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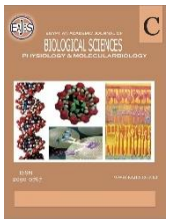

Variation in Antioxidant Enzymes and Lipid Peroxidation in Response to Gamma Irradiation in *Ocimum Basilicum* L.

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ABSTRACT

The present study was conducted to evaluate the effectiveness of different doses of gamma rays (25, 50, 100, 125 and 150) Gy on antioxidant enzymes and lipid peroxidase (MDA) of *Ocimum basilicum* L using C^{60} as an irradiation source. Radiation processing increased the total antioxidants of basil, this increase appeared at low doses (125 Gy) as shown in a significant increase in SOD, CAT, POD and MDA values. Defects in studied parameters increased with increasing gamma doses. Results indicated that the irradiation process can facilitate the utilization of basil as a preservative ingredient in the food and pharmaceutical industry.

INTRODUCTION

Basil is an annual, sometimes perennial, herb used for its leaves. It is an aromatic herb. Plants have basic nutritional importance because of their content of protein, carbohydrate, fats and oils, minerals, vitamins and water which are required for growth and development in man and animals. major nutritional constituents in Basile are (Carbohydrates, lipids, fiber contents, and protein), In addition to some elements and minerals including calcium, iron, phosphorus, magnesium, nitrogen, zinc, cupper, carotene, vitamins A, B6, and C as well potassium and sodium (Shuaib *et al.*,2015; Al-Aubadi, 2011; Dzida,2010)

Gamma radiation, more energetic than X-rays, is implemented from sources of radioactive isotopes, cesium- 137 or cobalt-60, and it is specified by the World Health Organization as a food preservation technique that enhances food safety without modifying the toxicological, biological, or nutritional quality of the food (Diehl, 2002; Datta, 2009; Farkas & Mohacsi-Farkas, 2011). Gamma irradiation was beneficial not only for the sterilization of medicine but also for the preservation of food and cereals in nutrition and agriculture (Mokobia & Anomohanran, 2005) indicating that

Antioxidants are essential substances that inhibit other compounds from being oxidized (Aqil *et al*., 2006). The antioxidants produced by spices and herbs usually act with free radicals created in the initiation phase of autoxidation (Lee *et al* ., 2005). MDA is a widely used marker of oxidative lipid injury caused by environmental stress. A number of studies have investigated MDA of plants under different stress conditions.

(Zhou *et al*.,2015), studied forest trees grown in soil that was exposed to Pb with different levels of water stress, and the results indicated that water stress significantly increased superoxide dismutase (SOD) and peroxidase (POD) activities and MDA content under different Pb concentrations. The data from (Jbir-Koubaa *et al* .,2015) suggested that salinity stress might cause a shock and photo-oxidative stress, which caused MDA accumulation in leaves.

MATERIALS AND METHODS

1. Enzymatic Estimation in Plants:

To extract an enzyme from the plant sample, leave a sample of 0.5 g was homogenized in 50 mm phosphate buffer (ph 7.5) in a prechilled mortar containing 0.5 mm edta (homogenization proceeded while keeping mortar on ice), later the mixture was centrifuged at 4°c in Beckman refrigerated centrifuge at 10000 rpm for 10 min. the supernatant was referred to as enzyme extract (esfandiari *et al.,* 2007). antioxidant enzymes activities included the following enzymes.

1.1. Superoxide Dismutase Activity (SOD):

According to (Marklund & Marklund,1974), a reaction mixture containing 50 µl of crude enzyme extract, 2 ml of Tris Buffer and 0.5 ml of pyragallol (0.2 mM) absorbs light at 420 nm. The control solution contains the same materials except enzyme extract that was replaced by dH_2O. As a blank, dH_2O was used.

A 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)

1.2. Catalase Enzyme Activity (CAT):

It was estimated according to the method of (aebi,1984). reaction mixture 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°c, and then change in optical absorbance at 240 nm wavelength was noted through a minute of time. decrease in absorbance through time was registered. a blank solution consisting of the same contents except for the enzyme extract that was replaced with pbs (phosphate buffers).

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 the quartz cuvette that was used with the spectrophotometer, the volumes will be doubled, so r = 2.04 in order to enable the spectrophotometer light beam to hit the reaction mixture.

• 0.001: a constant

1.3. POD Enzyme Activity:

The 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. The activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (E = 26.6 mM^{-1} cm^{-1}). 55

2 .Malondialdehyde(MDA) Content Determination in Plants :

It was estimated according to(Kramer *et al*., 1996). The reaction mixture consisted of 1 ml of a crude extract with 2 ml of 0.6% TBA, while the supernatant was taken to the spectrophotometer to read at (450, 532, 600) nm. MDA content was calculated by the following equation:

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

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

7. Statistical Analysis:

Data were analyzed by investigating the change of (ANOVA)table Noteworthy contrasts between medications were considered by SSPSS measurable program was utilized to calculate measurable investigations level of importance utilizing Fishers ensured slightest critical contrasts (LSD) Test.

RESULTS AND DISCUSSION

3.1. Effect of Gamma Rays on SOD enzyme of *Ocimium basilicum* L:

SOD activity in *Ocimum basilicum* L. was significantly 13.37 ($P < 0.05$) higher in 125 units than in other doses. The higher SOD activity was 65 units in 125 Gy and the lower value was 29.5 units in 150 Gy treatments. SOD is a metalloenzyme that catalyzes the dismutation of superoxide radicals that accumulate in response to environmental stress factors such as gamma radiation. Enzymes such as SOD and POD play a role in plant antioxidant defense systems and are the most important electron scavengers. As a member of the antioxidant defense system, (Cakmak, 2000)

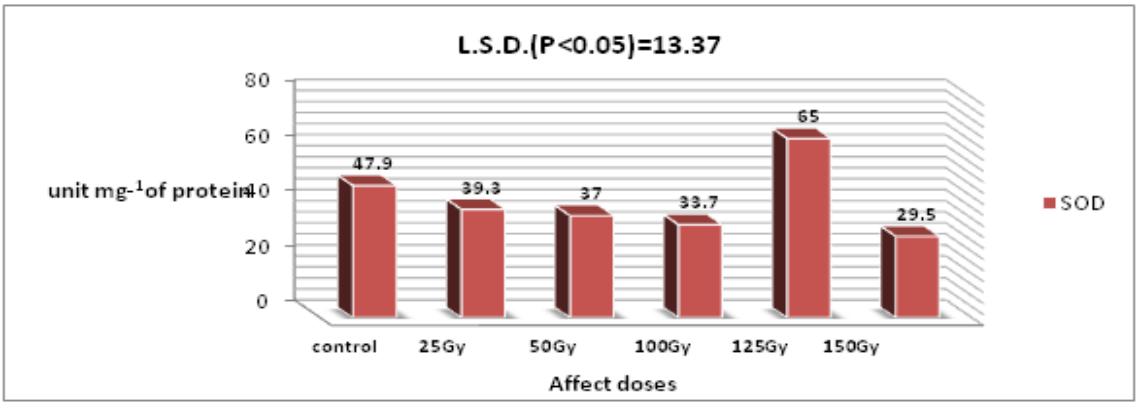


Fig. 2: The variation in the effect of irradiated doses on the SOD enzyme effectiveness

3.2. Effect of Gamma rays on Catalase CAT enzyme activity of *Ocimium basilicum* L.

Figure (3) shows variation in enzyme activity values. Studied treatments increased the effectiveness of enzymes by increasing radiation dose. The highest value in 125 Gy was 99.2 units and the lowest in control treatments was 63.3 units and the significant difference between radiation doses is 18.61 ($P \leq 0.05$). Because gamma irradiation could raise CAT activity and eliminate the accumulation of poisonous free radicals and prevent lipid peroxidation, that's agree with (Jan *et al.*, 2012).

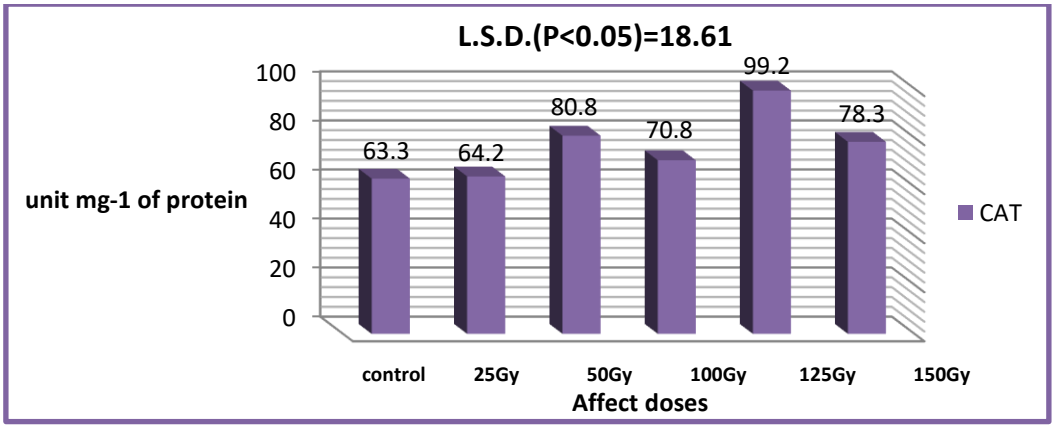


Fig. 3: The variation in the effect of irradiated doses on the CAT enzyme effectiveness.

3.3. Effect of Gamma rays on Peroxidase (POD) enzyme activity of *Ocimium basilicum* L.

In **Figure (5),** the highest value in 125Gy treatment was 18.9 units and lowest in 25Gy was 14.3 units and the significant difference between radiation doses is 9.66 (P <0.05). The studied treatments showed that enzyme activities increased by increasing doses of radiation. This result is in good agreement with previous studies reporting increased POX activity in plants under oxidative stress (Zaka *et al*.,2002; Malecka *et al*.,2001and Ferreira *et al*.,2002).

Kim *et al*. (2004) also characterize stimulatory effects of low-dose gamma radiation on early plant growth; they investigated alterations in the photosynthesis and antioxidant capacity of red pepper (*Capsicum annuum* L.) seedlings produced from gamma-irradiated seeds. For two cultivars (Yeomyung and Joheung), three irradiation groups (2, 4, and 8 Gy, but not 16 Gy) showed enhanced development. They revealed that irradiation altered the compositions of photosynthetic pigments (chlorophylls and carotenoids) as well as the activities of antioxidant enzymes.

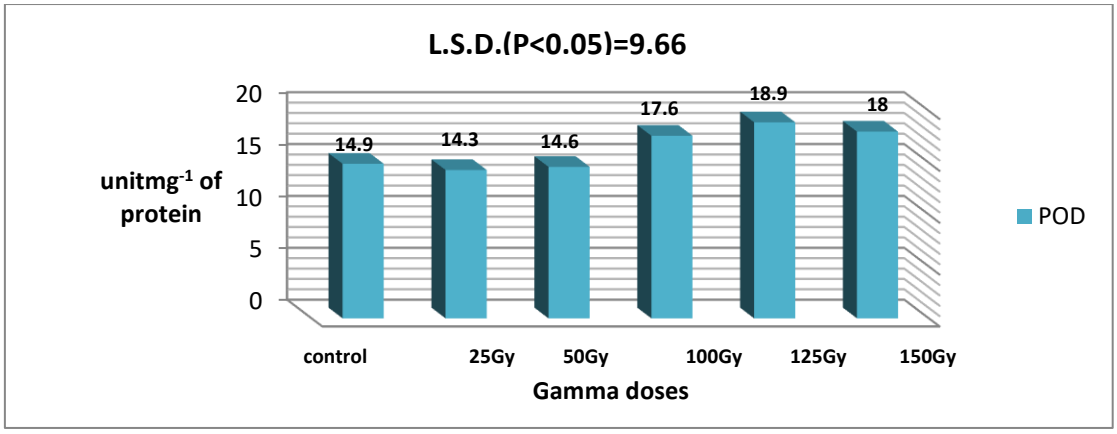
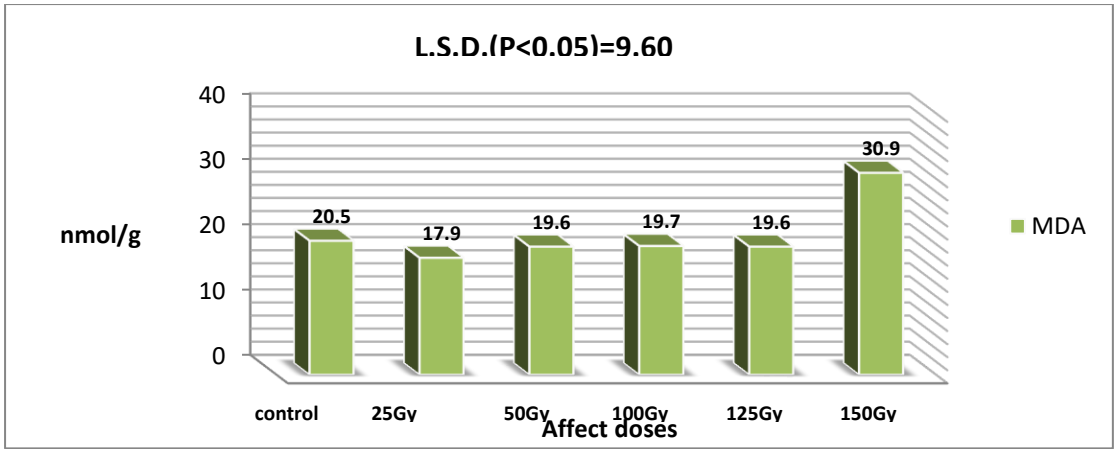


Fig. 4: The variation in the effect of irradiated doses on the POX enzyme effectiveness.

3.4. Effect of Gamma rays on Malondialdehyde Contents of *Ocimium basilicum* L.

Figure (5), the highest value of MDA in 150 GY was 30.9 mmol/g and the lowest value was 17.9 mmol/g in 25Gy treatment. The results were significantly different between the doses (LSD = 9.60) within a significant level (P <0.05). This is in good agreement with previously published results (Chaoui and Ferjani, 2005 and Chen *et al*., 2000)

Generally, Gamma irradiation is physical mutagens that have been reported by many researchers to induce mutations in plants. Radiation exposure can interact with cellular molecules, especially water to produce free radicals (Dehgahi and Joniyasa 2017; Kemal *et al*. 2018). According to Taheri *et al.* (2013), free radicals can combine to form toxic substances such as hydrogen peroxide (H2O2) which will cause damage to the cells. This is very important, especially in vegetative cells, because the cytoplasmic component is 80% water (Kovac and Keresztes 2002). The damage that occurs in cells directly affects the morphology, biochemistry, and physiology of plants. Generally, the damage is highly dependent on the sensitivity of cells or tissues in plants. (Due *et al*.,2019).



Fig; 5: The effect of a different dose of gamma rays on MDA content nmol/g of *Ocimum basilicum* L.

4. Conclusion

In conclusion, the present data suggest that relatively high doses of gamma rays increase the antioxidant enzymes of basil plants. Gamma irradiation at 125 Gy was superior in the enhancement of these parameters, whereas, a high dose of gamma irradiation (150 Gy) caused a decrease in these antioxidant enzymes, but an increase in lipid peroxidase MDA.

Declarations:

Ethical Approval:Ethical Approval is not applicable.

Competing interests: The authors declare no conflict of interest.

Authors Contributions:I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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