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Gamma-Irradiation Primed Thyme for Enhanced Antioxidant and Antiaging Properties: A Delayed Cultivation Study

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ABSTRACT

This study examined the effects of low-dose gamma irradiation (1, 3, and 5 Gy) on the antioxidant compounds and properties of *Thymus vulgaris* compared with those of nonirradiated control plants. The persistence of irradiation effects was evaluated through pot experiments conducted immediately following seed treatment and again six months later, with analyses performed at the initial and subsequent cultivation stages. The results revealed that irradiation at 1 and 3 Gy significantly reduced lipid peroxidation and hydrogen peroxide levels, whereas 5 Gy irradiation significantly increased these levels. A dose-dependent increase in total phenolic and flavonoid content was observed up to 3 Gy, after which a decrease was noted at 5 Gy. Antioxidant activity, measured using the ABTS, DPPH, and FRAP assays, was greater in all treatment groups than in the control group during the first cultivation. In the second cultivation, antioxidant activity, particularly in the 3 Gy treatment group, significantly increased and maintained its effectiveness over six months. These findings suggest that low-dose can sustainably increase the production of bioactive compounds in *T. vulgaris*. This study revealed that low-dose gamma irradiation has a hormetic effect on *T. vulgaris*, promoting the production of antioxidant compounds and improving its antioxidant properties.

Introduction

Cancer (Kruk and Aboul-Enein, 2017), diabetes (Nogueira et al., 2018), neurological disorders (Hassan et al., 2022), respiratory diseases (Di Stefano, 2020), and premature aging (Hajam et al., 2022) are among the many acute and chronic degenerative diseases that can develop when there is an imbalance between the production of ROS and RNS and the body's antioxidant defense mechanisms. Antioxidants, whether produced internally or externally, play crucial roles in lowering oxidative stress and improving immunological function, as the detrimental effects of ROS and RNS demonstrate. Immune cells, such as macrophages and lymphocytes, are particularly susceptible to damage by reactive oxygen species (ROS) and reactive nitrogen species (RNS). These antioxidants protect cellular structures against oxidative damage (Adwas et al., 2019).

An all-encompassing defense strategy against infections may involve the use of antioxidants, according to previous studies (Wu and Meydani, 2019; Ma et al., 2003), because they modify signaling pathways critical for immune responses, affect cytokine production, stimulate immune cells, and regulate inflammation. This fine line emphasizes how important it is to balance one's redox status for optimum health and illness avoidance.

Phytochemicals, including plant-based compounds, polyphenols, terpenes, and alkaloids, can fight oxidative stress. Free radical scavenging is one of the many benefits of these all-natural substances (Chiocchio et al., 2021). The powerful antioxidant capabilities of polyphenols, a diverse class of

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compounds that include flavonoids and phenolic acids, have been the subject of much research (Rudrapal et al., 2022).

Because of their beneficial effects on health, phytochemicals have been widely used in traditional medicine and have even been incorporated into modern beauty products, perfumes, and preservatives. The importance of secondary plant metabolites in human health promotion has been widely acknowledged in recent years, with research demonstrating their remarkable anticancer, antibacterial, and antidiabetic activities (Gadnayak and Dehury, 2023).

Antioxidant activity is a notable characteristic of *Thymus vulgaris* L. or garden thyme. This activity is attributed to the presence of many chemicals, including thymol, carvacrol, and p-cymene, in addition to several flavonoids and phenolic compounds (Hammoudi Halat et al., 2022; Dauqan and Abdullah, 2017). The Mediterranean plant has robust antioxidant properties and possible therapeutic advantages, such as antimicrobial, antifungal, and antiviral effects, making it widely used in the kitchen, medical field, and several industrial contexts (Kowalczyk et al., 2020). Agricultural and food technology fields have investigated gamma irradiation applications, especially from Co60 sources, because of its potential to increase genetic diversity, improve food safety, and cause mutations (Beyaz and Yildiz, 2017). According to Wang et al. (2022), seed priming with low radiation levels may improve seed viability, germination, seedling strength, and resistance to stress.

Gamma irradiation significantly affects seed priming, germination, and seedling vigor in *Thymus vulgaris*, with effects varying by dose. Optimal doses, such as 3-5 Gy, improved shoot and root lengths and shoot and root fresh weights by enhancing enzyme activation (kordrostami et al., 2024). Similar benefits were observed, including significantly improved germination speed to 2.40% d^{-1}, whereas low-dose -irradiation resulted in the highest dry weight of shoots and leaf number (19.40 leaf $plant^{-1}$) at 20 Gy, indicating increased seedling vigor in *Thymus vulgaris* (Taher et al., 2021). However, excessive doses negatively affect growth, reducing chlorophyll content, photosynthetic functionality, and overall vigor, despite potential increases in phytochemical content (Mohammadi et al., 2024). These findings underscore the need to balance irradiation doses carefully to enhance seedling vigor and seed priming without compromising plant health.

This research examined the hormetic effects of gamma rays on the antiaging capabilities of seeds, with a specific focus on how priming with gamma rays influences the antioxidant activities of *Thymus vulgaris*. By evaluating the time-dependent effects of post-irradiation cultivation, this study seeks to expand the application of thyme in the pharmaceutical, nutraceutical, and cosmetic industries.

Material and methods

Plant materials

Seeds of *Thymus vulgaris* Cv: Varico 3 were acquired from Pakan Bazr TM, which is located in Esfahan, Iran. The chemicals used in this investigation were analytical high-performance liquid chromatography (HPLC) grade and were obtained from Merck (Germany).

Seed preparation and irradiation

Thymus vulgaris Cv: Varico 3 seeds were first prepared for irradiation by separating and identifying healthy, uniform seeds to ensure consistency across treatments. A total of 240 seeds were selected and divided into four groups: control (no irradiation), 1 Gy, 3 Gy, and 5 Gy. Each group contained 60 seeds (20 seeds per replication).

Seed irradiation procedure

The seeds were subjected to γ-irradiation at the Nuclear Science and Technology Research Institute in Karaj, Iran. The irradiation was performed using a cobalt-60 γ-irradiator at a dosage rate of 0.018 Gy s^{-1}. Dosimetry was performed using a Fick reference standard dosimetry system (Gamma-cell Issledovatel, PX-30) to ensure precise delivery of radiation doses.

Cultivation

After irradiation, the treated seeds were divided into two groups. The first group was cultured immediately following the irradiation procedure whereases the second group was stored at room temperature for 6 months prior to cultivation. The subsequent treatments and experimental procedures were identical for both groups.

The tests were categorized as "1st cultivation" or "2nd cultivation" according to their respective cultivation durations. The seeds were surface sterilized with a 5% sodium hypochlorite solution for 5 min and then rinsed with sterilized water simultaneously. The seeds were then sprouted on Whatman-grade 181 filter papers in Petri plates. The resultant seedlings were then transferred into pots placed in cell trays with sterilized peat moss and left undisturbed for three weeks to establish. After the seedlings had taken root, they were placed in 3-L pots filled with a uniform blend of well-mixed loamy clay soil. This process occurred in a controlled greenhouse environment. The greenhouse maintained a consistent temperature of 24 °C/12 °C, with relative humidity levels fixed at 50-50%. The duration of light exposure was 16 h, and the light

intensity for photosynthesis varied from 850 to 950 μmol m^{-2} s^{-1}.

Indicators of oxidation

The content of H_2O_2 in the leaf samples was assessed using the colorimetric technique outlined by Velikova and Yordanov (2000). The aboveground portions of the plants were collected and immediately preserved by freezing in liquid nitrogen. The shoot samples were pulverized and mixed well in trichloroacetic acid (TCA) at a concentration of 0.1%. The mixture was then centrifuged at 10,000 × g for 20 min. The supernatant was combined with phosphate buffer (pH 7.0) and 1 mL of potassium iodide (KI). The absorbance was determined at a wavelength of 390 nm using pure water as the reference solution.

The evaluation of lipid peroxidation in shoot samples was conducted by measuring the concentration of malondialdehyde (MDA). The MDA test involved extracting frozen shoot powder using a solution containing TCA and TBA (20% (w/v)) trichloroacetic acid and 0.5% (w/v) thiobarbituric acid. The samples were subjected to heat treatment in a water bath at 95 °C for 30 min. The mixture was then promptly cooled and subjected to centrifugation at a speed of 10,000 × g for 10 min at 4 °C. The absorbance at 532 nm was measured for each component. In contrast, the absorbance at 600 nm was measured for non-specific components. The MDA concentration was calculated by subtracting the nonspecific absorbance from the specific absorbance using an attenuation value of 155 mM^{-1} cm^{-1}.

Extraction process

A botanical extract was derived from recently dehydrated aboveground portions of the samples. 2 g of plant material were mixed with 15 mL of 70% methanol and subjected to maceration with continuous agitation for 24 h. After incubation, the sample extracts were centrifuged at 1000 the acceleration force due to gravity for 15 min. The obtained supernatants were collected and stored at -20 °C until further analysis.

Measurement of total phenol content (TPC)

The concentrations of polyphenolic chemicals in the extracts were measured using the Folin–Ciocalteu reagent technique. A 20 μL portion of the suitably diluted extract was combined with 20 μL of recently made Folin-Ciocalteu reagent and allowed to sit at room temperature for 6 min. Two hundred μL of a 7% sodium carbonate solution and 10 μL of deionized water were added to each well. The solutions were vigorously combined and incubated in the dark at ambient temperature for 120 min. The absorbance was measured at 750 nm using a microplate reader. The reference standard gallic acid was used at different concentrations for external calibration.

Measurement of total flavonoid content (TFC)

The flavonoid content in the extracts was quantified via a colorimetric approach that relies on the aluminum chloride reagent. This method was derived from Koley et al. (2018), with a minor adjustment. Exactly 50 μL of the sample extract or a reference solution was carefully added to a 96-well plate. Additionally, 10 μL of 10% aluminum chloride, 100 μL of 96% ethanol, and 10 μL of 1 M sodium acetate were added to the plate. The plate was placed in the dark and incubated for 40 min at room temperature. The light absorption level at 415 nm was then determined using a nanospectrophotometer.

A standard curve was created for quercetin solutions in 96% ethanol. The solution concentrations ranged from 0 to 500 μg mL^{-1}, and absorbance readings were recorded. The total flavonoid content (TFC) was quantified as milligrams of quercetin equivalents per gram of dry weight (mg QE g^{-1} DW).

FRAP analysis

The FRAP test was performed using the potassium ferricyanide-ferric chloride technique, following the protocol outlined by Ustundag et al. (2016). This procedure involves the conversion of Fe^{3+} in the Fe^{3+}-TPTZ complex (a colorless complex consisting of ferric-2,4,6-tripyridyl-s-triazine) to Fe^{2+}-TPTZ (a blue-colored complex) by the use of electron-donating antioxidants in an environment with low pH. The reagents used consisted of a 300-millimolar solution of sodium acetate, a 10 mmol solution of TPTZ (40 mmol L^{-1} of hydrochloric acid), and $FeCl_3 \times 6 H_2O$ (20 mmol). The FRAP working solution was produced by combining 25 mL of acetate buffer with 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3 \times 6 H_2O$ solution. It is essential to develop a functional solution from abrasion frequently. In the test, a recently developed functioning FRAP reagent (280 μL) was mixed with the plant extract (20 μL) in a 96-well plate. The absorbance was measured at 593 nm after 10 min incubation at 37 °C. A calibration curve was generated using ferric sulfate ($FeSO_4 \times 7H_2O$). The antioxidant activity was quantified as milligrams of Fe2+ per milligram of dry weight (mmol Fe^{2+} mg^{-1} DW).

Evaluation of the DPPH radical scavenging activity

The plant extracts were evaluated for their antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical as a reagent, following the procedure described by Blois in 1985. DPPH is an enduring radical that is discolored by

exposure to antioxidants. A solution of DPPH in ethanol with a concentration of 0.2 mM was produced and then diluted until the absorbance readings were between 1.00 and 1.10. A daily ascorbic acid (ASC) solution was prepared in water at a concentration of 1 µg mL^{-1}. To initiate the reactions, 50 L of each sample were combined with 150 L of the DPPH solution in a 96-well plate. The plate was delicately agitated for 30 s and then left undisturbed in a lightless environment for 30 min. The light absorption level was quantified at a wavelength of 520 nm after the incubation period. The experiments were conducted in five repetitions. The antioxidant activity was determined using the following formula: (A.1) The radical scavenging activity was calculated using the following formula:

$$radical\ scavenging\ activity\ (\%) = \frac{AControl - ASample}{AControl} \times 100$$

ABTS radical cation scavenging

The antioxidant capacity of the alcoholic extracts of treated plants against ABTS was evaluated using a methodology similar to that outlined by a cited source (Giao et al., 2007). ABTS was first dissolved in deionized water until a concentration of 7 mM was reached. Afterward, the mixture was combined with a potassium persulfate solution at a concentration of 2.45 mM in equal proportions, and the resulting mixture was stored in the dark until a solution with a dark color, including ABTS radical cations, was created. The solution was then mixed with methanol until it reached an absorbance of 0.70 ± 0.1 at 734 nm. To assess the ability of the sample/standard (ascorbic acid) to remove free radicals, 190 µL of the ABTS working solution was combined with 10 µL of the sample/standard in a microcuv cuvette. The reduction in absorbance was measured at 734 nm. The scavenging activity percentage of each extract against the ABTS radical was determined using the following formula: (A.2) The radical scavenging activity percentage was calculated using the following formula:

$$\frac{AControl - ASample}{AControl} \times 100.$$

Statistical analysis

The analyses were conducted in triplicate, and the findings are presented as the means ± standard deviations. The statistical program SPSS version 23 was used to analyze variance (ANOVA), and Tukey's test was used to detect any disparities among means. Moreover, $P \leq 0.05$ was considered significant.

Results

In this study, *Thymus vulgaris* seeds were exposed to gamma radiation doses of 1, 3, and 5 Gy, and the impact of seed priming on various traits of the resulting plants was examined (Fig. 1). The results from the first cultivation indicated that the fresh weight of the aerial parts significantly increased to 4.463 g in the 3 Gy irradiation treatment group compared with 3.567 g in the control group. The fresh weight after 1 Gy irradiation did not significantly differ from that after 3 Gy irradiation. However, the fresh weight of plants irradiated with 5 Gy (1.447 g, representing more than a 59% reduction) was significantly lower than that of control plants. In the second cultivation stage, the fresh weight in the 1 and 3 Gy treatment groups was significantly greater than that in the control group. The fresh weight under the 5 Gy treatment (2.9367 g) was lower than that under the control treatment (3.713 g), representing a reduction of just over 36% (Fig. 2A). The dry weight measurements during the first cultivation period indicated that although the 3 Gy treatment (1.23 g) resulted in the highest weight among all the treatments, the difference between this treatment and the 1 Gy and control treatments was not statistically significant. The dry weight of the 5 Gy treatment (0.6768 g) was significantly lower than that of the control. At the second harvest, there were no significant differences in dry weight between the 1 and 3 Gy groups, with values of 1.133 g and 1.2 g, respectively. Compared with the control group, which had a dry weight of 0.93 g, both treatments resulted in significant increases in dry weight. However, the dry weight (0.8 g) of the 5 Gy treatment group did not differ significantly from that of the control group (Fig. 2B).

An investigation into the effects of gamma irradiation on hydrogen peroxide (H_2O_2) content in thyme plants over two cultivation periods yielded multifaceted results. Initially, the H_2O_2 content in the control group was measured at 1.046 nmol mg^{-1} FW, which then naturally increased to 1.53 nmol mg^{-1} FW in the second cultivation period, indicating potential environmental or developmental impacts on H_2O_2 levels. Seeds irradiated with 1 or 3 Gy exhibited slight, nonsignificant increases in H_2O_2 content (1.25 and 1.2 nmol mg^{-1} FW, respectively) in the first cultivation period (Fig. 3A). However, the second period resulted in a significant reduction in H_2O_2 for these treatments (1.1 nmol mg^{-1} FW for 1 Gy and 1.07 nmol mg^{-1} FW for 3 Gy) compared with the control, without a significant difference between the two doses. On the other hand, the 5 Gy treatment resulted in a notable increase in H_2O_2 levels during both cultivation periods (1.88 nmol mg^{-1} FW in the first and 1.99 nmol mg^{-1} FW in the second) (Fig. 3A), suggesting a dose-dependent relationship that may reflect a hormetic effect or an induction of stress responses at higher radiation levels.

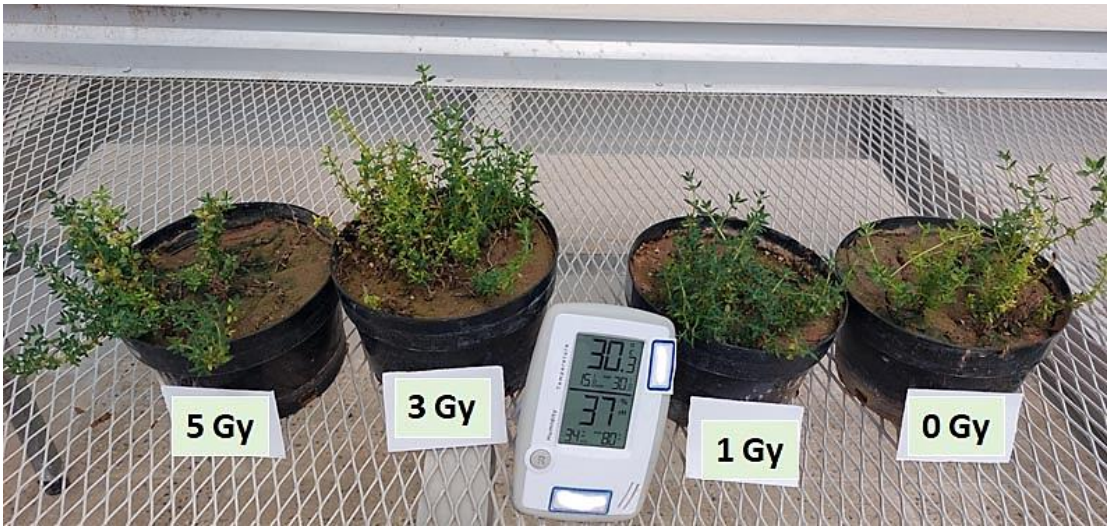

Fig. 1. Plants emerged from seeds irradiated with gamma radiation doses of 1, 3, and 5 Gy.

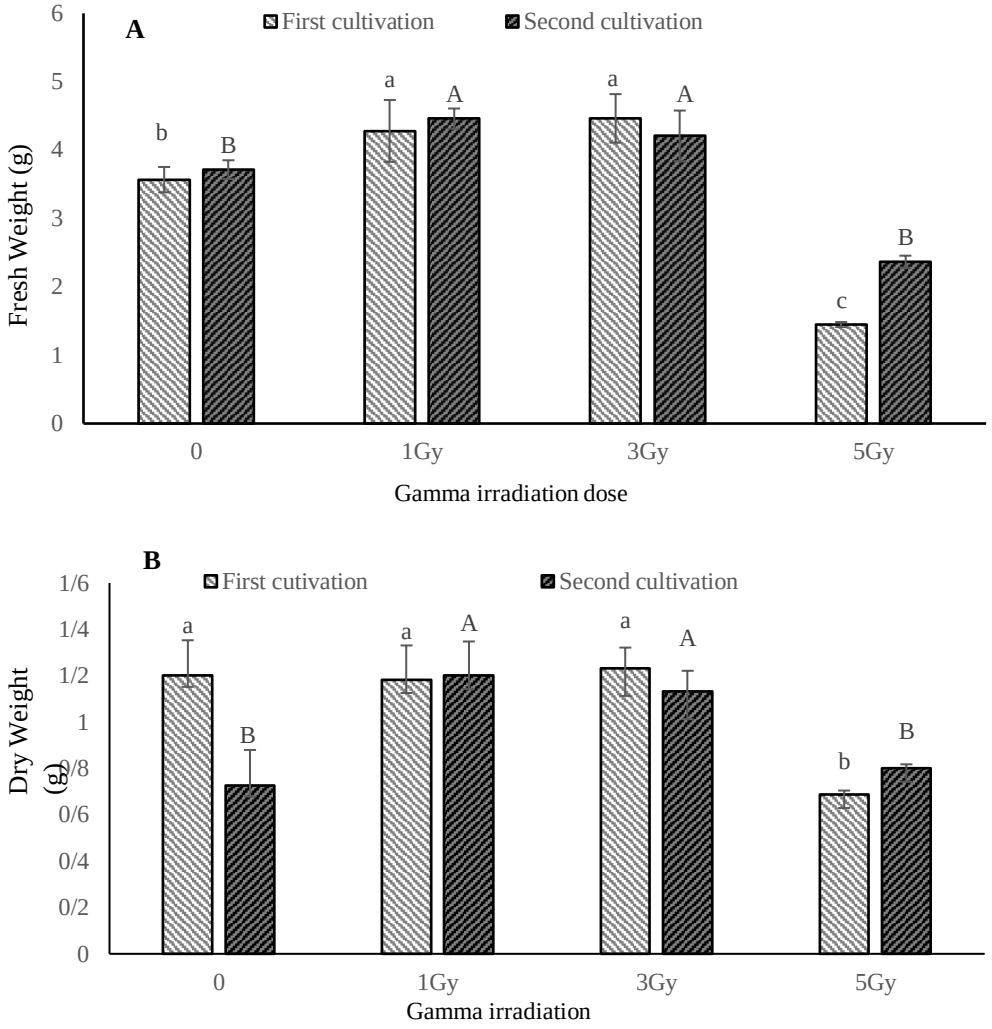

Fig. 2. Effects of gamma radiation on (**A**) fresh weight; and **B**) dry weight of *Thymus vulgaris* plants emerging from seeds irradiated with different doses of gamma rays during the first and second cultivations. The data from the first cultivation are represented by lowercase letters, whereas the data from the second cultivation are represented by uppercase letters. The error bars represent the SEs (n = 3). The mean value in the same series is significantly different when represented with different letters ($P \leq 0.05$).

These observations reveal that although moderate doses of gamma irradiation may confer temporary regulatory benefits to H_2O_2 content, potentially contributing to stress tolerance, higher doses appear to intensify H_2O_2 accumulation, which could lead to stress-related challenges for the plant.

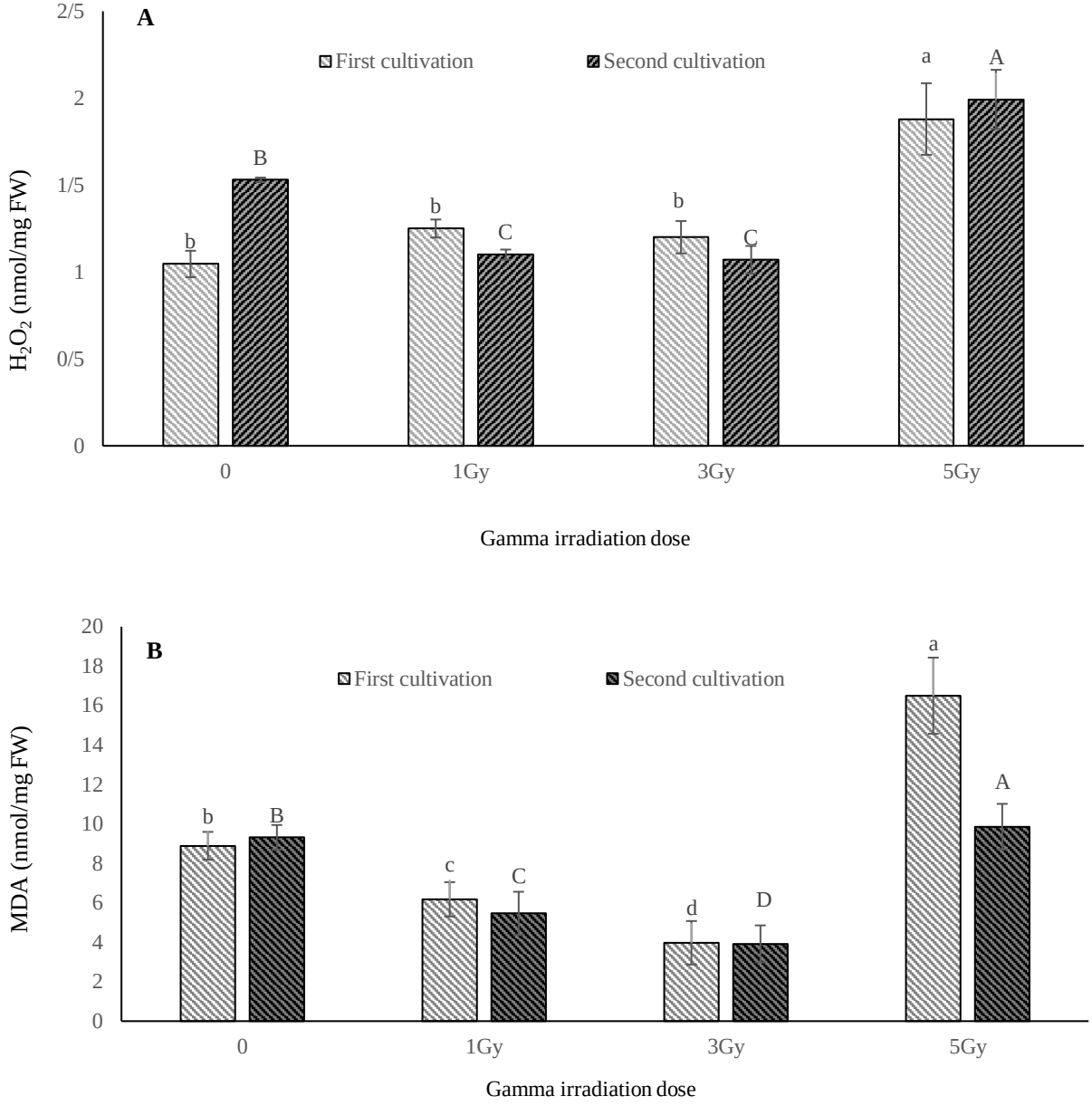

Fig. 3. Gamma radiation affects (**A**) the H_2O_2 concentration; and (**B**) the MDA content of *Thymus vulgaris* plants emerging from seeds irradiated with different gamma rays during the first and second cultivations. The data from the first cultivation are represented by lowercase letters, whereas the data from the second cultivation are represented by uppercase letters. The error bars represent the SEs (n = 3). The mean value in the same series is significantly different when represented with different letters ($P \leq 0.05$).

Investigating the effects of gamma irradiation on malondialdehyde (MDA) content in *T. vulgaris* over two cultivation periods revealed the relationship between irradiation dose and oxidative stress. The level of MDA, a marker of lipid peroxidation, decreased by 30.48% and 55.34% under the 1-Gy and 3-Gy treatments, respectively, in the first planting, whereas the 5-Gy treatment increased the MDA level by 85.6% (Fig. 3B). In the second planting, the 1 and 3 Gy treatments significantly decreased the MDA content by 41.3% and 58%, respectively, whereas the 5 Gy treatment slightly but nonsignificantly increased the MDA content by 5.8% (Fig. 3B). These findings are consistent with the notion that seed priming, including irradiation, can reduce reactive oxygen species (ROS) levels and

subsequent lipid peroxidation, as observed in various plant species under stress conditions. For example, different seed priming techniques decrease ROS and MDA levels in rice and pea plants, mitigating stress impacts. Conversely, some forms of priming, such as hydrogen peroxide treatment in cauliflower, can increase the levels of oxidative markers, such as H_2O_2, O_2^-, and MDA. Moreover, pretreatment of sweet osmanthus with -irradiation resulted in reduced superoxide production but elevated lipid peroxidation. In *Thymus vulgaris*, the baseline MDA content of the control group increased slightly across the cultivation period, suggesting increased oxidative stress. The 1 and 3 Gy treatments effectively reduced oxidative stress, with the most substantial reduction in MDA content observed at 3 Gy, which was maintained over time. The initial increase in oxidative stress at 5 Gy followed by a decrease in the second cultivation period indicates a complex response. Higher doses may initially intensify stress but trigger adaptive mechanisms that partially alleviate the effects. These observations underscore the delicate interplay between irradiation doses and plant oxidative status, where moderate doses enhance plant health by reducing oxidative stress. Nevertheless, higher doses may trigger stress responses that harm plant health. The dose-dependent effects of irradiation on MDA content in *Thymus vulgaris* highlight the potential for optimizing irradiation protocols to increase plant resistance and health. This strategy could benefit agricultural practices and plant-based pharmacology.

In this comprehensive study, the effects of gamma irradiation on the phenol content of *Thymus vulgaris* were meticulously explored over two cultivation periods, revealing a complex, dose-dependent response to irradiation. Initially, the control group established a baseline phenol content of 26.82 mg g^{-1} DW, which notably decreased by approximately 22.6% by the second cultivation period, suggesting environmental or developmental influences on phenolic synthesis. Conversely, irradiation treatments presented a dynamic increase in phenol accumulation: a significant increase to 32.20 mg g^{-1} DW was observed with 1 Gy irradiation, and an even more pronounced response was noted for the 3 Gy treatment, culminating in the highest recorded phenol content of 39.80 mg g^{-1} DW in the second period. The 5 Gy treatment also modestly increased the phenolic content, indicating a nuanced interplay between the irradiation dosage and phenolic biosynthesis. Owing to their antioxidative properties, the biosynthesis of phenolic compounds, which are vital for plant defense against reactive oxygen species (ROS), is markedly influenced by varying radiation doses. Compared with the 1 Gy and 5 Gy treatments, the first treatment had superior effects on increasing the phenolic content (34.73 mg GAE g^{-1} DW), indicating a dose-specific increase in phenolic accumulation but to a lesser extent. In the second cultivation, a significant increase in total phenolic content was recorded across all irradiation treatments, with the 3 Gy treatment again leading to phenolic enrichment (39.7 mg GAE g^{-1} DW), followed by the 1 Gy and 5 Gy treatments (Fig. 4A). This progression confirms the potential of moderate gamma irradiation doses, particularly at 3 Gy, to substantially increase phenolic content, potentially through a hormetic effect where mild stress invokes beneficial responses in plants, increasing their phenolic profiles and, by extension, their antioxidative capacity. These findings elucidate a critical observation: while the 1 Gy treatment effectively increased the phenolic content, suggesting an optimal threshold for enhancing phenolic synthesis without inducing stress, the 5 Gy treatment, despite its positive impact, resulted in a diminishing return at higher irradiation doses. This intricate relationship between gamma irradiation and phenolic compound synthesis not only highlights the potential for optimizing the phenolic profile of *Thymus vulgaris* for agricultural and pharmacological applications and underscores the importance of dose selection in the use of gamma irradiation as a tool for enhancing plant resistance and biochemical properties.

The investigation of the impact of gamma irradiation on the flavonoid content in *T. vulgaris* provides a comprehensive understanding of how different doses influence the production of crucial phenolic compounds, which are known for their potent antioxidant activity. Flavonoids are a significant subgroup of phenolic compounds characterized by low molecular weight, and they play pivotal roles in plant defense mechanisms against oxidative stress. This study revealed a dose-dependent response in flavonoid biosynthesis, with the total flavonoid content significantly increasing under 1 and 3 Gy treatments, highlighting an increase in the antioxidant capacity of the plants. Specifically, during the first cultivation period, the greatest increase in flavonoid content was observed at 3 Gy (16.1 mg QUE g^{-1} DW), whereas at 1 Gy (14.04 mg QUE g^{-1} DW), the flavonoid content was notably greater than that of the control (11.56 mg QUE g^{-1} DW). However, the 5 Gy dose led to a decreased flavonoid content (12.78 mg QUE g^{-1} DW), suggesting an optimal irradiation threshold for flavonoid biosynthesis. This study aimed to investigate the effects of seed priming on seedling establishment and seed aging, focusing on the effects of gamma-ray priming on physiological responses related to plant aging. Specifically, this study examines the hormetic effects of gamma rays and their impact on the anti-aging potential of seeds, with an emphasis on how gamma-ray priming enhances antioxidant activities in ***Thymus vulgaris***. By evaluating the time-dependent effects of post-

irradiation cultivation, this study expands the application of thyme in pharmaceutical, nutraceutical, and cosmetic industries. During the second cultivation period, the trend continued with the 3 Gy treatment, which resulted in the most substantial increase in flavonoid content (17.70 mg QUE g^{-1} DW), further confirming the efficacy of moderate gamma irradiation doses in promoting flavonoid accumulation, potentially through hormetic effects where mild stress triggers beneficial plant responses. Although the 1 Gy treatment also resulted in increased flavonoid content (14.2 mg QUE g^{-1} DW), the 5 Gy treatment (13.92 mg QUE g^{-1} DW) did not significantly differ, indicating a nuanced relationship between the irradiation dose and flavonoid synthesis (Fig. 4B). This observation underscores the hormetic zone in which irradiation acts as a positive stressor, increasing secondary metabolite production.

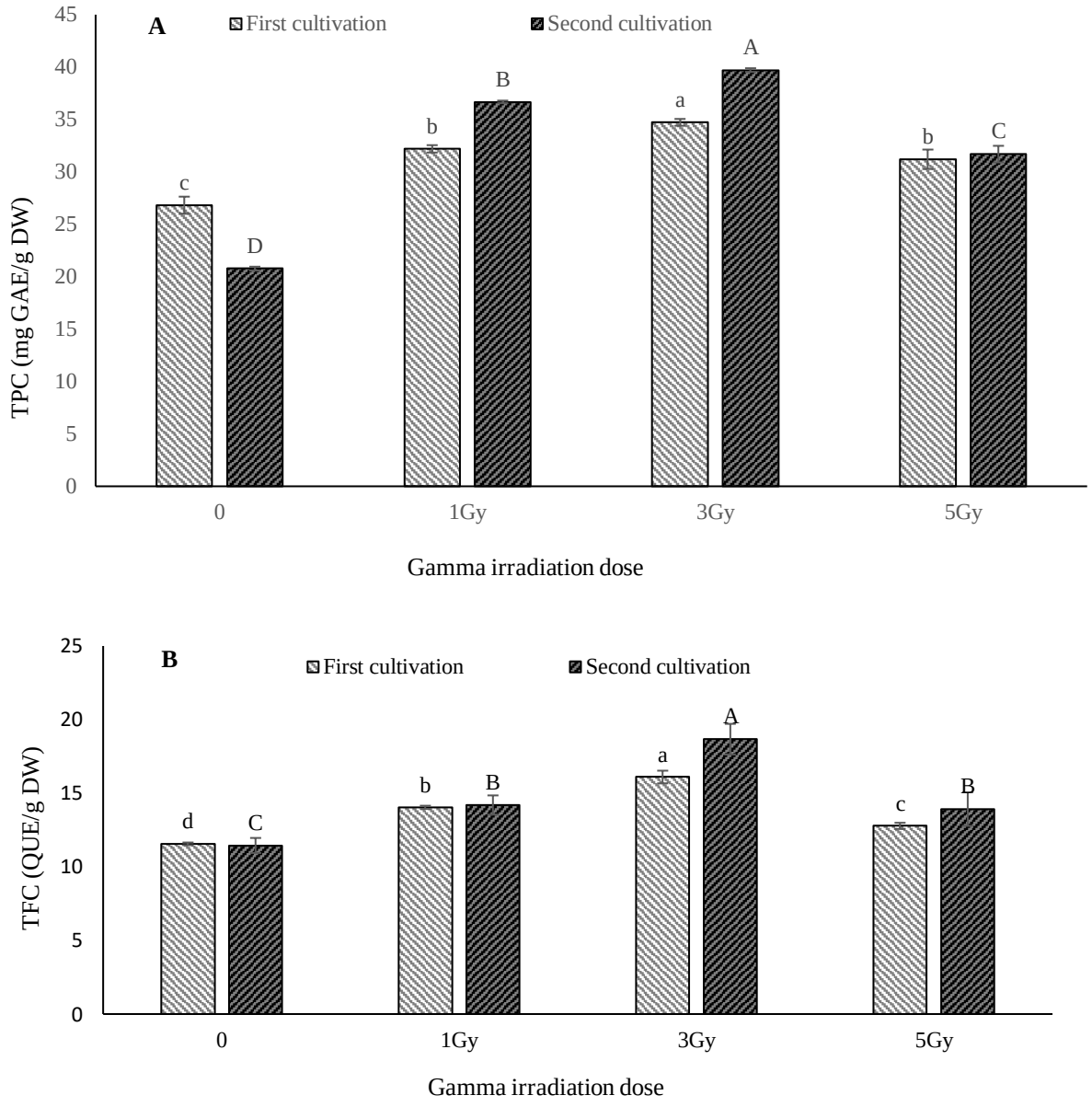

Fig. 4. Effect of gamma radiation on (**A**) total phenolic content and (**B**) total flavonoid content of *Thymus vulgaris* plants emerging from seeds irradiated with different doses of gamma rays during the first and second cultivations. The data from the first cultivation are represented by lowercase letters, whereas the data from the second cultivation are represented by uppercase letters. The error bars represent the SEs (n = 3). The mean value in the same series is significantly different when represented with different letters ($P \leq 0.05$).

Upon assessing the FRAP values as a measure of antioxidant capacity in *Thymus vulgaris*, it was found that while the control group and the initial 1 and 5 Gy treatments demonstrated similar antioxidant powers in the first cultivation period, the 3 Gy treatment resulted in a slightly reduced capacity. However, in the second cultivation period, the 1 and 5 Gy treatments resulted in increased FRAP values, indicating an improved antioxidant capacity. Notably, 3 Gy treatment resulted in a marked increase in antioxidant responses, suggesting that moderate gamma-irradiation may stimulate an antioxidant response over time. In contrast, the control group exhibited a decrease in the FRAP value, implying a natural decline in antioxidant capacity with cultivation progression (Table 1). These observations suggest a dose-dependent effect of irradiation on the antioxidant properties of *Thymus vulgaris*, with potential implications for optimizing plant health and stress resistance through controlled irradiation exposure.

Table 1. Effects of different dose of gamma irradiation on *Thymus vulgare* antioxidant capacity determined using different antioxidant assays.

	1st cultivation			2nd cultivation		
Irradiation	FRAP	DPPH	ABTS	FRAP	DPPH	ABTS
Control	0.08±0.001^{a}	64.79±2.5^{a}	62.17±0.25^{c}	0.07±0.001^{c}	62.95±0.85^{c}	43.90±1.19^{b}
1 Gy	0.08±0.01^{a}	68.44±2.6^{a}	66.39±1.3^{bc}	0.09±0.001^{b}	68.95±2.8^{b}	52.47±1.82^{a}
3 Gy	0.07±0.01^{a}	67.94±1.86^{a}	77.55±1.88^{a}	0.10±0.001^{a}	74.00±1.25^{a}	51.39±1.22^{a}
5 Gy	0.08±0.01^{a}	67.94±2.45^{a}	71.65±2.38^{ab}	0.09±0.00^{b}	68.89±1.13^{b}	49.54±0.70^{a}

Results are averages ± SE (n = 3)., average value in the same column with various superscript letters are significantly differ ($P \leq 0.05$).

The DPPH radical scavenging activity data for *Thymus vulgaris* across two cultivation periods revealed a nuanced response to gamma irradiation. Initially, the control group was the baseline for antioxidant capacity, which slightly decreased over time. In contrast, the 1-Gy irradiation treatment improved DPPH activity, which was sustained into the second cultivation period, suggesting enhanced antioxidant capability. The 3-Gy and 5-Gy treatments resulted in similar increases in the antioxidant capacity during the first period. However, the 3 Gy treatment exhibited the highest DPPH activity in the second period, indicating the most potent antioxidant response. Although the plants in the 5-Gy treatment group also improved, this improvement was not as pronounced as that in the 3-Gy treatment group (Table 1). These results suggest a dose-dependent effect of -irradiation on the antioxidant properties of *Thymus vulgaris*, with moderate irradiation potentially optimizing the plant's antioxidant defenses, which could be advantageous for agricultural practices aimed at enhancing plant resistance to oxidative stress.

When the antioxidant activity of *Thymus vulgaris* was assessed via the ABTS assay, a clear trend emerged in response to gamma irradiation. Initially, the control group presented a baseline activity level, which substantially declined in the second cultivation period. The 1 Gy treatment improved the baseline antioxidant activity, and despite a reduction in the second period, it maintained higher activity than the control. The 3 Gy treatment resulted in the most significant increase in ABTS activity in the first period but a notable decrease in the second period, although it still exceeded the control level. Compared with the control treatment, the 5-Gy treatment resulted in a similar pattern, with a decrease across periods but higher activity (Table 1). This pattern suggests that although gamma irradiation can initially increase antioxidant activity, the effects may diminish over time, with moderate doses (1 Gy and 3 Gy) potentially offering a greater sustained antioxidant capacity than higher doses. These results highlight the complex interplay between gamma irradiation doses and the temporal antioxidant response in plants, with implications for agricultural strategies aimed at improving plant stress tolerance and health.

Discussion

Gamma irradiation is a useful tool for improving crop yield and enhancing medicinal plant value by influencing various physiological, biochemical, and genetic traits. Low to moderate doses of gamma irradiation can stimulate seed germination, enhance seedling vigor, and improve overall plant growth, thereby increasing crop yield (Waqas et al., 2019). In thyme plants, low doses increased shoot and root lengths, whereas higher doses decreased shoot and root lengths, indicating a threshold effect. Correspondingly, both shoot and root fresh weights showed an initial increase followed by a decrease as the dose increased (Kordrostami et al., 2024). In medicinal plants, gamma irradiation enhances the

biosynthesis of secondary metabolites, such as phenolics and flavonoids (Kordrostami et al., 2024; Mohammadi et al., 2024). These metabolites contribute to the plant's antioxidative capacity and resistance against environmental stress, making gamma irradiation a valuable technique for producing high-quality medicinal plants with superior pharmacological properties. Moreover, gamma irradiation is employed for mutation breeding, enabling the development of plant varieties with desirable traits, such as higher yield, improved disease resistance, and enhanced adaptability to adverse environmental conditions (Ahloowalia et al., 2023). Additionally, gamma irradiation can be used for decontamination purposes, ensuring the microbiological safety of medicinal plant products without compromising their bioactive compounds (Rahman et al., 2021). These applications highlight the potential of gamma irradiation to connect agricultural productivity with pharmaceutical innovation, making it an indispensable tool for sustainable crop and medicinal plant production.

Reactive oxygen species (ROS), notably hydrogen peroxide (H_2O_2), primarily arise within plant chloroplasts and mitochondria as byproducts of photosynthesis and respiration, respectively. These molecules are integral to plant growth, development, stress hormone signaling, acclimation processes, and programmed cell death, contributing to the defense mechanisms against pathogens through hypersensitive responses and systemic acquired resistance. However, ROS overaccumulation can have harmful effects, leading to oxidative damage to nucleic acids, proteins, and lipids. Plants maintain a delicate redox balance through a complex network of enzymatic and nonenzymatic antioxidants to mitigate this potential damage (Dumanovic et al., 2023).

Physiologically, the observed initial slight increase in H_2O_2 levels after 1 and 3 Gy irradiation may indicate the activation of the plant's innate defense mechanisms. This response is consistent with the hormetic effect. In this context, low-dose stress can bolster a plant's resistance to subsequent environmental challenges without causing significant damage. At the molecular level, gamma irradiation has the potential to upregulate genes associated with antioxidative pathways, enhancing the activity of enzymes such as superoxide dismutase, catalase, and peroxidases, which are crucial for detoxifying ROS such as H_2O_2. The notable decline in H_2O_2 concentration during the second cultivation phase following the 1 and 3 Gy treatments underscores the enduring nature of these molecular adaptations, which contributed to improved tolerance to oxidative stress. The increased tolerance likely stems from irradiation-induced gene expression modifications that increase the efficiency of the ROS-scavenging machinery. In contrast, the increase in H_2O_2 observed with the 5 Gy treatment across both cultivation periods suggests that high irradiation levels may enhance antioxidative defenses, tipping the scale toward oxidative stress and cellular damage. Elevated H_2O_2 levels in this case could transition from a signaling molecule to toxic agents, causing lipid peroxidation, protein oxidation, and DNA damage, thereby disrupting cellular homeostasis. The varied responses to different gamma-irradiation doses emphasize the importance of dose optimization for effectively harnessing the stress response mechanisms of plants. Moderate irradiation appears to be prime the antioxidative system, thereby increasing plant health, whereas excessive irradiation can induce harmful stress. These insights into the dose-dependent molecular responses to gamma irradiation are invaluable for refining irradiation protocols for plant stress management. Additionally, they carry profound implications for agricultural methodologies, where controlled irradiation can serve as a preconditioning tool, allowing cultivating crops with superior stress tolerance and potentially higher yields.

Research on the effects of gamma irradiation on the malondialdehyde (MDA) content of *Thymus vulgaris* has provided insights into the physiological, molecular, and biochemical aspects of the oxidative stress response of plants. MDA, a crucial marker of lipid peroxidation, reflects the oxidative stability and integrity of cellular membranes. The first planting phase significantly reduced MDA levels by 30.48 and 55.34% under the 1 and 3 Gy treatments, respectively, whereas the 5 Gy treatment led to an 85.6% increase in MDA levels. This pattern persisted into the second cultivation period, albeit with a slight, nonsignificant increase in MDA levels following 5-Gy irradiation, highlighting the dose-dependent influence of gamma irradiation on lipid peroxidation and oxidative stress markers. These observations support the literature indicating that seed priming, including irradiation, can modulate reactive oxygen species (ROS) levels, thereby reducing lipid peroxidation and increasing stress tolerance in seedlings across various species (Sen and Puthur, 2020; Khan et al., 2022; Arafa et al., 2021). The decreases in MDA content following 1 and 3 Gy treatments suggest a hormetic effect in which low-dose irradiation triggers an adaptive, protective response, possibly by activating antioxidant defenses that mitigate lipid peroxidation. This effect is supported by the upregulation of genes encoding antioxidative enzymes such as superoxide dismutase, catalase, and peroxidases, which are instrumental in detoxifying peroxides and neutralizing free radicals. The sustained reduction in MDA levels across cultivation periods indicates long-term genetic and biochemical adaptations that

increase the plant's capacity to scavenge ROS, thereby reducing oxidative damage.

Conversely, the initial increase in MDA content following 5 Gy treatment suggests that n overwhelmed the antioxidative system, possibly due to excessive ROS generation that exceeds the plant's neutralizing capacity. The subsequent decrease in MDA levels during the second cultivation period may reflect the activation of delayed antioxidative mechanisms or repair systems that address lipid peroxidation. This nuanced response to varying doses of irradiation emphasizes the importance of dose optimization in improving plant health and stress resistance. The observed dynamics of MDA content, which decreased at moderate doses and increased at high doses, highlight the critical interplay between gamma irradiation and oxidative stress management in *T. vulgaris*. These findings support the strategic use of controlled irradiation to boost antioxidative defenses, offering a promising strategy for enhancing agricultural productivity and stress tolerance in crops. Moreover, the implications for plant-based pharmacology, where oxidative stability is paramount, are significant, suggesting that gamma irradiation can be harnessed to develop plant materials with optimized antioxidative properties.

Phenolic compounds are the most abundant specialized metabolites in plants and play pivotal roles in regulating growth, development, and responses to environmental stresses through various physiological functions. These compounds are synthesized primarily via the shikimic acid pathway and pentose phosphate/phenylpropanoid metabolism. In response to abiotic and biotic stresses, plants intensify the phenylpropanoid biosynthesis pathway, producing phenolic compounds to mitigate the adverse effects of such conditions. The antioxidant activity of phenolic compounds, which are crucial for scavenging harmful reactive oxygen species (ROS), forms a primary defense mechanism against oxidative stress (Šamec et al., 2021; Kumar et al., 2023; Paul et al., 2023). Research has revealed variable effects of gamma irradiation on the phenolic content of different plant species. For example, in *Curcuma alismatifolia*, a 10 Gy gamma radiation dose did not significantly alter the total phenolic and flavonoid content, whereas doses of 15 and 20 Gy markedly increased both (Taheri et al., 2014).

Similarly, red radish roots exhibited increased phenolic and flavonoid content up to 40 Gy, with a subsequent decrease observed at 80 Gy (El-Beltagi et al., 2022). In fenugreek plants from two generations, gamma irradiation ranging from 25 Gy to 100 Gy progressively increased the total flavonoid content. Moreover, doses exceeding 200 Gy adversely affected the total phenol content, indicating that a dose-dependent response was observed in the total phenol content (Hanafy and Akladious, 2018). The effect of gamma irradiation extends to increase the phenolic content in eggplant fruits at 50 Gy and decreases at 100 Gy (Aly et al., 2019). The mechanism underlying these changes involves high-energy gamma photons breaking the chemical bonds within polyphenols and between phenols and glycosides, thereby releasing soluble phenols. Furthermore, the induction of ROS by gamma irradiation can upregulate enzymes such as phenylalanine ammonia-lyase (PAL), which is pivotal in polyphenol biosynthesis, establishing a direct link between increased PAL activity and elevated levels of phenolic compounds (Vardhan et al., 2017; Jan et al., 2012; Mishra et al., 2012). The observed decrease in phenolic and flavonoid content at 5 Gy suggests disruption of ROS homeostasis, where excessive ROS generation exceeds the antioxidative capacity of the plant, leading to potential oxidative damage and cell death (Van Breusegem and Dat, 2006). The observed increases in phenolic and flavonoid content, particularly under 3 Gy treatment, along with the decrease in MDA content following 1 and 3 Gy treatment, highlight the significance of these compounds in mitigating oxidative stress. Phenolic compounds, known for their ability to scavenge reactive oxygen species (ROS), likely contributed significantly to the reduction of both H_2O_2 and MDA levels, thereby enhancing the plant's overall stress tolerance.

The effects of gamma irradiation on the antioxidant activity of *T. vulgaris* extracts, as observed through FRAP, DPPH, and ABTS assays, provide a comprehensive overview of how different irradiation doses influence plant defense mechanisms against oxidative stress. Initially, no significant changes were noted in the FRAP and DPPH activities of the irradiated samples compared with those of the controls in the first cultivation period. These findings suggest that there is a threshold at which -irradiation does not significantly alter the antioxidant capacity of the seedlings. However, the ABTS assay revealed a notable increase in antioxidant activity across all irradiated samples, indicating a dose-responsive increase in free radical scavenging ability, although no significant differences were observed among the treated groups. This finding aligns with the hormetic effect of gamma irradiation, in which low-to-moderate doses may stimulate antioxidant defenses without causing detrimental stress (Hong et al., 2018; Aly et al., 2019; El-Beltagi et al., 2022). In the second cultivation period, the antioxidant responses varied, with 3 Gy treatment resulting in the most pronounced increase in antioxidant activity, as measured by both DPPH and FRAP assays, underscoring the potential of specific irradiation levels to enhance plant antioxidative mechanisms over time.

Interestingly, the highest ABTS values were recorded for the 5 Gy treatment, suggesting a complex interaction between the irradiation dose and the activation of antioxidant pathways. These observations are supported by studies indicating that antioxidant activity can increase with increasing gamma irradiation doses up to a certain point, beyond which the capacity to inhibit free radicals decreases (Zani et al., 2017). The different outcomes of the ABTS, DPPH, and FRAP assays are primarily due to the diverse reaction mechanisms and sensitivities inherent to each method. The ABTS assay evaluates the capacity of antioxidants to quench the $ABTS^{\bullet+}$ radical cation through both single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms, accommodating a wide range of antioxidant compounds, including hydrophilic and lipophilic substances. In contrast, the DPPH assay predominantly involves the SET mechanism, using the DPPH• radical, which is soluble in organic solvents, thereby preferring lipophilic antioxidants. The FRAP assay measures the reducing potential of antioxidants by converting ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions under acidic conditions, which operate mainly through a SET mechanism and are more selective toward antioxidants with strong reducing power. Therefore, differences in results among these assays can be attributed to the specific chemical properties of antioxidants present in the samples and their interaction with the reactive species and conditions unique to each method (Danet, 2021; Shah and Modi, 2020). The significant correlation often found between total phenolic content and antioxidant activity highlights the role of phenolic compounds as primary antioxidants, alongside other crucial nonenzymatic antioxidants, such as ascorbic acid and tocopherols (Kasote et al., 2015).

The present study focused on the effects of gamma irradiation on the oxidative status, antioxidant compounds, and activities of thyme seedlings after priming, reflecting the broader implications of seed aging. Seed storage conditions leading to increased ROS levels and decreased antioxidant enzyme activity contribute to oxidative stress and aging, and gamma irradiation-induced seed priming can be mitigated by modulating ROS levels and increasing plant antioxidant defenses (Kurek et al., 2019; Ren et al., 2023). Overall, the nuanced response of *T. vulgaris* to gamma irradiation, as evidenced through variations in antioxidant activities across assays and cultivation periods, illustrates the potential of gamma irradiation as a seed-priming technique to increase plant resistance against oxidative stress. By potentially optimizing the balance between ROS production and antioxidant defense activation, this approach can improve seed quality and longevity, offering promising strategies for agricultural practices and the pharmacological utilization of plant extracts with enhanced antioxidative properties.

Conclusion

To summarize, this study provided convincing evidence that relatively low doses of gamma radiation, particularly approximately 3 Gy, significantly enhanced the antioxidant properties of thyme plants. The observed accumulation of antioxidant components and improved antioxidant capacity in the emerging plants can be attributed to the hormetic effect of low-dose of gamma irradiation. Hormesis, a beneficial response to low or moderate stress, activates defense mechanisms within the plant, leading to an overall improvement in resistance and vitality.

Moreover, gamma-irradiated seeds exhibit increased resistance to aging, as highlighted by the suppression of the production of reactive oxygen species (ROS). The accumulation of ROS during seed storage poses a challenge to seed quality and viability, suggesting that gamma irradiation boosts the plant's antioxidant defenses and acts as a preventative strategy against ROS-induced decline. This dual benefit of gamma irradiation is essential for seed preservation strategies and could significantly impact crop production by improving seed longevity and performance.

These findings emphasized the utility of low-dose gamma irradiation as a seed priming technique to increase antioxidant properties and bolster resistance to oxidative stress and aging in plants. This approach presents a novel strategy for enhancing plant health and productivity, with broad implications for agricultural practices and seed preservation.

Although this study provided valuable insights into the effects of gamma irradiation in *Thymus vulgaris*, several limitations must be considered. However, further investigations are necessary to elucidate the specific molecular pathways involved and improve gamma irradiation protocols for diverse plant species and agricultural contexts, with the aim of optimizing the benefits of this technique for sustainable crop production and seed management strategies. Additionally, future studies should investigate the molecular mechanisms by which gamma irradiation influences ROS regulation and secondary metabolite biosynthesis using transcriptomic and metabolomic approaches.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

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