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Effect of gamma radiation on antioxidant enzymes and biochemicals of coriander (Coriandrum sativum L)

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***Abstract*---**Coriander or Cilantro (Coriandrum sativum L.), is an annual herb that distinguishes with short life cycle, it displays broad adaptation as a crop around the world, growing well under many different types of soil and weather conditions. The analysis was carried out in 2022 in the laboratories of Biology Department, College of Science under stable variables such as temperature, humidity, sunlight, grains of Coriander were irradiated with gamma rays at dose levels (25,50, 100 and 150 Gy) in addition to control treatment (not irradiated) using Cobalt 60 as irradiation source and planted in nursery of AL-Kufa university to study the effects of gamma irradiation on the antioxidant enzymes (Antioxidant enzyme include Catalase (CAT), Superoxide dismutase (SOD) and Peroxidase (POD) in addition to several biochemicals (Chlorophyll a and b , α and β Carotenes , Proline and Malondialdehyde). In sum, the results here presented show that the effects of gamma radiation of Coriander depend on the irradiation dose. In parallel to dose increase, plant chlorophyll contents decreased ,Chlorophyll-a, chlorophyll-b, and total chlorophyll were negatively affected by radiation 0.43 , 0.22 and 0.86 respectively at 100 Gy , while α and β Carotenes , Proline and MDA contents, SOD, POD and CAT enzyme activities increased as a result of oxidative stress caused by radiation. Antioxidant activity (Scavenging activity %) of Coriander acetone and methanolic extracts using DPPH assay were showed relation to concentration (activity decrease with decrease extract concentration) and to level of irradiation (activity increase with increase level of irradiation) .These results may be used in estimation of optimal radiation doses and in planning of mutation studies.

Keywords---coriander, DPPH, antioxidant activity, antioxidant enzymes, proline content.

Introduction

Coriandrum sativum L. (Coriander) belonging to the family Umbelliferae / Apiaceae is one of the most useful essential oil bearing spices as well as medicinal plants,. in addition to its use as a seasoning in food preparation, it possess promising antibacterial, antifungal and anti-oxidative activities as various chemical components in different parts of the plant (Mandal and Mandal , 2015). Gamma rays are the most energetic form of electromagnetic radiation and they possess an energy level from 10 keV (kilo electron volts) to several hundred keV. They are considered the most penetrating radiation they source compared with other sources such as alpha and beta rays (Jan *et al* , 2012). Gamma rays fall into the category of ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect the morphology, anatomy, biochemistry, and physiology of plants differentially, depending on the irradiation level.

These effects include changes in the plant cellular structure and metabolism e.g., alteration in photosynthesis, modulation of the ant oxidative system, and accumulation of phenolic compounds (Wi *et al.* 2005). Regulation of cellular reactive oxygen species is important for protection against oxidative stresses. Thus, to combat oxidative stress induced by gamma radiation, plants employ antioxidant defense systems consisting of enzymatic [such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD)] and non-enzymatic antioxidants. The antioxidant defense systems play a major role in scavenging free radicals and regulating the cellular level of ROS, which result in protecting the plant against different biotic and abiotic stresses, including radiation (Amirikhah *et al.*, 2021). Gamma radiation can be useful for the alteration of physiological characters (Kiong et al., 2008). The relatively low-doses ionizing irradiation on plants and photosynthetic microorganisms are manifested as accelerated enzyme activity, stress resistance and crop yields (Chakravarty and Sen,2001)

Antioxidant activity is a parameter that can be used for characterizing plant materials, free radical inhibitor, oxygen scavenger, peroxide decomposer and metal inactivator are some features of mechanisms of antioxidant activity (Demir and Korukluoglu, 2020). An antioxidant is a molecule capable of terminating the chain reactions that damage cells by removing free radical intermediates, and inhibit other oxidation reactions by thereby reducing stress responsible for many degenerative disorders (Deepak *et al.*, 2014). The aim of this study was to determine the effect of gamma radiation on both antioxidant enzymes and biochemicals parameters that may in future play a role in plant breeding and improvement through mutation.

Materials and Methods

This study was conducted at laboratories of Biology Department, College of Sciences, Kufa University. Grains of Coriander were irradiated with gamma rays at dose levels (25, 50, 100 and 150 Gy) in addition to control treatment (not irradiated) using Cobalt ^{60} as irradiation source. Fresh plant material at age of 30 days (fully expanded and undamaged leaves), was separated from whole plants and kept in cool place 4c°for physiological-biochemical characteristics studies.

Physiological-Biochemical Studies of Plants

Determination of Antioxidant Enzymes Activities in Plants
Enzyme Extract

To extract the enzyme from the plant sample, Leave sample 0.5 g were homogenized with 50m M phosphate buffer (pH 7.5) in ice cold containing 0.5 mM EDTA with prechilled mortar. Then centrifuged at 4°C in Beckman refrigerated centrifuge at 10000 r.p.m. for 10 min. supernatant was referred to enzyme extract (Esfandiari *et al.,* 2007).

Superoxide Dismutase (SOD) Enzyme Activity Determination in Plants

According to Marklund and Marklund (1974), reaction mix is consisting of 50 µl crude enzyme extract with 2 ml of tris buffer and 0.5 ml of pyragallol (0.2 mM) which absorbs light at 420 nm. Control solution contains the same materials except for the enzyme extract that was replaced by dH_2O. As a blank, dH_2O was using. Single unit of enzyme is defined as the amount of enzyme that is capable of inhibiting 50% of pyragallol oxidation. SOD activity was calculated using the following equation:

$$SOD\ Activity\ (unit)=(\%P/50\%)\times R/T$$

Where:
• %P: percentage of the inhibition of pyragallol reduction
*Note: %P of every sample is calculated by comparing Δabs of the sample (X%) with Δabs of control (100%)
• R: Total reaction volume (2.55 ml)
• T: Time of reaction in minutes (2 minutes)

Catalase (CAT) (EC 1.11.1.6) Enzyme Activity Determination in Plants

It was estimated according to the method of Aebi (1984). Reaction mix consisted of 20 µl of crude enzyme extract with 1 ml of 30 mM H_2O_2 in a test tube, which was shaken rapidly for less than 5 seconds at 25^0C, and then change in optical absorbance at 240 nm wavelength was noted through a minute of time. Decrease in absorbance through time was registered. Blank solution was consisting of the same contents except for the enzyme extract that was replaced with PBS. Results expressed as CAT units mg^{-1} of protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1}protein). CAT activity was calculated using the following equation:

$Catalase\ activity\ (unit) = (\Delta abs/T) \times R\ /0.001$

Where:
• Δabs: Change in absorbance through time
• T: time in minutes
• R: Total reaction volume in ml (1.02)
*Note: Due to the volume of quartz cuvette that was using with the spectrophotometer, the volumes will doubled ,so R = 2.04 in order to enable the spectrophotometer light beam to hit the reaction mixture.
• 0.001: a constant

Peroxidase (POD) (EC 1.11.1.7) Enzyme Activity Determination in Plants

Activity was estimated according to Hemeda and Klein (1990). The reaction mixture contained 25 mmol. L^{-1} phosphate buffer (pH 7.0), 0.05% guaiacol, 10 mmol. L^{-1} H_2O_2 and enzyme. Activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (E = 26.6 mM^{-1} cm^{-1}). 55

Determination of Non-Enzymatic Activities
Chlorophyll a and b Content Estimation in Plants

Chlorophyll pigments in the leaves were estimated following the method of Arnon (1949).According with this method weight 0.5g fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The volume of acetone extract was made up to a known one and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer. The concentration of chlorophyll pigments was calculated using the following formula and the results are expressed in mg/g fresh weight.

Chlorophyll a = [(12.7 × OD at 663) – (2.69 × OD at 645)] × ml acetone / mg
Chlorophyll b = [(22.9 × OD at 645) – (4.68 × OD at 663)] × ml acetone / mg
OD= Optical degree

α and β Carotenes content estimation in Plants

Carotenoid content was determined per method of Duxbury and Yentsch, 1956. Weighted 0.5g of fresh plant leaf sample was taken, and homogenized in mortar and pestle with 10 ml of 80% acetone. Then centrifuge for 10,000 rpm. For 15min at 40C. The supernatant were separated and analyzed for carotenoids content in spectrophotometer (EMCLab, Germany). The results are expressed in mg/l fresh weight, and the equation using for the determined α and β carotenes concentration (mg/l) were:

α carotenes = [(OD at 424) × ml acetone ×100] / 2500 ×100
β carotenes = [(OD at 429) × ml acetone ×100] / 2500 ×100

Proline (Pro) Content Determination in Plants

Free proline content in leaves was determined in accordance with the method of (Bates *et al.*, 1973). 0.5 g of Leave samples were homogenized with 5 ml of

sulphosalycylic acid (3%) by mortar and pestle. 2ml of extract and 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added together. Boiled in water bath at 100°C for 30 min. After cooling the reaction mixture, 6 ml of toluene was added, then separated and absorbance at 520 nm in spectrophotometer against toluene blank. A standard curve using to estimated concentration of proline.

Malondialdehyde (MDA) Content Determination in Plants

It was estimated according to Kramer *et.al.* (1996). Reaction mixture was consisting of 1 ml of crude extract with 2 ml of 0.6% TBA, then tubes was sealed tightly and moved to boiling water bath of 100^0C for 15 minutes. Thereafter, tubes were cooled down using ice bath, then centrifuged at 4000 rpm. for 10 min, protein containing residue (because of TCA) was neglected, while supernatant was taken to the spectrophotometer to read at (450, 532, 600) nm. Blank contained same materials except for crude extract that was replaced with phosphate buffer. MDA content was calculated by the following equation:

$MDA\ \mu mol \bullet g^{-1} = [6.45 \times (A532 - A600) - 0.56 \times A450] \times R/W$

Where:

- A532: Absorbance at wavelength 532 nm
- A600: Absorbance at wavelength 600 nm
- A450: Absorbance at wavelength 450 nm
- R: Total reaction volume (3 ml)
- W: Fresh weight of sample

Offline DPPH Assay for Antioxidant Activity Evaluation

The DPPH radical cation method (Pellegrini *et al.*, 1999) was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The DPPH reagent was DPPH (8 mg) dissolved in MeOH (100 mL) for a solution concentration of 80μL/mL. To determine the scavenging activity, 100μL DPPH reagent was mixed with 100μLofsampleina96-well microplate and was incubated at room temperature for 30 min. After incubation, the absorbance was measured 514 nm using an ELISA reader (TECAN, Gr¨ oding, Austria), and 100% methanol was used as a control. The DPPH scavenging effect was measured using the following formula:

Radical scavenging (%)

$$= \left[\frac{(A)\text{control} - (A)\text{sample}}{(A)\text{control}}\right] \times 100. \quad (1)$$

The IC_{50} DPPH values (the concentration of sample required for inhibition of 50% of DPPH radicals) were obtained through extrapolation from regression analysis. The antioxidant was evaluated based on this IC_{50} value.

Results and Discussion

Antioxidant enzymes activities in plants
Superoxide Dismutase (SOD) enzyme activity

SOD enzyme activity consider digital scale to resist free radicals in the plant. The higher value of the enzyme activities, mean more resistant and tolerant the stress. SOD activity in Coriander *(Coriandrum sativum L.)* was significantly 0.09 ($P < 0.05$) higher in 50 unit than other dose Figure (1). The higher SOD activity was 1.98 unit in 50 GY and lower value was 1.08 unit in control treatments. Aerobic organisms have developed various antioxidant mechanisms to prevent damage by ROS. Enzymes such as SOD and POD play a role in plant antioxidant defense systems are the most important electron scavengers. As a member of antioxidant defense system, SOD is a metalloenzyme that catalyzes dismutation of superoxide radicals that accumulate in response to environmental stress factors such as gamma radiation (Cakmak, 2000).

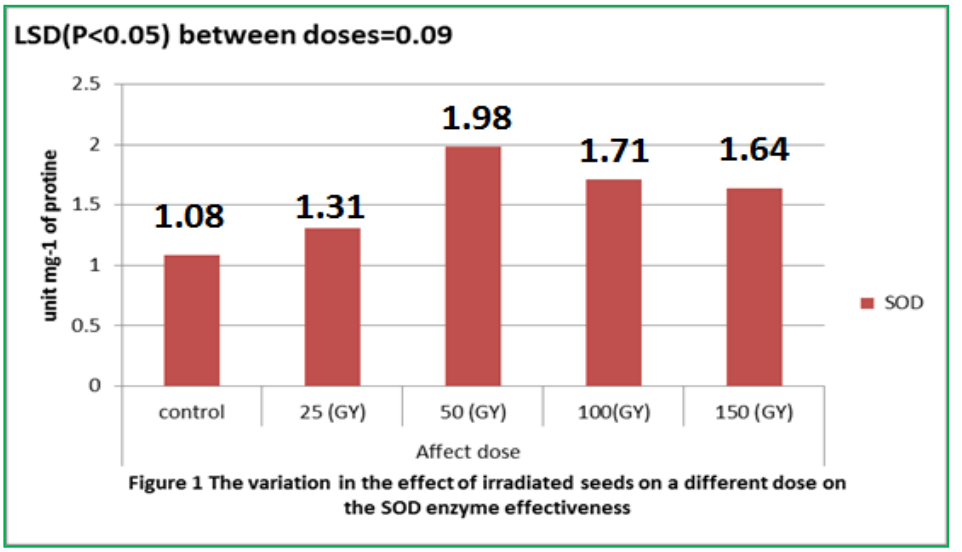

Figure 1 The variation in the effect of irradiated seeds on a different dose on the SOD enzyme effectiveness

Catalase CAT enzyme activity

Figure (2) showed variation in enzyme activity values. Studied treatments increased effectiveness of enzyme by increasing radiation dose. The highest value in 50 GY was 36.2 unit and lowest in control treatments was 32.6 unit and significant differences between radiation doses is 1.27 ($P \leq 0.05$) ,because gamma irradiation could raise CAT activity and could eliminate the accumulation of poisonous free radicals and prevent lipid peroxidation , that's agree with (Jan *et al.* , 2012).

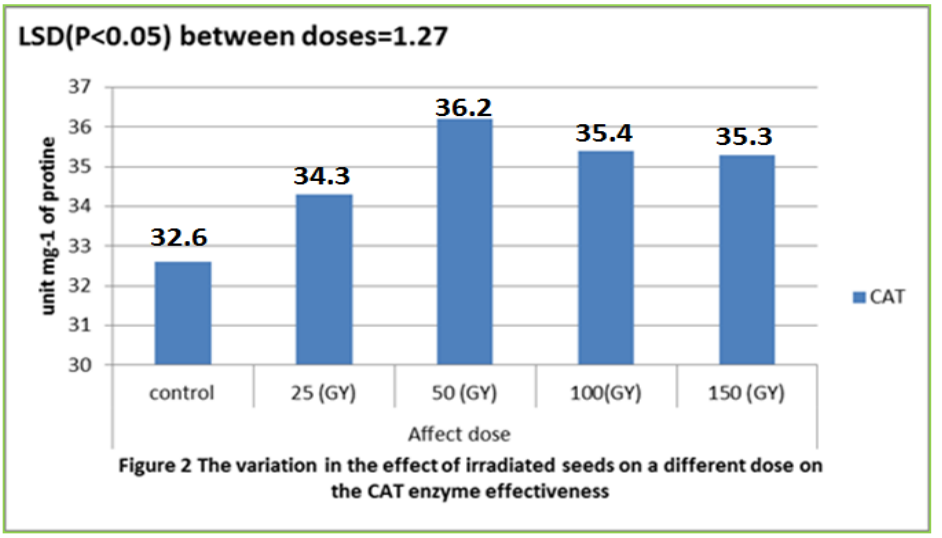

Figure 2 The variation in the effect of irradiated seeds on a different dose on the CAT enzyme effectiveness

Peroxidase (POD) enzyme activity

In the Figure (3), the highest value in 150 Gy treatment was 0.65 unit and lowest in control is 0.23 unit and significant differences between radiation doses is 0.7 ($P \leq 0.05$) . The studied treatments showed that enzyme activities increased by increasing doses of radiation (Fig. 3). This result is in good agreement with previous studies reporting increased POD activity in plants under oxidative stress (Zaka *et al.* ,2002 ; Malecka *et al.* ,2001and Ferreira *et al.*,2002).

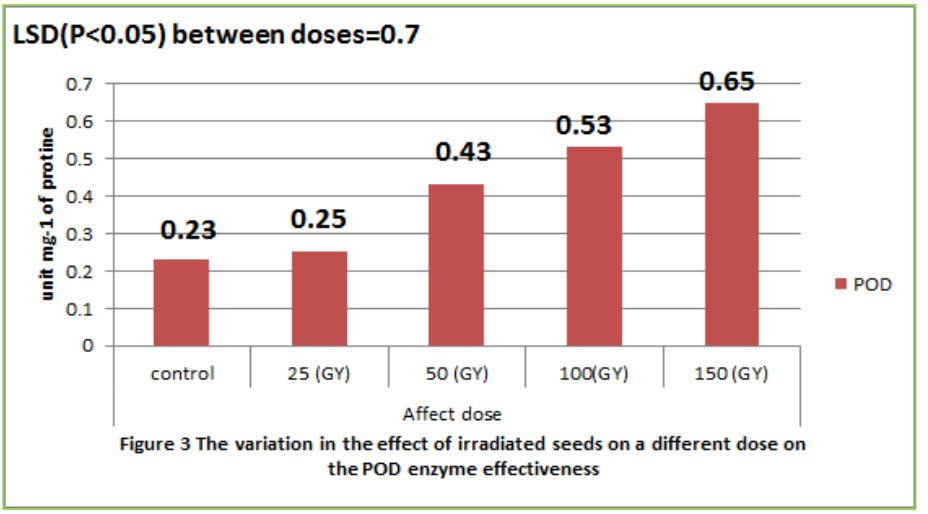

Figure 3 The variation in the effect of irradiated seeds on a different dose on the POD enzyme effectiveness

Antioxidant non enzymatic contents in plants a, b and total chlorophyll contents

The results at Figure (4) showed that a, b and total chlorophyll contents was significantly different between the different dose 0.16 at ($P < 0.05$). The highest

value of chlorophyll content in control was 1.23 mg/l and lowest in 150 GY was 0.87 mg/l. The studied treatment showed the chlorophyll contents were decreased by increasing doses of radiation that's agree with (Alikamanoglu *et. al* ,2011) , these results are in agreement with the findings of Wi *et al.*, (2007) , because of the ultra-structural observations of the irradiated plant cells showed that prominent structural changes of chloroplasts after radiation gamma rays revealed that chloroplasts were more sensitive to a high dose than the other cell organelles, plastids were affected by irradiation in two ways: (i) inhibition of senescence and (ii) dedifferentiation into agranal stage (Kim *et al.* 2004). Gamma radiations also damaged the photosynthetic pigments that reduced photosynthetic capabilities of plants (Kiong *et al.* 2008). A high dose of gamma rays decreases chlorophyll content and decreases the organized pattern of grana and stroma thylakoid (Alikamanoglu *et al.* 2011).

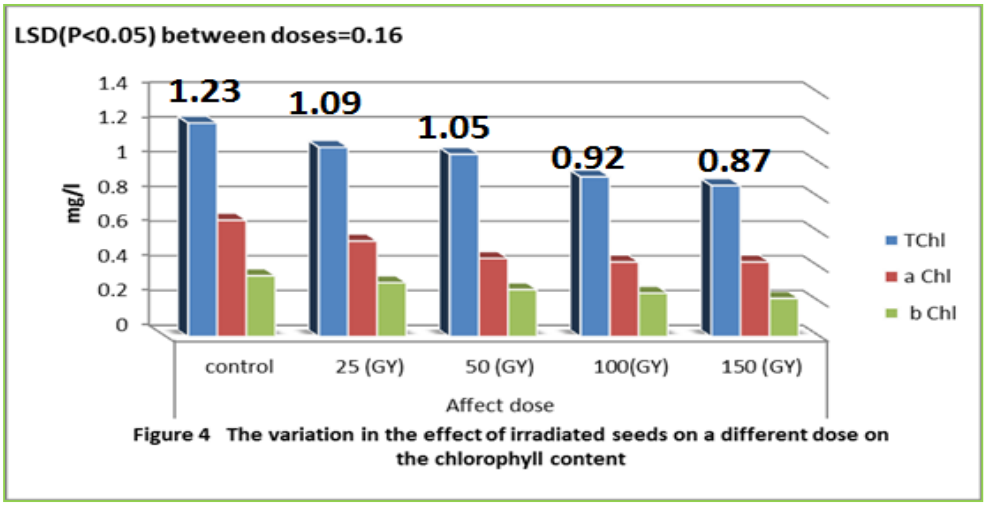

Figure 4 The variation in the effect of irradiated seeds on a different dose on the chlorophyll content

α and β Carotenes

Results showed that the α and β Carotenes content were significantly different between different doses (LSD = 0.06) (P <0.05). The highest value of α and β Carotenes content in 100 GY is 1.54 and 1.21 mg/l respectlly and lowest in control is 0.87 mg/l and 0.55 mg/l respectlly Figure (5). The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kovacs and Keresztes, 2002). These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf, 2009). These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (Ashraf, 2009).
Carotenoids possess dual functions like harvesting light pigment and scavenging of free oxygen radicals at abnormal irradiance levels (Muley *et al.*, 2019).

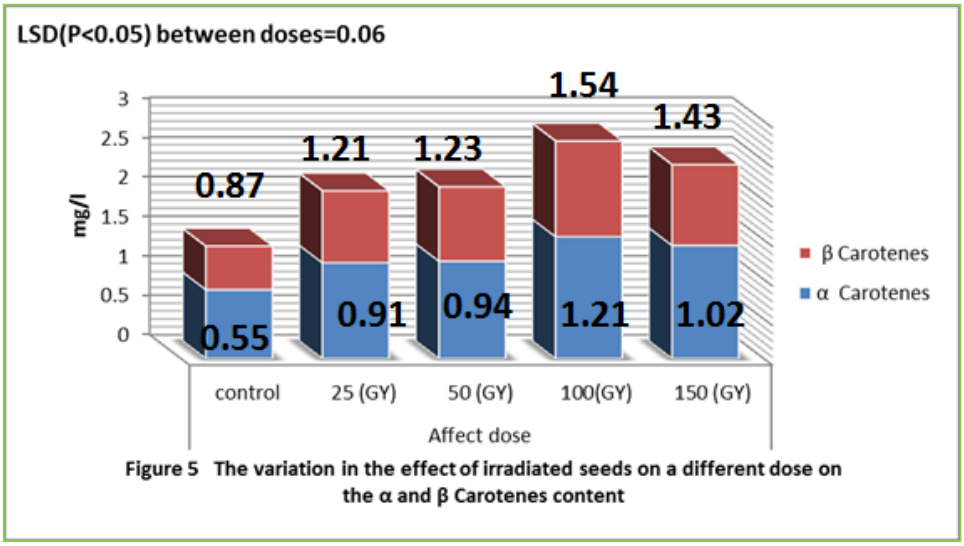

Figure 5 The variation in the effect of irradiated seeds on a different dose on the α and β Carotenes content

Proline content

When the radiation dose was increased in seeds until reach 150 GY, the proline content was increased. The most highest value was observed in 100 GY was 1.41 mmol/g and the lowers value was 0.67 mmol/g in 25 YG .Figure (6). The results showed that the proline content was significantly different between the doses (LSD = 0.8) within a significant level (P <0.05). Proline is a hydrophilic solute that helps in water shortages by replacing water around nucleic acid, protein, and membrane, where proline and nonaqueous tails of protein surface interaction help in increasing protein stability (Irigoyen *et al.*, 1992). Prolines are important solutes that act as osmoregulators by contributing in stress tolerance, protection, hydrophobicity, active oxygen scavenging, and maintaining cell pH (Kuznetsov and Shevyakova 2007). Proline functions to scavenge the hydroxyl radical and acts as a cytosolic osmoticum that helps in regulating and stabilizing various structure and functions such as DNA, protein, and membranes (Kishor et al. 2005). Proline is not the only component involved in stability; it along with other compounds is referred to as “compatible solutes” to maintain the osmolality of cells in various organisms (Yancey 2005). Therefore, the more high proline content is present in a cell, the more it is protected against various stresses, Al-Enezi and Al-Khayri (2012). Our present result was supported by Esfandiari *et al.* ,(2008) who reported that the increase in proline content observed in irradiated plants due to the protective mechanism in the synthesis of osmolytes was essential to plant growth.

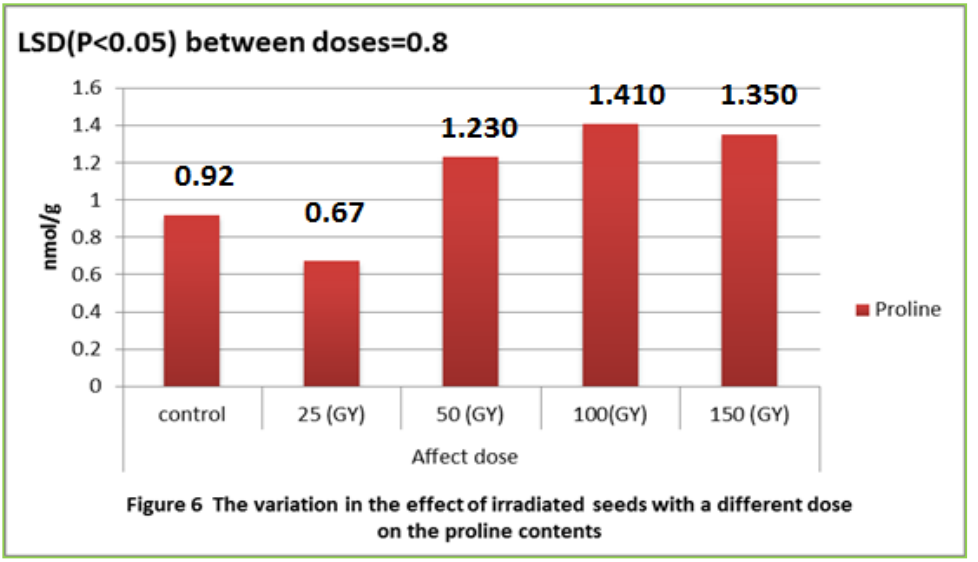

Figure 6 The variation in the effect of irradiated seeds with a different dose on the proline contents

Malondialdehyde (MDA) Contents

Malondialdehyde is a cytotoxic product of lipid peroxidation, MDA was used as an indicator of membrane lipid oxidation. However, the increase in the level of radiation doses caused changes in the levels of MDA concentration, which increased the dose of radiation in the studied plants. This is a guide for all plants but in varying degrees. Figure (7). Where the highest value of MDA in 150 GY was 1.97 mmol/g and the lowest value was 1.32 mmol/g in control treatments.The results were significantly different between the doses (LSD = 0.27) within a significant level (P <0.05). This is also in good agreement with previously published results (Chaoui and Ferjani, 2005 and Chen *et al.* , 2000)

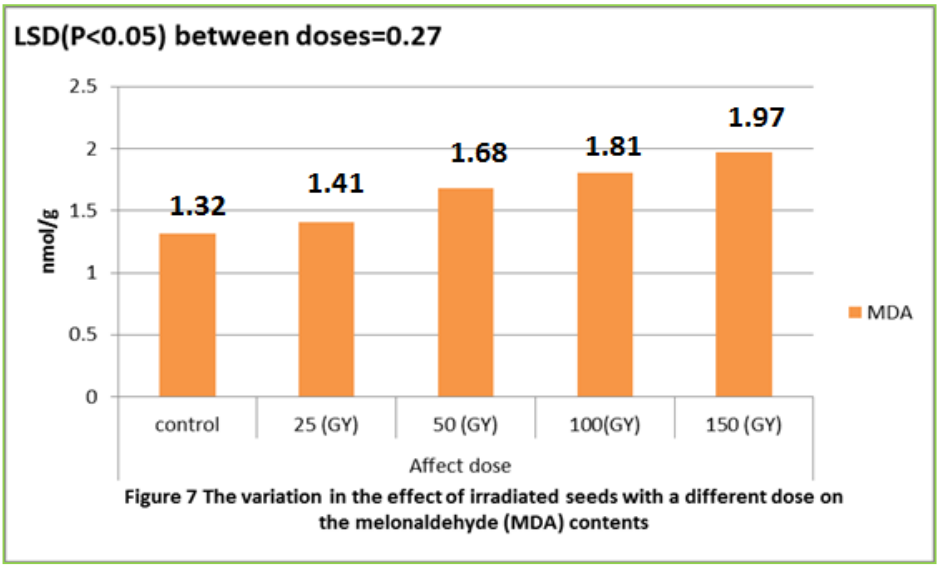

Figure 7 The variation in the effect of irradiated seeds with a different dose on the melonaldehyde (MDA) contents

Offline DPPH Assay

Table 1
Antioxidant activity (Scavenging activity %) of Coriander acetone extract using DPPH assay

Dose of gamma ray / Conc.	control	25	50	100
16	84.5	87.5	88.7	86.8
8	81.9	87	85.3	83.8
4	79.1	83.9	83.4	80.9
2	75.7	82.1	78.4	77.2
1	74.8	78.4	75.7	75.8
0.5	71.7	75.8	75.5	73.6

Table 2
Antioxidant activity (Scavenging activity %) of Coriander methanolic extract using DPPH assay

Dose of gamma ray / Conc.	control	25	50	100
16	83	85.6	87.5	86.1
8	80.9	83.1	83.2	81.8
4	77.8	81.5	77	80.2
2	75.5	77.8	75.9	75.7
1	70.3	75.7	74.2	73.6
0.5	68.7	74.8	72.2	70.4

The results in both tables (1), (2) were established that both acetone and methanolic Coriander extracts showed the variation in their antioxidant activity with diverse concentration and level of irradiation . Also, all extracts exhibit antioxidant activity. And there was variation in the activity in relation to concentration (activity decrease with decrease extract concentration) and to level of irradiation (activity increase with increase level of irradiation) .Table (1) Coriader extract with acetone was showed antioxidant activity in highest percentage at 16 concentration and dose of ray 50 Gy, which reached 88.7, while the lowest value 71.7 at 0.5 concentration and control treatment (not iraadited). As shown in table (2) Coriader extract with methanol gave an antioxidant effect , the highest value at 16 concentration and dose of ray 50 Gy, which reached 87.5 while the lowest percentage 68.7 in the concentration 0.5 and at control treatment (not iraadited). These results combat with study of Hwang *et al.* (2021) ,that was showed as the irradiation dose increased, antioxidant contents (total phenol and flavonoid contents) and antioxidant activities (DPPH radical scavenging activity, ferric reducing power ability, and nitrite scavenging capacity) increased .

Conclusions

According to this study, there was effectiveness of gamma ray on antioxidant activity parameters (enzymatic, non- enzymatic and DPPH) of Coriander.

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Conflict of interest

There is no conflict of interest in this work.

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