randomize complete block. The Stat View 5.1 statistical program (SAS, program) was used to perform Fisher's PLSD and analysis of variance (0.05 significant level).

RESULTS AND DISCUSSION

Morphological characteristics:

Table (2) provides a summary of the experiment's results. The highest rate of survival, at 100%, was noticed with the use of chemical treatment. On the other hand, exposure to gamma rays at 1.5 k gray resulted in the lowest survival rate, at 60%. Caffeine at a concentration of 150 mg/l led to a high number of shoots, while caffeine at concentrations of 50 mg/l and 100 mg/l resulted in longer shoots. However, gamma rays at 0.5 k gray, 1 k gray, and 1.5 k gray resulted in lower shoot numbers and shoot lengths. Furthermore, every treatment increased the number of leaves, with the highest number of leaves produced by colchicine at 50 mg/l, 100 mg/l, and 150 mg/l concentrations and that showed in figure (1).

Table 2. Effect of different media composition and concentrations on survival, shoot number, shoot length, leaves number, rooting, root length, and callus formation of *Capparis spinosa*

Mutation treatment	Survival %	Shoot No (shoot/ explant)	Shoot Ln (cm)	Leaves No (Leaf/ shootlet)
Control	100.00	2.78	2.81	4.30
Colchicine 50 mg \l	100.00	2.00	2.30	6.70
Colchicine 100 mg \l	100.00	2.80	3.40	7.10
Colchicine 150 mg\l	100.00	2.79	3.60	7.40
Caffeine 50 mg\l	100.00	3.57	4.30	6.50
Caffeine 100 mg\l	100.00	3.20	4.10	6.10
Caffeine 150 mg \l	100.00	4.60	3.50	6.30
Gamma ray 0.5 KG	80.00	1.29	1.90	5.40
Gamma ray 1 KG	70.00	2.00	0.60	5.70
Gamma ray 1.5 KG	60.00	1.17	2.20	4.90
UV-C 2 H	90.00	2.50	0.62	6.40
UV-C 4 H	100.00	2.83	0.66	6.00
UV -C 6 H	100.00	2.43	0.75	5.80
LSD 5 %	9.56	0.4592	0.28	1.04

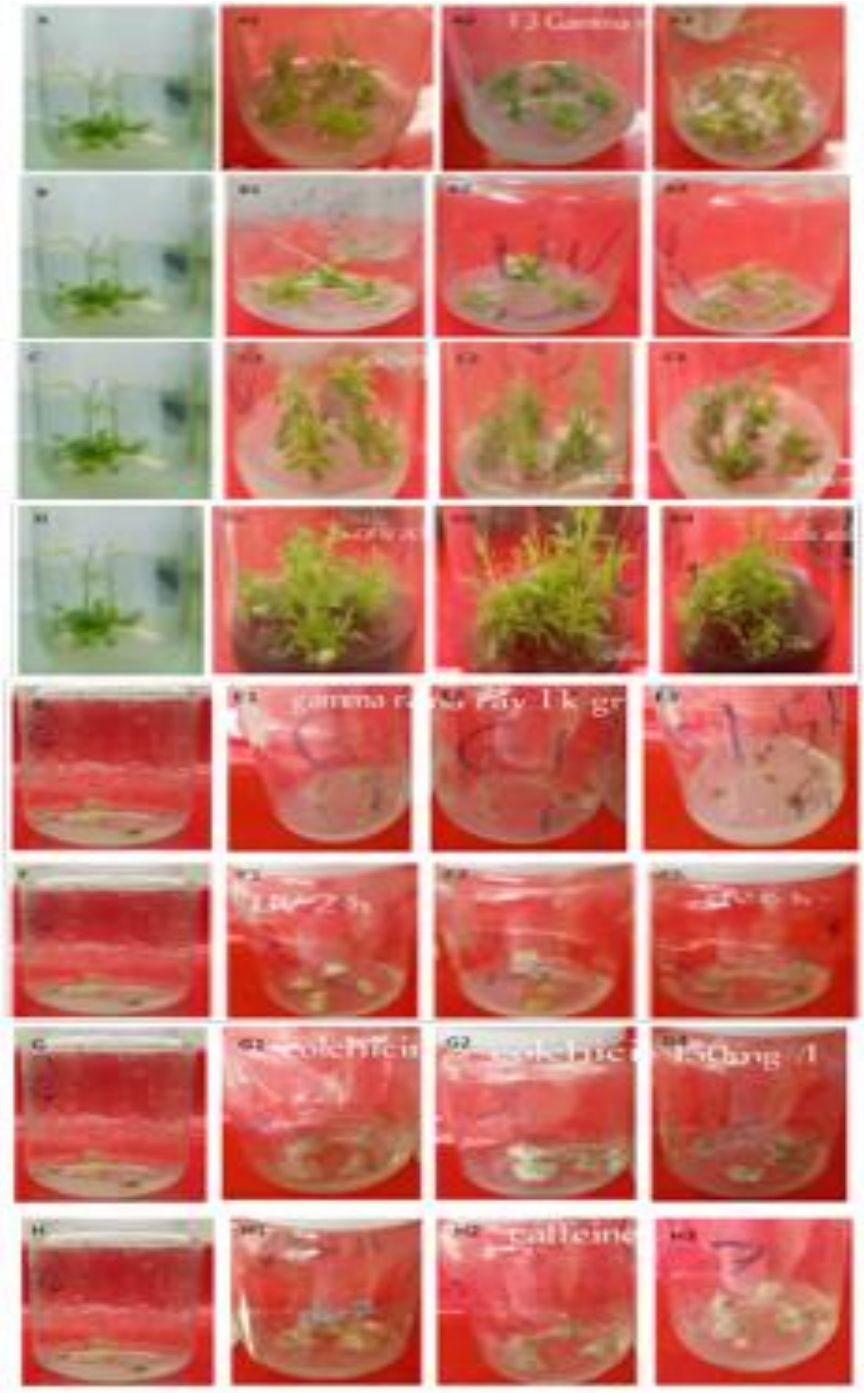

Fig. 1. Effect of various Gamma radiation dosages on survival, shoot number, shoot length, and leaves number of *Capparis spinosa.*

(A)explant untreated media ,(A1) explant media treated by 0.5 k Gray of gamma ray ,(A2) explant media treated by 1 k Gray of gamma ray , (A3) explant media treated by 1.5 k Gray of gamma ray, (B1) explant media treated by ultraviolet ray 2 hour , (B2) explant media treated by ultraviolet ray 4 hour, (B3) explant media treated by ultraviolet ray 6 hour, (C1) explant media with colchicine 50mg/l , (C2) explant media with colchicine 100 mg/l and (C3) explant media with colchicine 150mg/l , (D1) explant media with caffeine 50mg/l , (D2) explant media with caffeine 100 mg/l and (D3) explant media with caffeine 150mg/l , (E) callus untreated media , (E1) callus media treated by 0.5 k Gray of gamma ray , (E2) callus media treated by 1 k Gray of gamma ray , (E3) callus media treated by 1.5 k Gray of gamma ray ,(F1) callus media treated by ultraviolet ray 2 hour , (F2) callus media treated by ultraviolet ray 4 hour, (F3) callus media treated by ultraviolet ray 6 hour,(G1) callus media with colchicine 50mg/l , (G2) callus media with colchicine 100 mg/l and (G3) callus media with colchicine 150mg/l , (H1) callus media with caffeine 50mg/l , (H2) callus media with caffeine 100 mg/l and (H3) callus media with caffeine 150mg/l .

Regarding callus formation, the average callus increment was significantly higher (2.13) when using MS treated with ultraviolet light for 4 hours. In contrast, MS supplemented with colchicine at 150 mg/l concentration showed the lowest significant average callus formation value (0.32). As shown in Table (3), the control group had brown-colored callus, while callus treated with colchicine at 50 mg/l and 100 mg/l concentrations exhibited a white color. Callus treated with caffeine at 50 mg/l and 100 mg/l concentrations displayed a brown/white color, as indicated in Table (3) and figure (1) as well.

Table 3. Effect of different media concentrations on weight and color of callus of *Capparis spinosa*

Mutation treatment	fresh weight (g)	dry weight (g)	Growth increment	callus color
Control	2.60	1.92	0.68	brown
Colchicine 50 mg\l	4.55	4.00	0.67	white
Colchicine 100 mg\l	4.36	3.84	0.53	white
Colchicine 150 mg\l	4.38	4.06	0.32	white\brown
Caffeine 50 mg\l	4.37	3.95	0.43	brown\white
Caffeine 100 mg\l	4.30	3.81	0.49	brown\white
Caffeine 150 mg \l	4.64	3.98	0.66	brown
Gamma-ray 0.5 KG	1.25	0.23	1.02	brown
Gamma-ray 1 KG	1.14	0.32	0.82	brown
Gamma-ray 1.5 KG	1.09	0.12	0.97	brown
UV-C 2 H	2.36	0.23	1.85	brown
UV-C 4 H	2.16	0.31	2.13	brown
UV -C 6 H	2.16	0.36	1.80	brown
LSD 5 %	0.33	0.109	0.054	

The findings of this study are in agreement with those of previous research. *Abdulhafiz,F. et al. (2018)* investigated the impact of gamma irradiation on Musa cv Tanduk and found that the highest survival rate, at 74%, was observed at a gamma ray dose of 10 Gy, while the lowest survival rate, at 20%, was observed at 70 Gy. Similarly, *Elkhateed et al. (2016)* examined the impact of various gamma radiation doses (0.5, 2, 4, and 8 krad) on philodendrons. They observed a gradual decrease in survival percentage as they increased the gamma dose. With increasing dosages of gamma radiation, plants' height, leaf count, fresh weight, and weight of both leaves and stems all decreased.

In another investigation by *Azizan, N.I. et al. (2020),* the impact of various concentrations of colchicine (ranging from 0.5% to 2.5%, with a control group at 0.0%) on Stevia plants was evaluated. It was found that a colchicine concentration of 2.0% resulted in higher average plant height, leaf length, and leaf thickness. This indicates that the formation of mutants in Stevia plants depends on increased colchicine concentration.

Furthermore, *Rosmaina et al. (2021)* investigated the impact of different concentrations of colchicine (ranging from 0.03% to 0.05%, with a control group) over a three-month period on *Ananas comosus* L. Merr. They noticed that colchicine affected genotype and lengthened stomata. In particular, a colchicine concentration of 0.05% caused a 26.67% rise in plant height and a 48.98% increase in the number of leaves.

SRAP analysis

Sequence-Related Amplified Polymorphism (SRAP) is a widely used PCR-based marker technique for studying genetic relationships and polymorphisms within a species. It amplifies coding sequences using forward primers with a specific sequence and selective nucleotides, targeting GC-rich regions, and reverse primers with another specific sequence, targeting AT-rich regions. *Dhutmal,R.R. et al. (2018)* have shown the effectiveness of SRAP in various applications.

According to the results mentioned in Table (4), the highest number of bands, reaching 91 bands, was observed in the control group (untreated sample). This was produced by exposure to gamma rays at 1.5 k gray and ultraviolet rays for 2 hours, using ten SRAP primers. On the other hand, the lowest number of bands, with a count of 72 bands, was obtained from samples treated with caffeine at a concentration of 100 mg/l.

In gamma-ray treatments, a dose of 1.5 k gray resulted in the highest number of bands (91), while lower doses of gamma rays (0.5 k gray and 1 k gray) produced fewer bands (81). For the ultraviolet ray treatments, exposure for 2 hours yielded the highest number of bands (91), followed by 4 hours of exposure (88). However, 6 hours of exposure resulted in the lowest number of bands (70). These results were compared to the untreated control group.

Table 4. Total number of bands in treated samples of *capparis spinosa* Compared to control.

No.	Primers	Control	CL50	CL100	CL150	CA50	CA100	CA150	GA0.5	GA1	GA1.5	UV2	UV4	UV6
A	SRAP-01	6	5	7	7	7	5	7	7	6	7	9	9	9
B	SRAP-02	11	12	12	7	10	9	9	7	10	10	10	10	10
C	SRAP-03	9	10	10	10	11	8	7	9	9	8	10	10	9
D	SRAP-04	5	8	7	8	8	6	10	8	4	6	6	8	6
E	SRAP-05	7	5	5	5	6	5	5	8	10	8	10	7	6
F	SRAP-06	9	10	7	11	11	8	9	10	7	13	11	9	9
G	SRAP-07	6	7	8	7	6	5	8	7	8	10	6	7	7
H	SRAP-08	11	6	5	7	10	5	8	6	9	9	9	8	6
I	SRAP-09	10	12	11	11	9	10	9	10	9	9	11	10	10
J	SRAP-10	12	10	12	10	11	11	9	9	9	11	9	10	10
TOTAL		86	85	84	83	89	72	81	81	81	91	91	88	82

TNB = Total number of band, control = untreated plant, CL50=Colchicine 50mg/l, CL100=Colchicine 100mg/l, CL150=Colchicine 150mg/l, CA50=Caffeine 50 mg/l, CA100=Caffeine 100 mg/l, CA150=Caffeine 150 mg/l, GA0.5 = Gamma ray 0.5 k gray, GA 1 = Gamma ray 1 k gray, GA1.5 = Gamma ray 1.5 k gray, UV2= ultraviolet 2 hour , UV4= ultraviolet 4hour , UV6= ultraviolet 6hour .

In the colchicine treatments, different doses (50 mg/l, 100 mg/l, and 150 mg/l) led to relatively low numbers of bands, with 85, 84, and 83 bands respectively. Regarding caffeine treatments, the highest number of bands (89) was observed when samples were treated with a concentration of 50 mg/l. Conversely, the lowest number of bands (72) was found in both the untreated control group and samples treated with a concentration of 100 mg/l.

SRAP analysis, the SRAP-06 primer produced one unique positive marker which was treated by Gamma-ray 1.5 k Gray at 1050 bp, SRAP-09 primer gave one unique positive marker which was treated by colchicine 50 mg/l at 550 bp and also SRAP-10 produced one unique positive marker which treated by caffeine 50 mg/l at 350 pb as shown in Fig (2).

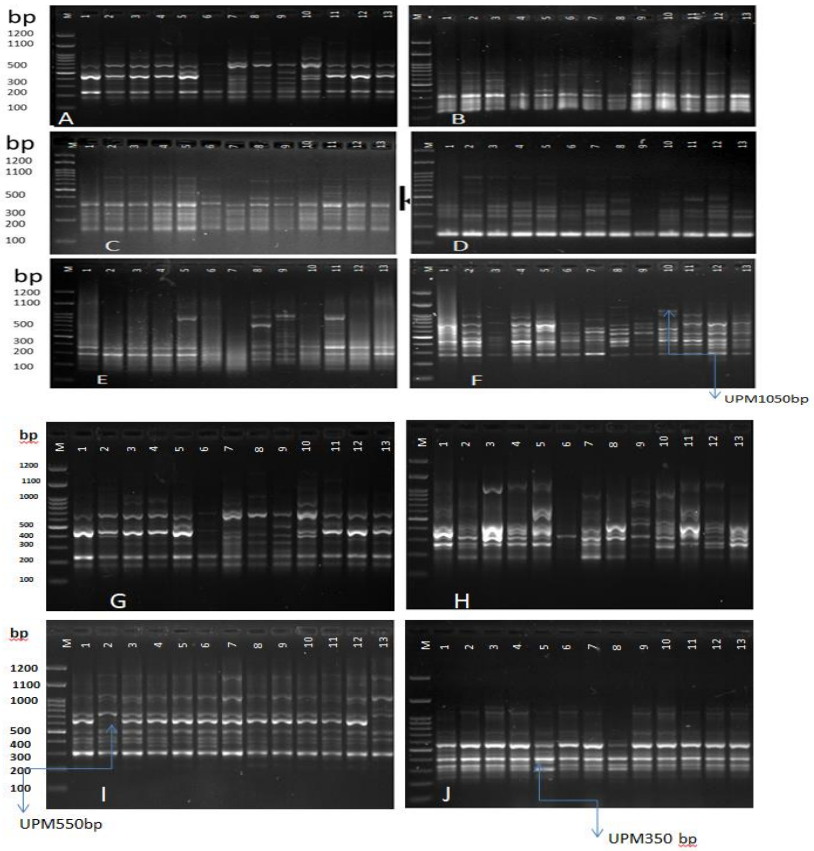

Figure 2. SRAP profiles of 13 *Capparis spinosa* samples (1 - 13) as detected with primers (A) SRAP-01, (B) SRAP-02, (C) SRAP-03, (D) SRAP-04, (E) SRAP-05, (F) SRAP-06, (G) SRAP-07, (H) SRAP-08, (I) SRAP-09, and (J) SRAP-10 as illustrated in table (4).

Genetic similarity within and among treated samples of *Capparis spinosa.*

We used Dice's similarity coefficients (DSCs) to measure the genetic similarity among treated samples of *Capparis spinosa*. Based on the SRAP data, a similarity matrix was created and the UPGMA method was used to construct a dendrogram.

The analysis of the SRAP data reported that the genetic similarity among the treated samples of *Capparis spinosa* ranged from 79% to 97%. On average, there was 88% similarity, indicating a relatively low level of DNA-level variation due to the use of *Capparis spinosa* treatments. Among the treatments, the highest similarity (97%) was noticed between the accessions Gamma-ray 1.5 k gray and Gamma-ray 1 k gray, both receiving gamma ray treatment according to table (5).

Table 5. Genetic similarity matrix within and among thirteen treatments of *Capparis spinosa* according to Dice's similarity coefficient from SRAP generated data.

MW	Control	CL50	CL100	CL150	CA50	CA100	CA150	GA0.5	GA1	GA1.5	UV2	UV4	UV6
Control	1.00												
CL50	0.89	1.00											
CL100	0.87	0.90	1.00										
CL150	0.84	0.87	0.86	1.00									
CA50	0.87	0.86	0.86	0.86	1.00								
CA100	0.84	0.85	0.86	0.86	0.83	1.00							
CA150	0.85	0.87	0.82	0.85	0.82	0.88	1.00						
GA0.5	0.81	0.85	0.81	0.86	0.81	0.86	0.90	1.00					
GA1	0.84	0.79	0.81	0.82	0.87	0.81	0.82	0.84	1.00				
GA1.5	0.85	0.80	0.81	0.81	0.87	0.80	0.82	0.84	0.97	1.00			
UV2	0.86	0.82	0.83	0.84	0.88	0.82	0.81	0.86	0.94	0.95	1.00		
UV4	0.84	0.86	0.88	0.84	0.86	0.83	0.83	0.85	0.89	0.90	0.91	1.00	
UV6	0.85	0.84	0.89	0.85	0.87	0.84	0.83	0.83	0.88	0.89	0.90	0.93	1.00

control = untreated plant, CL50=Colchicine 50mg/l, CL100=Colchicine 100mg/l, CL150=Colchicine 150mg/l, CA50=Caffeine 50 mg/l, CA100=Caffeine 100 mg/l, CA150=Caffeine 150 mg/l, GA0.5 = Gamma ray 0.5 k gray, GA 1 = Gamma ray 1 k gray, GA1.5 = Gamma ray 1.5 k gray, UV2= ultraviolet 2 hour , UV4= ultraviolet 4hour , UV6= ultraviolet 6hour.

On the contrary, the lowest genetic similarity (79%) was found between the accession colchicine 50 mg/l and the Gamma-ray 1 K Gray treatment, likely due to their different treatments.

According to table (5) The highest genetic similarity (89%) was found in the colchicine 50 mg/l treatment among the untreated samples, while the lowest similarity (81%) was observed in the Gamma-ray 0.5 K gray treatment. For radiation treatments, the highest genetic similarity (97%) was seen in the gamma-ray 1.5 k gray and gamma-ray 1 k gray treatments, while the lowest similarity (83%) was found in the gamma-ray 0.5 k gray and ultraviolet ray 6-hour treatments.

This observation was in agreement with that reported by *Hanafy,R.S. and Akladious,S.A. (2018).* They investigated five primers and discovered that gamma irradiation doses altered the DNA and generated DNA polymorphic bands with

variable intensities to be created. These doses were 25, 50, 100, 200, and 400 G. In addition, *Tilwari, A. and Sharma, R.* (2021) used 6 ISSR markers and found that, it was possible to analyze the genetic variation in Gloriosa superba L in the presence of colchicine and the ISSR primers generated 328 fragments, 298 of which were polymorphic, with an average of 49.7 bands per primer and 91.83% polymorphism.

Genetic relationships within and among treated samples of *Capparis spinosa.*

Genetic relationships among treatments of *Capparis spinosa* were analyzed using similarity matrices and the UPGMA method to create dendrograms. The dendrogram clearly distinguished between *Capparis spinosa* treatment accessions and other treatments.

The dendrogram separated the treated *Capparis spinosa* samples into two main clusters based on their geographic distributions. The first cluster had two subclusters: one containing the control, colchicine 100 mg/l, and colchicine 150 mg/l treatments, and the other containing Gamma-ray 0.5 k gray, caffeine 150 mg/l, caffeine 100 mg/l, and colchicine 150 mg/l treatments. The second cluster could also be divided into two subclusters: one containing Gamma-ray 1.5 k gray, Gamma-ray 1 k gray, and ultraviolet ray (2, 4, 6 hours) treatments, and the other containing caffeine 50 mg/l. The colchicine (100 mg/l and 50 mg/l) treatments showed the highest similarity to the control, while the Gamma-ray 0.5 k gray treatment exhibited the lowest similarity. Refer to Figure (3) for a visual representation.

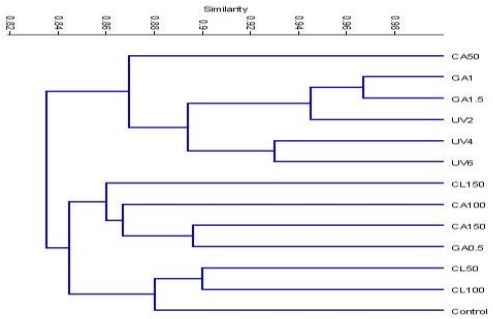

Figure 3. Dendrogram for the 13 samples of *Capparis spinosa* treatments constructed from the SRAP produced data using the UPGMA method and similarity matrices computed using DSC's.

Molecular analysis based on SDS- PROTEINS

According to Figure (4), the main protein in *Capparis spinosa* is flavonol-3-O-glucoside L-rhamnosyl transferase, with a molecular weight of 6.3 kDa. This protein facilitates the transfer of rhamnose from UDP-rhamnose to the 3-OH position of kaempferol and quercetin.

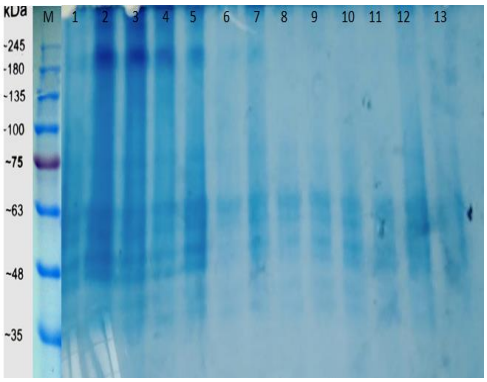

Figure 4. SDS-protein banding patterns among treated samples of *Capparis spinosa*.

(1) Control, (2) colchicine 50 mg/l,(3) colchicine 100 mg/l,(4) colchicine 150 mg/l, (5)caffeine 50mg/l, (6) caffeine 100mg/l,(7) caffeine 150mg/l,(8) Gamma 0.5 kG, (9) Gamma 1 kG,(10) Gamma 1.5 kG , (11) ultraviolet ray 2h, (12) ultraviolet ray 4h and (13) ultraviolet ray 6h

According to table (6), the analysis of the SDS-protein data reported that the genetic similarity among the treated samples of *Capparis spinosa* ranged from 71% to 100%. The highest similarity (100%) was noticed the accessions colchicine 50mg/l and colchicine 100 mg/l while the lowest genetic similarity (71%) was found the accession Gamma ray 0.5 k gray, ultraviolet ray 2 hour and ultraviolet ray 4 hour treatments according to untreated plant.

Additionally, *Hanafy, R. S. and Akladious, S. A. (2018)* observed that a concentration of 0.1% colchicine resulted in the highest diameter of S. platensis (12.57 µm), while a concentration of 0.025% colchicine led to high protein content (0.091 mg/ml) after treatment with multiple doses of colchicine. Furthermore, low gamma radiation doses were found to enhance growth, yield characteristics, leaf-soluble protein, and the contents of phenolic and flavonoid compounds in fenugreek.

Table 6. Genetic similarity matrix within and among thirteen treatments of *Capparis spinosa* according to Dice's similarity coefficient from SDS-protein generated data.

MW	Control	CL50	CL100	CL150	CA50	CA100	CA150	GA0.5	GA1	GA1.5	UV2	UV4	UV6
Control	1.00												
CL50	1.00	1.00											
CL100	1.00	1.00	1.00										
CL150	0.95	0.95	0.95	1.00									
CA50	0.95	0.95	0.95	1.00	1.00								
CA100	0.78	0.78	0.78	0.82	0.82	1.00							
CA150	0.90	0.90	0.90	0.84	0.84	0.88	1.00						
GA0.5	0.71	0.71	0.71	0.75	0.75	0.77	0.67	1.00					
GA1	0.78	0.78	0.78	0.82	0.82	0.86	0.75	0.92	1.00				
GA1.5	0.78	0.78	0.78	0.82	0.82	0.86	0.75	0.92	1.00	1.00			
UV2	0.71	0.71	0.71	0.75	0.75	0.92	0.80	0.83	0.92	0.92	1.00		
UV4	0.71	0.71	0.71	0.75	0.75	0.92	0.80	0.83	0.92	0.92	1.00	1.00	
UV6	0.78	0.78	0.78	0.71	0.71	0.71	0.88	0.77	0.71	0.71	0.77	0.77	1.00

control = untreated plant , CL50=Colchicine 50mg/l, CL100=Colchicine 100mg/l, CL150=Colchicine 150mg/l, CA50=Caffeine 50 mg/l,CA100=Caffeine 100 mg/l, CA150=Caffeine 150 mg/l, GA0.5 = Gamma ray 0.5 k gray, GA 1 = Gamma ray 1 k gray, GA1.5 = Gamma ray 1.5 k gray, UV2= ultraviolet 2 hour , UV4= ultraviolet 4hour , UV6= ultraviolet 6hour.

Genetic relationships within and among treated samples of *Capparis spinosa.*

Genetic relationships among treatments of *Capparis spinosa* were analyzed using similarity matrices and the UPGMA method to create dendrograms. The dendrogram clearly distinguished between *Capparis spinosa* treatment accessions and other treatments.

The dendrogram separated the treated *Capparis spinosa* samples into two main clusters based on their geographic distributions. The first cluster had two subclusters:

one containing the control, colchicine 100 mg/l, colchicine 150 mg/l, caffeine 50mg/l and caffeine 150mg/l treatments, and the other containing ultraviolet –ray 6 hour treatment. The second cluster could also be divided into two subclusters: one containing Gamma-ray 0.5 k gray, Gamma-ray 1 k gray, and Gamma-ray 1.5 k gray treatments, and the other containing caffeine 100 mg/l, ultraviolet-ray 2 hour and ultraviolet-ray 4 hour treatments. The colchicine (100 mg/l and 50 mg/l) treatments showed the highest similarity to the control, while the Gamma-ray (0.5,1,1.5) k gray treatments exhibited the lowest similarity. Refer to Figure (5) for a visual representation.

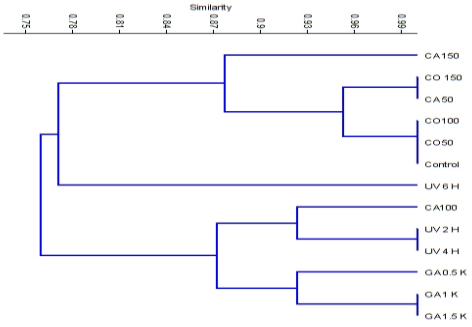

Figure 5. Dendrogram for the 13 samples of *Capparis spinosa* treatments constructed from the SDS-Protein produced data using the UPGMA method and similarity matrices computed using DSC's.

Optimization of chromatographic condition

The High-performance liquid chromatography (HPLC) technique is commonly used to separate, identify, and quantify components in mixtures like Rutin. The UV-Vis spectrum analysis successfully detected Rutin at a wavelength of 210 nm, which provided clear separation and accurate retention time (*Nkwocha, C.C.et al, 2022*)

According to Table (7), the highest amount of rutin (4.483 mg/ml) was produced after exposure to ultraviolet radiation for 4 hours. The lowest amount of rutin (1.213 mg/ml) was found in the caffeine 150 mg/l treatment and that showed in figure (6)

Table 7. The amount of Rutin of treated samples by using HPLC method validation with wavelength at 210 nm.

NO.	Samples	Amount of Rutin (mg/ml)
0	Standard	1
1	Control	1.394
2	Colchicine 50 mg/l	2.212
3	Colchicine 100 mg/l	1.924
4	Colchicine 150 mg/l	1.308
5	Caffeine 50 mg/l	2.997
6	Caffeine 100 mg/l	1.695
7	Caffeine 150 mg/l	1.213
8	Gamma - ray 0.5 k Gray	3.237
9	Gamma - ray 1 k Gray	3.831
10	Gamma - ray 1.5 k Gray	3.997
11	Ultraviolet 2 hour	3.976
12	Ultraviolet 4 hour	4.483
13	Ultraviolet 6 hour	3.569

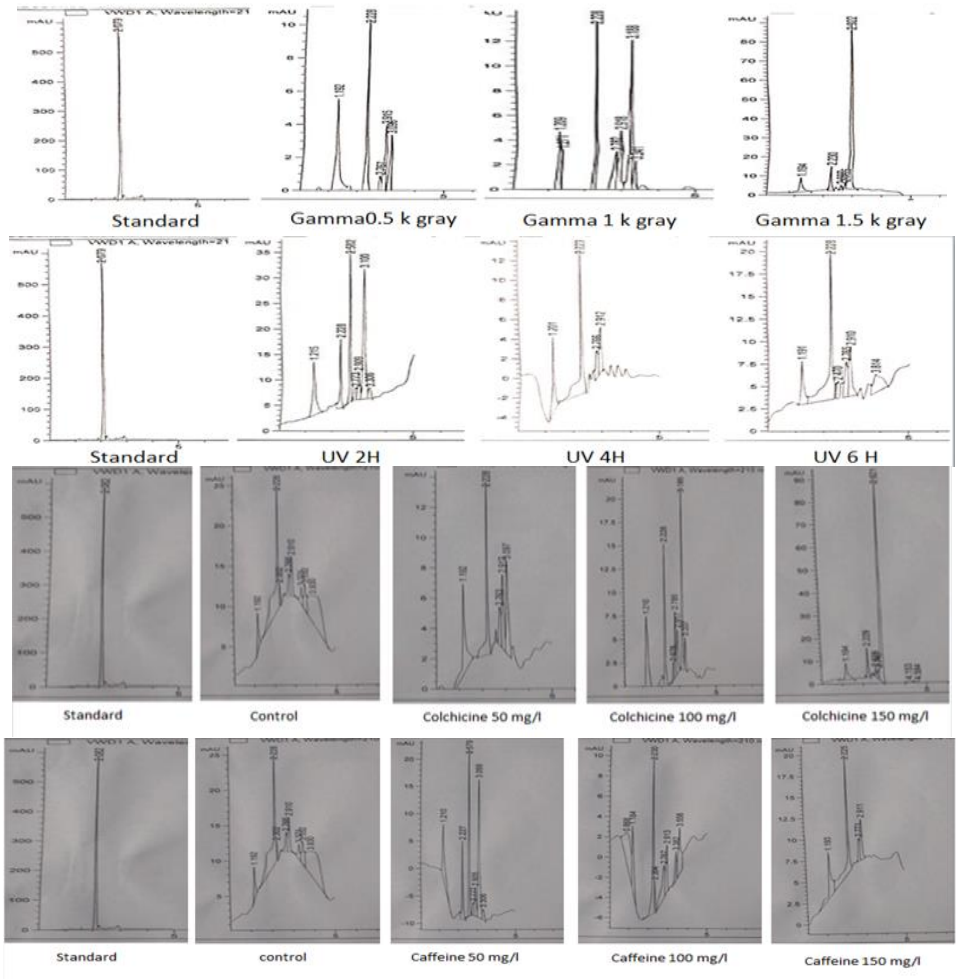

Figure 6. The amount of rutin compound under the effect of Gamma-ray (0.5,1,1.5 KG), ultraviolet ray (2, 4, 6 h), colchicine (50,100,150 mg/l), caffeine (50,100,150 mg/l) using HPLC chromatogram.

This finding aligns with the results reported by Gupta, G. et al. (2021). Similarly, *Hanafy, R.S. and Akladious, S.A. 2018* observed that a 0.025% concentration of colchicine increased biochemical parameters such as total flavonoids, total phenolics, total carotene, and total antioxidants compared to the control. *Zhang,H. and Ma,Z.F. (2018)* mentioned an increase in total flavonoids, rosmarinic acid, caffeic acid, hyperoside, and phenolic levels in P. vulgaris under the influence of UV-B radiation.

Antibacterial assay

The standard reference antibiotic Chloramphenicol at 30 µg per disc showed that the zone of inhibition against *Bacillus subtilis* bacteria range between 28 to 37 mm.

According to table (8), the highest zone of inhibition (33 mm) was recorded by the treatment of *capparis spinosa* explants with gamma ray 1.5 K gray against *Bacillus subtilis* bacteria and also, the treatment of *capparis spinosa* explants with ultraviolet 4 hour against *Bacillus subtilis* bacteria gave (31 mm). as well as the inhibition zone decrease with increase in dilution while the treatment of *capparis spinosa* explants with colchicine 50mg/l against *Bacillus subtilis* bacteria doesn't produce any inhibition zone that due to the difference in the carbohydrate side of rutin synthesis, difference in the stereoscopic shape of rutin, the mutation occurred that led to the degradation of the carbohydrate part of rutin and that showed in figture (7).

Table 8. Antibacterial activity of methanolic elicitated explants Extracts of *Capparis Spinosa* against *Bacillus subtilis* bacteria

	Zone of inhibition (mm)#			
	Std.*	**0 ppm**	**1:1 ppm**	**1:2 ppm**
Control	29	15	12	10
UV. 4h	31	31	20	12
Gamma1.5 k gray	37	33	21	13
Colchicine 50mg/1	32	0	0	0
Caffeine 50 mg/l	28	25	14	11

 # Values are mean of three replicates; $ - Control (without treatment); *Std. - Standard antibiotic, Chloramphenicol (30 µg); 1:1 and 1:2 dilution extract: methanol.

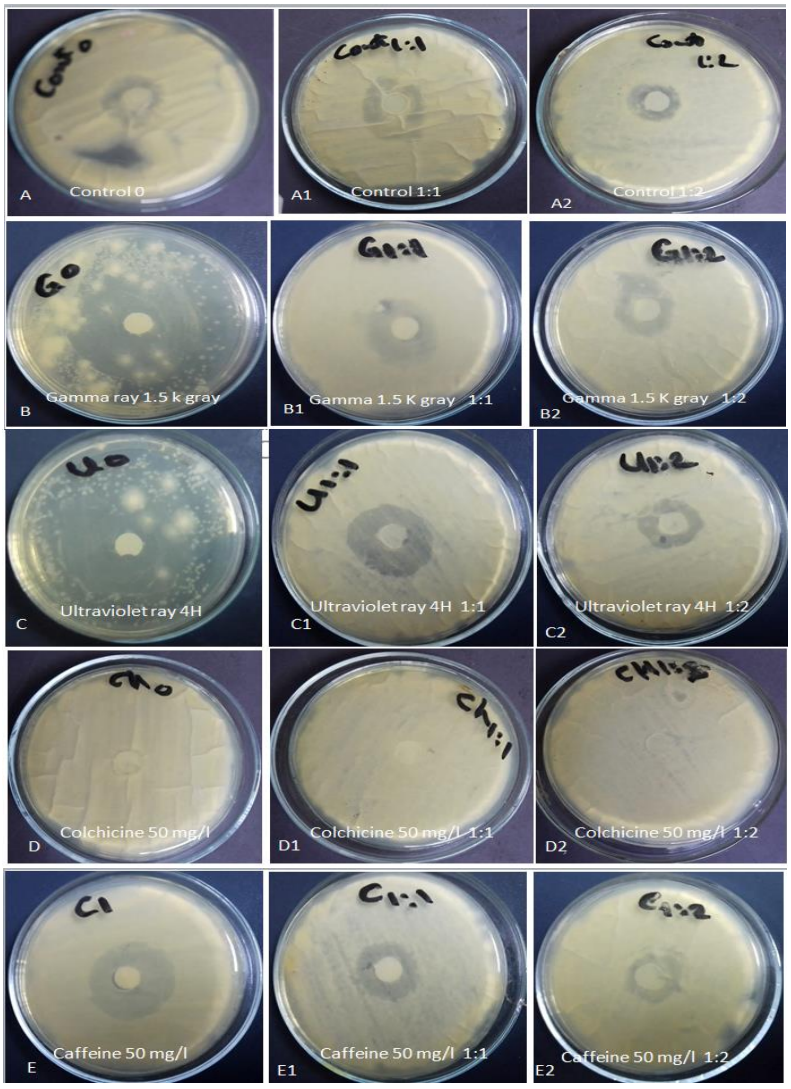

Figure 7. Antibacterial activity of methanolic elicitated explants Extracts of *Capparis Spinosa* against *Bacillus subtilis* bacteria.

(A) untreated media (control) ,(A1) untreated media dilution 1:1 ,(A2) untreated media dilution 1:2, (B) media treated by gamma ray 1.5 k gray (B1) media treated by gamma ray 1.5 k gray dilution 1:1 (B2) media treated by gamma ray 1.5 k gray dilution 1:2 ,(C) media treated by ultraviolet ray 4 hour , (C1) media treated by ultraviolet ray 4 hour dilution 1:1 (C2 media treated by ultraviolet ray 4 hour dilution 1:2, (D) media treated by colchicine 50mg/l , (D1) media treated by colchicine 50mg/l dilution 1:1 (D2) media treated by colchicine 50mg/l dilution 1:2 , (E) media treated by caffeine 50 mg/l , (E1)) media treated by caffeine 50 mg/l dilution 1:1 and (E2) media treated by caffeine 50 mg/l dilution 1:2

This observation was in agreement with that reported by *Prakash, M. and Karmegam, N.* (2012). They showed that, the inhibition of bacterial isolate x. campestris by some of the solvent plant extracts in different concentrations similar to the standard antibiotic (Bacteriomycin) and that mean plant extracts can serve as a good alternative to chemical bactericides

CONCLUSION

The effect of ultraviolet and gamma rays on *Capparis spinosa* led to the change in DNA, this mutation was transmitted to RNA and a difference occurred in the protein product which led to an increase in rutin at a large rate the responsible of flavonol-3-Oglucoside Lrhamnosyl transferase (RT).

The result of the dense protein in the colchicine and caffeine treatments is an increase in the activity of the rutinase enzyme responsible for breaking down rutin into quercetin, while radiation inhibits this enzyme, that is why rutin is high in radiation treatments and low in chemotherapy.

The highest value of rutin production was exposed to ultraviolet rays for four hours, this treatment gave high inhibition zone against *Bacillus subtilis* bacteria (31mm) and low similarity rate was 84 % according to untreated plant (control) that using ten SRAP primers and appearing morphological differences gave the highest callus growth, flattening of the leaf and increasing in number of leaves while dwarfing the shoot length.

Finally, we recommended that the use of ultraviolet-C rays for four hours with short a wavelength linear tube (254 nm) and a distance of 10 cm from the UV-C lamp is the best treatment to produce a high frequency of useful mutations in *Capparis spinosa*.

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التقييم الجزيئي لنبات الكبارس سبينوزا وتاثير كمضاده بكتيري علي نشاط بكتريا *Bacillus subtilis*

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الملخص

الكبارس سبينوزا تنتمي إلى عائلة Capparaceae ، وتم تقييم تأثير أنواع مختلفة من المطفرات (أشعة جاما ، الأشعة فوق البنفسجية ، الكولشيسين ، الكافيين) على الصفات المورفولوجية والتركيب الكيميائي الحيوي والتباين الوراثي لنبات كبارس سبينوزا. أظهرت جرعات الأشعة فوق البنفسجية انخفاضًا في عدد الفروع وطول النبتة ولكن وجد زيادة في عدد الأوراق بينما أظهرت المطفرات الكيميائية بالكولشيسين ،والكافيين زيادة عدد الفروع وعدد الأوراق. أوضح التباين الوراثي للعينات باستخدام عشرة بادئات بتقنية SRAP-PCR حيث ظهر أعلى تشابه (89٪) عند استخدام كولشيسين 50 مجم / لتر بينما وجد أقل تشابه (81٪) مع جاما 0.5 كيلو جرام. أوضحت الدراسة عند قياس محتوى الروتين باستخدام HPLC إنتاج أعلى كمية روتين (4.483 مجم / مل) عند تعرض النبات للأشعة فوق البنفسجية لمدة 4 ساعات ، بينما تم الحصول على أقل كمية (1.213 مجم / مل) من النباتات المعالجة بالكافيين بتركيز 150 مجم / لتر. واوضحت الدراسه لنشاط المضاد للبكتريا المستخرجه من الكبارس اسبينوزا ضد بكتريا *bacillus subtilis* ان اعلي تثبيط (33 مليمتر) بمستخلص النباتات المعرضه لاشعه جاما 1.5 كيلو/جراي ويليها بمستخلص النباتات المعرضه للاشعه الفوق بنفسجيه لمده اربع ساعات اعطت (31 مليمتر) ،بينما لم يحدث تثبيط بمستخلص النباتات المعالجه بكولشيسين 50 مجم/لتر . بالإضافة لذلك كانت هذه المعاملة (المعاملة بالأشعة فوق البنفسجية لمدة 4 ساعات) هي الأفضل حيث أعطت عددًا مرتفعًا من الفروع (2.83 ساق / النبته) ، طول النبتة (0.66 سم) ، عدد كبير من الأوراق (6 أوراق / النبته) ونمو الكالس (1.849 جم) بالإضافة قيمة الروتين العالية (4.483 مجم / مل) .