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Studying the Influence of Gamma Irradiation on Germination, Vegetative Growth, Photosynthetic pigments and Biochemical Characteristics of *Dodonaea viscosa* L. Plant

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# ABSTRACT

**Background**: *Dodonaea viscosa* has a fibrous expansion root system and is effective in soil stabilization, which reduces erosion of sand dunes. It has green leaves and deep red capsules, which make it pleasing to see and can also be used as a hedge, specimen plant, or maybe a small patio tree. Gamma rays are considered a source of some desirable mutations in plants. Therefore, the goal was to study the effect of different concentrations of gamma rays to obtain the best concentration that produces the best and strongest specifications for the plant. **Results:** The results obtained through the study showed that the seeds exposed to the dose of 200 Gy had a positive effect on FGP, GRI, and CVG, while the GI and TSG were affected by the dose of 300 Gy. On the other hand, the seeds treated with the dose of 400 Gy had a significant effect on most of the vegetative characteristics, photosynthetic pigments, and the activity of PPO and POD isoenzymes. Data analysis of (SDS-PAGE) showed a change in protein metabolism, which is one of the functional aspects of the effects of radiation. **Conclusions:** The results showed that the doses of 200 and 300 Gy had a positive effect on germination characteristics, while the morphological and chemical differences appeared mostly in plants produced from seeds treated with 400 Gy.

*Keywords:* Dodonaea viscosa, gamma ray, vegetative growth, photosynthetic pigments, Native-PAGE, SDS-PAGE, superoxide dismutase, peroxidase, polyphenol oxidase.

# 1. Introduction

Dodonaea genus belongs to the family Sapindaceae and comprises 68 species, most of which are small trees and shrubs (Muhammad *et al.*, 2016). Dodonaea viscosa (L.) Jacq., common name is hopbush. It is a flowering evergreen shrub, multi-stemmed shrub, or single-stemmed small tree up to 5–7 m tall (Mothana *et al.*, 2010). Dodonaea viscosa originated in Australia and is available throughout tropical and subtropical countries(AL-Oraimi and Hossain, 2016), D. viscosa has a fibrous expansion root system and is effective in soil stabilization, which reduces erosion of sanddunes. It can be tolerated during a drought(Lira-Caballero *et al.*, 2018; Ilyas *et al.*, 2020). D. viscosa is an aesthetically pleasing plant. It has green leaves and deep red capsules, which make it pleasing to see and can also be used as a hedge, specimen plant, or maybe a small patio tree. The wood is suitable for use as thatching to cover the roofs of houses (Al-Snafi, 2017).

More studies have shown that *D. viscosa* plants include most of the main secondary metabolites. Numerous chemical components were isolated (Mostafa *et al.*, 2014), which have numerous pharmaceutical properties and are conventionally used around the world. It is used to treat

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skin diseases (Priya *et al.*, 2021), and is also used as an antimalarial (Clarkson *et al.*, 2004; Mengiste *et al.*, 2012), antidiabetic (Hossain, 2019), and antibacterial agent (Anandan *et al.*, 2019).

The gamma ray is a kind of electromagnetic radiation with a short wavelength that has a good penetration effect on molecules, causing ionization of materials through the excitement of their electrons (Majeed *et al.*, 2018). The host genetic material was disturbed in the ionized cells, which caused significant changes in the inherited traits. Cobalt-60 is one of the main sources of gamma rays used in radiobiological work at present (Moussa *et al.*, 2006). At the level of medicinal plant studies, radiation is a good inducer for many useful improvements. Good results have been obtained at the level of germination, yield growth parameters, and enhanced active ingredients (Borzouei *et al.*, 2010; Mali *et al.*, 2011; Silva *et al.*, 2013; Kumagai and Takahashi, 2020)

Gamma rays represent an effective mutagenic tool for plant breeders who want to add new traits to commercially valuable crops and develop new varieties (Wi *et al.*, 2007). Gamma-rays directly interact with the cell parts, reaching the membranes, nucleic acids, and proteins (Araujo *et al.*, 2016). However, an indirect action is also recorded through the reactive oxygen species (ROS) generation from water radiolysis (Qi *et al.*, 2015; Tanabe *et al.*, 2022). The physiological and metabolic processes are tremendously changed due to gamma radiation, which leads to the induction of antioxidants and polyphenolic compounds that act as primary defense mechanisms (Khan *et al.*, 2018).

The disturbance of DNA could be constant, which leads to serious impacts, or might be temporal as a result of the ability of DNA to restore itself after the injury following the mechanism of nucleotides in restoring themselves (Shi *et al.*, 2017; Tiwari and Wilson III, 2019).

Determination of the optimum dose of gamma rays to induce mutation in each species is very important in order to get a higher mutant frequency. Hence, the aims of this study were to determine the radio sensitivity and investigate the effects of various doses of gamma irradiation on germination traits, plant growth, morphological variation, and biochemical genetic characteristics, and discuss their potential for *Dodonaea viscosa* ornamental. Hitherto, there is no previous report of an induced mutation in this species.

# 2. Materials and Methods

# 2.1. Seeds collection and experiment location

Seeds of *Dodonaea viscosa* were obtained from the Agricultural Research Center, Giza, located at 30°01'12.5"N 31°12'24.2"E, Egypt. The field experiment was carried out during 2020/2021season in the experimental farm of Horticulture Research Institute of Agricultural Research Center, Cairo, Egypt. The chemical analysis were conducted in the laboratory of Ornamental Plants and Woody Trees Department, National Research Centre.

# 2.2. Procedure

The uniform healthy seeds of *Dodonaea viscosa* were sterilized for 15 minutes in a 3.75% sodium hypochlorite solution (Telci *et al.*, 2011) and then Irradiation of seeds was performed using a 60Co (Cobalt 60) gamma source (Gamma Chamber 900) in ambient conditions at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The doses of exposure were (0, 100, 200, 300, 400, and 500 Gray), where every treatment contains 20 seeds. The irradiated seeds were cultivated individually in March 2020 in plastic bags containing the soil mixture of clay and sand (1:1 v/v). Physical and chemical analysis were carried out according to Jackson (1973) and tabulated in Table (1), and the obtained seedlings were harvested in March 2021.

Soil sample	Coarse sand%			Fine sand%		Silt%		Clay%		
	61.44			9.36		12		17.	.20	
Sandy	E.C.(1:1)	рН	O.M. (%)	Anion (meq/l)		Cation (meq/l)				
loam	(dS/m)			HCO3 <sup>-</sup>	Cŀ	SO4 <sup>-</sup>	Ca <sup>++</sup>	$Mg^{++}$	Na <sup>++</sup>	$\mathbf{K}^{+}$
	0.48	8.1	1.36	6.88	3.25	2.47	6.00	1.82	2.79	0.78

Table 1: The physical and chemical properties of the soil mixture

## 2.3. Data recorded

## 2.3.1. Germination parameters

## Final Germination percentage (FGP)

According to Anjum and Bajwa (2005), Final Germination percentage (FGP) was calculated as follows:

FGP= (NT/N)\*100

Where NT is the number of germinated seeds at each time to count and N is the total seeds in each treatment.

## **Germination index (GI)**

The germination index is a quantitative expression of germination that relates the daily germination rate to the maximum germination value. The germination index (GI) was calculated as described by the Association of Official Seed Analysts (Dezfuli *et al.*, 2008) by the following formula:

## GI = N1/1 + N2/2 + N3/3 + ... + Nn/n

Where; N1, N2, N3, ... Nn are number of germinated seeds, (1, 2, 3, ...) are days of the count and n is day of final count

## **Germination Rate Index (GRI)**

The Germination Rate Index (GRI) was determined as described by Esechi, 1994:

GRI (% / day) =
$$G1/1 + G2/2 + \cdots + Gx/x$$
,

Where; G1 is germination percentage at the first day after sowing and G2 is germination percentage at the second day after sowing.

#### Time Spread of Germination (TSG)

TSG (day) is the time in days between the first and last germination occurring in a seed part, was described by Kader (1998)

#### **Coefficient of Velocity of Germination (CVG)**

The Coefficient of Velocity of Germination (CVG) Jones and Sanders (1987) gives an indication of the rapidity of germination. Its value increases when the number of germinated seeds increases and the time required for germination decreases (Talská *et al.*, 2020).

 $CVG=N1 + N2 + \cdots + Nx/100 \times N1T1 + \cdots + NxTx$ 

Where; N is No. of seeds germinated each day and T is No. of days from seeding corresponding to germination

## Mean germination time (MGT).

The Mean Germination Time (MGT) (Mavi *et al.*, 2010) computes the day of average germination; accordingly, the lower the MGT, the faster a population of seeds reaches germination.

MGT (day) = 
$$\Sigma NiTi / \Sigma Ni$$

Where Ni is number of seeds used in the treatment and Ti represents the number of days since the beginning of the experiment.

#### 2.3.2. Vegetative growth parameters

Plant height (cm), root length (cm), stem diameter (cm), leaf area (cm<sup>2</sup>), number of leaves/plant and number of branches/plant.

# 2.3.3. Photosynthetic pigments

Photosynthetic pigments including chlorophyll a, b, and carotenoids (mg. g<sup>-1</sup> F.W.) were

determined according to Saric et al., (1967).

# 2.3.4. Gene expression (POD, PPO, and SDS-PAGE)

# Native Polyacrylamide Gel Electrophoresis (Native-PAGE)

Electrophoresis was performed to identify isoenzyme differences between control and treatments in the second season.

Peroxidase isoenzmes POD (E.C. 1.11.1.7) in leaf samples were assessed by the procedure defined by (Barceló *et al.*, 1987).

Polyphenol oxidase isoenzymes PPO (E.C. 1.10.3.1) in leaves (100 mg fresh weight) samples were estimated as described by Thipyapong *et al.*, (1995).

The relative distance (Rf-value) of the bands on the gel was calculated as described by (Manganaris and Alston, 1992) using rf = 1.0, distance to the fastest band, and Rf = 0.0, the starting point.

## Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

*Dodonaea viscosa* leaf protein fingerprints were analyzed using SDS-PAGE Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) based on the method of (Laemmli, 1970) as modified by (Studier, 1973). The molecular weight of proteins was then obtained relative to the marker, a large variety of molecular weight proteins (Gene Direx com).

## 2.4. Experimental layout and statistical analysis

The experimental layout was set in randomized complete design (RCD). The data were analyzed through analysis of variance ANOVA and the treatments' means were compared for significance by Least Significant Difference (LSD) test at the 5% level as described by Little and Hills (1978). Standard deviation (±SD) was calculated.

## 3. Results

### 3.1. Germination parameters

Gamma rays treatment at doses 0, 100, 200, 300, 400, and 500 Gy showed different effects on the germination parameters of *D. viscosa* seeds, where FGP, GRI, and CVG were raised in the seeds exposed to dose 200 Gy giving values  $95\pm 2.65\%$ ,  $6.81\pm 0.68$  and  $6.46\pm 0.89$ , respectively, while at the same dose MGT day gave the lowest value  $15.44 \pm 1.31$  when compared with untreated seeds. GI and TSG gave the highest value in the seeds exposed to 300 Gy as compared with untreated seeds.

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Dose	FGP (%)	GI	TSG (day)	GRI (%/ day)	CVG	MGT (day)
Control (0.0)	$80{\pm}\ 2.65$	$996{\pm}4.58$	$21\pm2.00$	$4.45\pm0.53$	$5.06\pm0.35$	$19.75\pm2.13$
100Gy	$82{\pm}\;3.00$	$1386{\pm}\ 8.19$	$24{\pm}~2.00$	$5.7{\pm}~1.08$	$5.85{\pm}0.54$	$17.09 \pm 1.67$
200Gy	$95{\pm}\ 2.65$	$1573\pm7.55$	$22\pm2.65$	$6.81{\pm}~0.68$	$6.46{\pm}~0.89$	$15.44 \pm 1.31$
300Gy	$93{\pm}\ 2.65$	$1692 \pm \! 10.44$	$27\pm1.00$	$6.43{\pm}~0.95$	$5.95{\pm}1.02$	$16.81\pm2.01$
400Gy	$90{\pm}~2.65$	$1535{\pm}\ 10.44$	$24\pm4.36$	$5.91{\pm}\ 0.94$	$3.72{\pm}~0.86$	$17.94 \ \pm 1.44$
500Gy	$85{\pm}~4.36$	$1437{\pm}\ 7.81$	$25 \pm 4.36$	$5.33{\pm}~0.50$	$5.53{\pm}~0.73$	$18.09{\pm}\ 1.16$
LSDat 5%	5.43	18.37	5.34	1.48	1.36	2.96

**Table 2:** The effect of gamma rays treatment with different doses on germination parameters of D.

 viscosa seeds

#### **3.2.** Vegetative growth parameters

The obtained results in Table (3) and Fig. (1) Showed that the effect of different radiation doses was evident on plants, as it showed significant differences in all morphological characteristics. It was clear that the radiation treatment at 400 Gy gave the highest values in the following traits: plant length (cm), number of leaves per plant, number of branches per plant, leaf area (cm<sup>2</sup>), and stem diameter (cm), which were (41.62, 53.33, 9.33, 6.52 and 0.38, respectively), while the highest value was in the root length when seeds were treated with 200 Gy (28.69 cm) when compared to the non-irradiated plants.

# 3.3. Photosynthetic pigments

The data presented in Fig. (2) Stated that all gamma rays treatments increased the content of the photosynthetic pigments. Where; the seeds were exposed to gamma rays at 400Gy produced the highest content of chlorophyll a, b and carotenoids giving values 1.53, 0.40 and 0.83 mg.g<sup>-1</sup> F.W., respectively, followed by treatment 200Gy which gave 1.44, 0.37 and 0.77 mg.g<sup>-1</sup> F.W., respectively, as compared to Non-Irradiated Seeds.



- Fig. 1: The differences between morphological traits of irradiated *D. viscosa* with gamma rays (Gy) where; C: control (0.0), 1:100, 2: 200, 3: 300, 4:400 and 5: 500.
- **Table 3:** The effect of gamma rays treatment with different doses on the vegetative growth traits of *D*.

   *viscosa* seedlings

Dose	Plant height (cm)	No. of Leaves/ plant	No. of branches/ plant	Leaf area (cm²)	Stem diameter (cm)	Root length (cm)
control	$22.5\pm1.80$	$28.33 \pm 2.35$	$1.00{\pm}0.00$	$2.05 \pm 0.09$	$0.22 \pm 0.03$	$20.80{\pm}1.61$
100Gy	$33.52\pm2.54$	$36.67 \pm 2.40$	5.33±1.15	$4.28 \pm 0.10$	$0.30 \pm 0.04$	$23.75 \pm 2.91$
200Gy	$34.27 \pm 1.68$	$48.00 \pm 2.21$	$7.00 \pm 1.00$	$4.50 \pm 0.26$	$0.34 \pm 0.04$	$28.69 \pm 1.57$
300Gy	$30.57\pm2.81$	35.67±2.39	4.67±1.15	$3.33 \pm 0.30$	$0.28{\pm}0.05$	$22.43{\pm}1.99$
400Gy	$41.62\pm2.42$	53.33±2.81	$9.33 \pm 2.08$	$6.25 \pm 0.08$	$0.38 \pm 0.04$	26.49±3.37
500Gy	$28.50 \pm 1.56$	$30.33 {\pm} 2.58$	$2.67 \pm 0.58$	$2.92{\pm}0.10$	$0.27{\pm}0.04$	21.53±1.63
LSD at 5%	3.89	4.38	2.09	0.32	0.072	4.07



**Fig. 2:** The effect of gamma rays treatment with different doses on the photosynthetic pigments (chlorophyll a, b and carotenoids mg.g<sup>-1</sup> F.W.) of *D. viscosa* seedlings

## 3.4. Gene expression

# 3.4.1. Native Polyacrylamide Gel Electrophoresis (Native-PAGE Antioxidant Isoenzyme 3.4.1.1. Polyphenol Oxidase isoenzymes

The electrophoretic pattern and diagram of polyphenol oxidase isoenzymes are illustrated in Fig. (3 A&B) and Table (4), showed the activity stain for isoenzymes, gamma irradiation effects appeared on both band numbers and intensity (faint, moderate and high intensities) when compared with non-irradiation plant. The PPO isozyme total bands of *Dodonaea viscosa* a plant leaves showed six PPO isoenzymes at Rf (0.209, 0.392, 0.462, 0.621, 0.717 and 0.830). The effect of gamma irradiation on number of bands was clear at both 200 and 300Gy, which gave the highest value of band numbers (6 bands), and that the third and sixth bands at Rf (0.462 and 0.830) are positive markers at the 200 and 300Gy doses compared with untreated plant which gave 4 bands. The intensity of the PPO activity bands at 300 and 400 Gy produced three highly intense bands at Rf (0.209, 0.392 and 0.621), while a control got only one highly intense band Rf at (0.392). The activity (number and intensity bands) of the PPO increased with increased gamma irradiation up to 400 Gy then decreased at 500 Gy.



Fig. 3: Effect of different doses of gamma rays (Gy) where; 1: Control (0), 2: 100, 3: 200, 4: 300, 5: 400, 6: 500 (A) polyphenol oxidase isoezyme and (B) ideogram analysis of polyphenol oxidase isoenzyme of *D. viscosa* plants

**Table 4:** Isomers of polyphenol oxidase isoenzymes (+/-) and their retention factor (Rf) in response to<br/>different doses of gamma rays (Gy) Scheme, where; 1: Contro (0) , 2: 100, 3:200, 4: 300, 5:<br/>400, 6: 500

, .						
RF	1	2	3	4	5	6
0.209	+	++	++	+++	+++	+++
0.392	+++	+++	+++	+++	+++	
0.462	_		+	+		
0.621	++	+++	+++	+++	+++	+
0.717	++	++	++	++	++	++
0.830		+	+	+	+	++

# 3.4.1.2. Peroxidase isoenzyme

The influence of different gamma rays doses on the activity of Peroxidase isoenzyme of *Dodonaea viscosa* plants is shown in Fig. (4 A & B) and Table (5) The POD isozyme total bands of *D.viscosa* plant leaves inducted 7 POD isozymes at Rf (0.295, 0.371, 0.735, 0.784, 0.818, 0.871, and 0.939). The highly POD activity of gamma irradiation based on number of bands was clear at 300 gy, which gave the highest number of bands (7 bands), then treatment with gamma irradiation at 400 Gy, which induced 6 bands, while the control showed 5 bands. The highly of the POD activity bands recorded at 100 Gy gave 1 highly intense band at Rf (0.295) and three moderate intensity bands at Rf (0.818, 0.871, and 0.939) while a control recorded 1 highly intense band at Rf (0.295), two moderate bands at Rf (0.818 and 0.871), and two faint bands at Rf (0.735 and 0.784). The lowest intensity

bands were at irradiation at 200 Gy, which produced three bands one moderate band at Rf (0.295) and two faint bands at Rf (0.784 and 0.871).



Fig. 4: Effect of different doses of gamma rays (Gy) where; 1:Contro (0), 2: 100, 3: 200, 4: 300, 5: 400Gy, 6: 500 (A) peroxidase isoenzyme and (B) ideogram analysis of Peroxidase isoenzyme of *D. viscosa* plants

**Table 5:** Isomers of peroxidaseisoenzymeisoenzymes (+/-) and their retention factor (Rf) in responseto different doses of gamma rays (Gy) Scheme, where; 1:control (0), 2: 100, 3:200, 4: 300,5: 400, 6: 500

RF	1	2	3	4	5	6
0.295	+++	+++		+++	+++	++
0.371		+	_	+	_	
0.735	+		—	+	+	+
0.784	+		+	+	+	+
0.818	++	++		+	++	++
0.871	++	++	+	++	++	++
0.939		++		+	+	

# 3.4.1.3. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)S protein banding pattern

As shown in Fig. (5) and Table (6), The total proteins bands from the leaves generated from IS (Irradiated Seeds) and NIS (Non-Irradiated Seeds) were analyzed by SDS-PAGE in the range of 13.005-131.906 kDa, four monomorphic bands (Similar protein bands in all treatments) were observed with molecular weights (14.849, 17.689, 19.143 and 131.906 kDa), only 2 unique bands were different between the control and the treated sample showed up in (0.0 gy) with molecular weights (35.7 and 25.8 kDa. A 101.173 kDa protein bands was present in (0.0, 400 and 500 gy) and absent in the rest of the treatments, also protein band of 70.701 kDa was present only with treatments (0.0, 300, 400 and 500 gy).



Fig. 5: Protein banding pattern between 1: control (0.0), 2: (100), 3: (200), 4: (300), 500 (400) and 6: (500) gy *Dodonia viscosa* protein banding pattern by SDS-PAGE analysis.

Protein band at 61.571 does not appear in control (0.0 gy) but appears in (100,300, 400 and 500 gy). It is observed that four bands were monomorphic, while six bands were polymorphic and unique, giving 60% polymorphism. This is due to the irradiation effect on the protein profile.

MW	1	2	3	4	5	6	Polymorphism
131.906	1	1	1	1	1	1	Monomorphic
101.173	1	0	0	0	1	1	Polymorphic
70.701	1	0	0	1	1	1	Polymorphic
61.571	0	1	0	1	1	1	Polymorphic
35.715	1	0	0	0	0	0	Unique
25.745	1	0	0	0	0	0	Unique
19.143	1	1	1	1	1	1	Monomorphic
17.689	1	1	1	1	1	1	Monomorphic
14.849	1	1	1	1	1	1	Monomorphic
13.005	1	0	0	0	1	1	Polymorphic

**Table 6:** Gamma rays doses (Gy) (1):control (0.0), (2):100, (3): 200, (4): 300, (5):400 and (6): 500 *Dodonia viscosa* protein banding pattern by SDS-PAGE analysis.

# 4. Discussion

It was observed that the germination percentage increased from the beginning of the radiation doses, where the highest percentage was at 200Gy and then decreased gradually again with increasing the dose up to 500Gy. These findings positively agree with germination traits studies in *Lathyrus chrysanthus* (Beyaz *et al.*, 2016), *Ocimum basilicum* (Asgari Lajayer *et al.*, 2019), and *Pavonia* sp. (Yue and Ruter, 2020). The stimulating effects of gamma rays on germination may be attributed to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seeds were irradiated (Abdel-Hady *et al.*, 2008); (Araujo *et al.*, 2016) or may be attributed to the effects of radiation on genes controlling these traits, stimulating hormones, activating enzymes involved in germination processes, and accelerating DNA repair. In addition, it may improve plant germination by accelerating cell division in meristematic tissues (Dhakshanamoorthy *et al.*, 2011); (Katiyar *et al.*, 2022).

Exposure to high doses led to a decrease in germination traits, this decrement might be due to the effect of mutagens on meristematic tissues of the seed as well as chromosomal aberrations and interruptions in DNA replication and growth regulators (Layek *et al.*, 2022; Wang *et al.*, 2017). On the other hand, the inhibition of seed germination at high doses could be due to the damage to seed tissue, chromosomes, and subsequent mitotic retardation, and the severity of the damage depends on the doses used(Li *et al.*, 2018); (Volkova *et al.*, 2020).

The results obtained through this study showed different effects of the gamma ray doses used on the morphological characteristics of *D. viscosa* seedlings, with the highest values of the traits under study obtained from treatment with the 400Gy dose. The increased plant growth caused by gamma irradiation treatment could be explained by the stimulation of the biosynthesis of some amino acids (Hanafy and Akladious, 2018), acceleration in cell division rates (Majeed *et al.*, 2018) as well as activation of auxin (Qi *et al.*, 2015). The stimulatory effect on the morphological traits is in line with Beyaz *et al.* (2016) on *Lathyrus chrysanthus*, (Ryu *et al.*, 2019) on *Chrysanthemum morifolium*, Altay *et al.* (2019) on *Ocimum basilicum* and Cahyaningsih *et al.* (2022) on *Echinacea purpurea*.

The results revealed that gamma rays had a positive effect on the content of photosynthetic pigments, as the highest content appeared when the treatment was 400gy. El-Beltagi *et al.* (2022) attributed the stimulating effect of irradiation on chlorophyll to stabilizing the enzyme active site and photosynthetic reactions. The highly effective effects of gamma radiation on chemical content may be induced by irradiation induced stimulation of plant growth at low doses, particularly during the early genetic stages, which may be regulated through changes in plant growth hormones. The effect of radiation on increasing endogenous hormones in plants and the involvement of auxin and cytokinin in regulating growth and photosynthetic attributes are known (Xiong *et al.*, 2020). The present results are generally in agreement with those of (Patil *et al.*, 2017) on *Dendrathema grandiflora*; (Wang *et al.*, 2017)

al., 2020) on Chrysanthemum morifolium 'Donglinruixue; Asgari Lajayer et al., (2019) on Ocimum basilicum, Hajizadeh et al. (2022) on Lilium longiflorum; and Ghosh et al. (2020) on J. auriculatum.

The resulted PPO expression activity increased with increasing radiation dose up to 400 gy compared with the control (0.0) Gy, in agreement with Mohamed *et al.*, (2021),while POD activity declined at 200 gy and increased at 100, 300, and 400 gy, in agreement with Li *et al.*, (2022) they said that gamma radiation is a stress that leads to the creation of reactive oxygen species (ROS) such as superoxides, hydrogen peroxide, and hydroxyl radicals, which are likely to cause oxidative stress. Increased enzyme activity is primarily responsible for the plant's improved antioxidant capacity and activity following irradiation (Bitarishvili *et al.*, 2018).

It has been shown that gamma-radiation generates active species such as hydrated electrons and hydroxyl radicals (OH.) through its impact on water molecules, which then react with protein molecules (Hassan *et al.*, 2018). The appearance and disappearance of protein bands may be associated with radiation that affect gene expression alterations agreement with (Singh and Datta, 2010) that used a Cobalt-60 gamma source to irradiate wheat seeds and observed an increase in qualitative changes in the protein profiles of the irradiated seeds. Irradiation-induced chemical changes in proteins include degradation, cross-linking, disturbance of the ordered structure of protein molecules, and aggregation of polypeptide chains produced by oxygen radicals Ariraman *et al.*, (2016).

# 5. Conclusions

Treatment with gamma rays at different concentrations had positive effects on germination, vegetative characteristics, photosynthetic pigments in leaves, enzymatic activity of antioxidant enzymes and plant protein content. Seeds exposed to the dose of 200 Gy showed positive effects on some germination characteristics, while others were affected by the dose of 300 Gy. Whereas, the vegetative characteristics, photosynthetic pigments and enzymatic activity were positively affected by the dose of 400 Gy.

# List of Abbreviations

*D. viscosa: Dodonaea viscosa*, FGP: Final Germination percentage, GI: Germination index, GRI: Germination Rate Index, CVG: Coefficient of Velocity of Germination, MGT: Mean Germination Time SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis, Native-PAGE: Native Polyacrylamide Gel Electrophoresis, PPO: Polyphenol Oxidase POD: Peroxidase, SOD: Superoxide Dismutase.

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# **Authors' contributions**

Amr Said Mohamed carried out the field experiment and recorded the morphological data. Samah Mostafa El-Sayed performed the chemical analysis for obtained samples. Ramez Saber Thabet analyzed the obtained data statistically and wrote the manuscript. All authors read and approved the final manuscript.

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