

Effect of lemon juice in the growth, physiological traits, proline and antioxidants of Basil (*Ocimum basilicum*) plant growing in water stress

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Abstract

Objective

Basil (*Ocimum basilicum* L.) is a widespread annual plant cultivated in various parts of the world. Water stress is one of the most significant environmental challenges facing plants, including basil, in their growth and development. The aim of this study was to investigate the effect of lemon juice in mitigating the harmful effects of water stress on basil.

Materials and methods

This study was conducted during the autumn season of 2025 in a private nursery in Najaf, Iraq. Seeds were sown on 27/10/2025 in plastic containers in a two-factors factorial experiment. The first factor was water stress, with plants being irrigated (once, twice, and three times a week). The second factor was lemon juice at four concentrations (0, 2, 4, and 6 mL/L). Morphological and physiological parameters were calculated. Relative water content, chlorophyll content, proline and enzymatic antioxidants, proline concentration, peroxidase estimation, catalase enzyme activity, ascorbic acid, total phenolic content (TPC), and total flavonoids content were estimated. The results were analyzed using Genstat 2012 according to the ANOVA method.

Results

The results of the study indicated the negative effect of reducing irrigation intervals on basil plant growth indicators such as plant height, stem diameter, leaf area and some physiological characteristics such as relative water content, chlorophyll, and shoot fresh weight, with an increase in the leaf content of proline and enzymatic and non-enzymatic antioxidants. The experiment also showed the effect of lemon juice concentrations as a biostimulant that improves the growth indicators under study, enhances enzymatic and non-enzymatic antioxidants, and reduces the harmful effects of water stress.

Conclusion

The results of this study showed that water stress can reduce the vegetative growth of basil, but it can increase the defense compounds (proline, enzymes and antioxidants). Irrigation three times a week showed the best growth performance. While, less irrigation enhanced the stress responses. Foliar spraying of lemon juice, especially at concentrations of 2 to 6 ml/l, improved growth and

physiological traits and increased stress tolerance. Overall, it can be concluded that the use of lemon juice can be an effective strategy to reduce the negative effects of water stress in basil.

Keywords: catalase, flavonoids, peroxidase, phenols, vitamin C

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Introduction

Phytobiotics and medicinal plants have gained considerable attention in recent years due to their potential as natural alternatives to synthetic additives in nutrition (Amirteymoori et al., 2021). These natural products are rich in bioactive compounds such as essential oils, alkaloids, flavonoids, and phenolic acids, which contribute to their antimicrobial, antioxidant, and anti-inflammatory properties (Mohammadabadi et al., 2025a). Consequently, phytobiotics play a crucial role in improving health, performance, and product quality (Safaei et al., 2025). The use of phytobiotics and medicinal plants as natural antimicrobial growth promoters in place of antibiotics in feed offers numerous advantages (Khezri et al., 2025). These benefits include improved zootechnical efficiency parameters, suppression of specific diseases (Mohammadabadi et al., 2025b), antimicrobial and antioxidant activities, hypocholesterolemic effects, enhancement of digestive enzymes, and improved liver function (Roudbar et al., 2015). Moreover, phytobiotics have been shown to modulate gut microbiota, which enhances nutrient absorption and supports overall immune function (Vahabzadeh et al., 2021). Studies have demonstrated that incorporating these plants into the diets can increase feed consumption, improve feed conversion ratios, and enhance yield (Vahabzadeh et al., 2020). Furthermore, phytobiotics are associated with reducing stress-related impacts, improving meat quality, and decreasing the environmental impact of production systems by optimizing nutrient utilization (Alhasoon et al., 2026; Mohammadabadi et al., 2025c). Given the growing global concerns regarding antibiotic resistance and the demand for safer and healthier products, the incorporation of phytobiotics and medicinal plants into feed presents a promising and sustainable alternative strategy (Mohammadabadi et al., 2022). Basil (*Ocimum basilicum* L.) is a typical annual plant that is grown both inside and outdoors all over the world. It is a member of the Lamiaceae family. Given that its name is derived from the Latin word *ozo*, which means smell, the genus name *Ocimum* is fitting for its well-known smell

(Khair-ul-Bariyah, 2012). It has several components that are useful in medicine and grows well in temperate, tropical, and subtropical climates. Due of its therapeutic qualities, researchers are now looking into the potential of genetically altering basil to further its therapeutic advantages. This herbaceous plant adds a unique flavor and taste to meals by using its leaves, either fresh or dried. Juices, drinks, and other liquids also contain it (Nadeem et al., 2020). Though it differs from variety to variety, it is regarded as a spice that has made its way into the culinary world because of its flavor and taste. It has long been a part of Italian, British, and American cuisine (Spence, 2024). Its seeds are high in dietary fiber, lipids, minerals, and vital amino acids, which are the building blocks of proteins. According to Calderón Bravo et al. (2021), the seeds have anti-inflammatory, heart-protective, and diabetes-prevention qualities. In traditional medicine, basil has been used to treat bronchial exhaustion, coughs, sore throats, respiratory conditions, and wounds. It possesses similar anti-inflammatory and immune-boosting properties as dexamethasone (Eftekhari et al., 2019). Because basil extract has antifungal properties that are effective against various plants, especially *Fusarium oxysporum* fungus, which infects many field crops, plant seeds are soaked in basil extract before planting (Neamah et al., 2024). One of the biggest environmental obstacles to basil's growth and development is water stress. The physiological traits of the plant, particularly the metrics and indicators of photosynthesis, water use efficiency, gas exchange, leaf water potential, and essential oils in basil, are obviously impacted, as is crop yield (Kalamartzis et al., 2020). Plant growth and development are severely inhibited by environmental stressors, which cause morpho-logical, physiological, metabolic, biochemical, and molecular alterations in plants (Khan et al., 2023). However, it has been overcome the functional imbalances that plants encounter during their growth stages under these arid environments by comprehending the mechanism via which water stress impacts plants and their productivity (Shaker, 2022). Saad-Allah et al. (2022) in their research on five maize genotypes, found that drought significantly decreased the growth parameters. They were examined plant height, shoots fresh, and dry weight. On the other hand, as the severity of the drought increased, they studied the level of carotenoids, catalase, peroxidase, and ascorbate in the leaves. Drought significantly reduces plant height, leaf area, relative leaf water content, and leaf chlorophyll a and b content, according to Abdelaal et al. (2022). According to a study by Ali et al. (2022) water stress caused a large increasing in amino acids such methionine, proline, and glycine betaine. All of these are suitable substances that help regulate the osmotic potential of stressed cells. Growth markers for basil plants, including plant height, branch count, fresh and dried weights, relative leaf water content, and photosynthetic pigments, were significantly decreased under water stress (Mafakheri et al., 2025). Biostimulants play a fundamental role in plant growth and enhance productivity compared with side effects of synthetic stimulants. They also improve nutrient absorption and efficiency, modulate hormonal and metabolic pathways, and serve as successful and environmentally friendly alternatives (Gonçalves et al., 2025). One of these biostimulants is fruit juice. The natural antioxidants included in these juices are intriguing due to their minimal risk of adverse effects and safety, particularly when combined. The antioxidant content of various juice mixes is higher than that of individual juices (Vichaibun & Kanchanaphu, 2019). Numerous secondary metabolites found in lemon juice, including terpenes,

polyphenols, flavonoids, and essential oils, are crucial for human health. Monoterpenes, linalool, methyl eugenol, methyl chavicol, and other antibacterial and antioxidant substances are also present (Kačániová et al., 2022). Lemons have important health benefits, including reducing swelling and anti-cancer properties, due to their high content of bioactive compounds (Badiche-El Hilali et al., 2024). However, lemon juice works well as a substitute for commercial and industrial plant growth regulators, which have become more popular recently, particularly in tissue culture research (Yahya et al., 2024). Due to the scarcity of studies on the effect of bioactive lemon juice in reducing the harmful effects of water stress and the resulting antioxidant effect, this study was conducted to investigate the effect of lemon juice in mitigating the harmful effects of water stress on basil.

Material and methods

The experiment was conducted in a private nursery in Najaf Governorate/ Iraq for the autumn season of 2025. Plastic planting containers with a diameter of 9 cm and a height of 10 cm were prepared. They contained a soil mixture of sand and peat moss in a 1:2 ratio. Basil seeds were sown on 23/10/2025 at a rate of 0.25 g per plastic container. After germination, shaped plastic boxes were placed over the plastic containers to protect the seedlings from birds. One month after planting, the plants were sprayed with a neutral NPK foliar fertilizer (20:20:20) manufactured by the Bavaria company (Germany). The plants were sprayed three times. The first time performed when the true leaves appeared. Then a week later the second spray was applied, and the third spray was applied a week after the last one as well, to give the plants better growth. After the appearance of the true leaves and the increase in the growing plants, the process of reducing the plants was carried out. And then the excess seedlings were removed to preserve the growth of the new seedlings using small scissors, and five plants were left for each pot. The pots were divided into three groups. The first group was watered 3 times a week, the second twice a week, and the third once a week. Lemon juice was prepared by taking lemons from local lemon trees and placing them directly in the refrigerator to prevent any potential metabolic processes that might affect the juice's effectiveness later. The juice solution was diluted by adding 0, 2, 4, and 6 mL of juice (pH=3) to distilled water, adjusting the volume to 1 liter, and allowing it to filter through filter paper for 3 hours in the refrigerator. The plants were sprayed early in the morning. One month after planting and after the emergence of true leaves, three times with the diluted lemon juice, with 50 mL applied to each pot until the leaves were completely wet. This was done using a hand sprayer prepared for this purpose, with the addition of a surfactant to reduce surface tension (anionic surfactants). To enhance the first spray, the area around the roots was injected.

Morphological and physiological traits: Ten days after the third spray, three plants were taken from each experimental unit to perform calculations for morphological and physiological parameters such as plant height, stem diameter, leaf area, shoot fresh and dry weights.

Estimation of relative water content: According to the method mentioned by Al-Dhalimi and Al-Ajeel (2020), three randomly plants were taken early in the morning. 2 months after cultivation, from each experimental unit 3 randomly leaves were cut from each plant. They were washed thoroughly, dried to remove excess water, and 1 cm diameter discs were taken and

weighed three times. The first time was while the discs were still moist. The second performed after being placed in distilled water for 24 hours to calculate the turgid weight. The third was done after drying in an electric oven at 60°C until the weight stabilized. The water content was then calculated using the following equation:

$$R.W.C = (\text{fresh water-dry weight}) / (\text{turgid weight} - \text{dry weight})$$

Estimation of chlorophyll content: The chlorophyll content of the leaves, which indicates the intensity of the greenness of the leaves, was measured using a Japanese-made SPAD device. The amount of light transmitted through the leaves was calculated for two different wavelengths. The fourth and fifth leaves from the top of each fully grown plant were taken, and three plants were measured in each experimental unit. Each leaf was read three times.

Estimation of proline and enzymatic antioxidants: Dry samples were prepared from the aerial parts of the basil plant (leaves and young stems). As basil extracts from the stems, as well as extracts from the leaves, are of nutritional and health importance due to the antioxidants that the stems also contain (Bensaid et al., 2022).

Estimation of proline concentration: The proline concentration in leaves was determined according to the method of Bates et al. (1973). 0.5 g of dry plant material is dissolved in 10 mL of 3% sulphosalicylic acid. The solution is then filtered through filter paper. 2 mL of the solution are placed in a test tube, and 2 mL of glacial acetic acid and 2 mL of ninhydrin solution are added. The mixture is heated in a water bath for 20 minutes (temperature was 95 °C). It was left to cool until a light red or pink color appeared as a result of the reaction of proline with ninhydrin. Then the proline layer was separated by 4 mL of toluene which is added to the reaction mixture. The absorbance of the solution was measured at a wavelength of 520 nm, and the proline concentration was calculated using the following equation:

$$\text{Proline}(\mu\text{g g}^{-1}) = \frac{\mu\text{g proline} \times \text{mL toluene}}{115.5} \times \frac{5}{\text{g sample}}$$

Where, $\mu\text{g proline}$: Proline concentration determined from the standard curve, mL toluene: Volume of toluene used, 115.5: Conversion factor, g sample: Weight of the plant sample

Estimation of peroxidase estimation: Peroxidase enzyme was estimated according to the method described by Pitotti (1994). Guaiacol reagent was prepared by taking 100 microliters of the dye and mixing it with 5 mL of 70% ethanol. Then diluting to 100 mL with distilled water. Next, H₂O₂ solution was prepared by taking 150 microliters of H₂O₂ and diluting to 100 mL with distilled water. Then, a phosphate buffer solution (pH=7) was prepared by dissolving 1.36 g of acidic KH₂PO₄ in a small amount of distilled water. Then adjusting the pH to 7 with a weak base, and finally diluting to 100 mL with distilled water. To estimate the enzyme activity, 1 g of the dry material was digested for 5 minutes with 10 mL of buffered KH₂PO₄ phosphate buffer in a ceramic mortar under refrigerated conditions. The mixture was filtered through filter paper and placed in the refrigerator at a temperature of 2°C and prepared for the purpose of estimating the enzyme activity. Then the absorbance was measured using a Spectrophotometer at a wavelength of 420 nm.

Estimation of catalase enzyme activity: Catalase enzyme activity was estimated according to the method of Aebi (1983). The reaction mixture consisted of 20 μL of the enzyme extract with

1 mL of 10 mM hydrogen peroxide solution (H₂O₂) prepared in potassium phosphate buffer (pH=7, 20 mM). The absorbance of this solution was then measured using a spectrophotometer at 240 nm, and a decrease in absorbance was observed over time. Two potassium phosphate buffer solutions were prepared. Solution A, by dissolving 0.0346 g of K₂HPO₄ in distilled water and adjusting the volume to 100 mL. Solution B, by dissolving 0.270 g of K₂HPO₄ in distilled water and adjusting the volume to 100 mL. Solutions A and B were then mixed, and the pH was adjusted to 7. A 10 mM H₂O₂ solution was then prepared by mixing 1.0306 mL of 30% hydrogen peroxide solution in potassium phosphate buffer (pH 7, 20 mM). Then the volume was brought up to 100 mL. To extract and calculate the enzyme activity, 0.5 mL of the dry samples were crushed with 5 mL of PPB solution. 0.15 g of polyvinylpyrrolidone (PVP) added during crushing using a ceramic mortar under ice shards. The extract was then filtered using filter paper and centrifuged at 10,000 rpm for 10 minutes at 4°C. 40 microliters of the enzyme extract were withdrawn and 2 mL of 10 mM hydrogen peroxide solution were added. The mixture was incubated for one minute, after which readings were taken to estimate enzyme activity at a wavelength of 240 nm. The enzyme activity was then calculated according to the equation mentioned by Frary et al. (2010).

$$\text{Catalase activity} = \frac{\Delta \text{abs}}{\text{min}} \times \frac{\text{reaction volum}}{0.001}$$

Estimation of non-enzymatic antioxidants-Estimation of ascorbic acid (Vitamin C):

The determination of ascorbic acid, based on the Pearson method (Pearson, 1976), relies on the principle of the reaction of ascorbic acid with the blue dye 2,6-Dichloro-phenolindophenol, reducing it to a colorless compound. Simultaneously, vitamin C is converted to hydroascorbic acid. The endpoint is the disappearance of the blue color of the dye, and the vitamin C concentration is calculated based on the amount of dye consumed. The procedure involves weighing 1 g of the dry sample and digesting it with 10 mL of the extraction solution, then filtering the solution through filter paper. For the DCPIP dye, 0.0125 g of the dye powder is dissolved in 100 mL of a 0.05% sodium bicarbonate solution with boiling water, and then stored in a dark container. The extraction solution is prepared by dissolving 4 g of oxalic acid in 100 mL of distilled water. Take 10 mL of acetic acid and dilute to 100 mL with distilled water. Then, take 0.9 mL of acetic acid and dilute to 200 mL with distilled water. Mix the acids above. Next, titrate the dye solution by taking 5 mL of this standard solution into a conical flask and centrifuging it with the dye until the pink color stabilized for 15 seconds. Repeat this process several times, and the resulting volume of dye was used to determine the amount of vitamin C needed to neutralize it. The dye strength was then estimated by dividing the volume of the standard solution by the volume of the burette. Next, take 5 mL of the filtrate plant extract and centrifuge it using the same titration method until the pink color stabilized. Record the volume of dye used. Finally, estimate the amount of vitamin C.

Estimation of total phenolic content (TPC): According to Chandra et. al. (2014) the dry sample was extracted using a 20% methanol solution containing 1% hydrochloric acid (HCl). The methanol solution was prepared by taking 20 mL of methane and adding 2.7 mL of HCl, then completing the volume to 200 mL with distilled water. The sample extract was placed on a Vortex Mixer for two hours at room temperature at 200 rpm. The samples were then centrifuged for 15

minutes at 1000 rpm. 200 mL of the filtrate was taken and 1.5 mL of Folin-Ciocalteu reagent (prepared by adding 10 mL of the original reagent to 100 mL of distilled water) was added. The filtrate and reagent mixture were left to stand for 5 minutes. Then, 1.5 mL sodium carbonate solution (8%) was added, and the mixture was left in the dark at room temperature for 90 minutes. The absorbance was then measured using a spectrophotometer at a wavelength of 765 nm. The concentration was estimated by comparing the absorbance of the sample to a standard calibration curve using another compound (gallic acid).

Estimation of total flavonoids content: According to Chandra et. al. (2014) the same initial steps mentioned in the estimation of phenols were used in the estimation of flavonoids until the filtrate was prepared after the centrifugation process. One milliliter of the clear liquid was taken and one milliliter of 1% aluminum chloride solution was added to it. They were mixed together and incubated for one hour at room temperature. After that, the absorbance was read at a wavelength of 420 nm. The standard calibration curve was prepared using quercetin (prepared by dissolving 5 mg in 1 mL of methanol), and then dilutions of the stock solution were prepared to set the calibration curve.

Statistical analysis: The factorial experiment was designed using completely randomized blocks (CRB) for the two study factors, water stress and lemon juice concentrations. The results were analyzed using Genstat 2012 according to the ANOVA method, and the rates were compared using Duncan's multiple range test at a probability level of 5%.

Results and discussion

Shoot traits: The results showed in Table 1. Water stress had an adverse effect on the shoots traits, with the exception of the shoot dry weight. Basil was impacted by reduction irrigation intervals. In significant differences to the other treatments, plants that received three weekly irrigations had the highest values for plant height, stem diameter, and shoot fresh weight (14.52 cm, 2.570 mm, and 1.659 g, respectively, compared to plants irrigated once a week). plants irrigated once a week gave the lowest values in plant height, stem diameter, and shoot fresh weight (10.68 cm, 1.422 mm, and 1.036 g, respectively). At the same time, the shoot dry weight didn't show any significant differences between the three irrigation periods. Although plants irrigated twice a week gave the highest value (0.1278 g). The results of the lemon juice effect show that foliar application improves shoot traits including fresh weight, stem diameter, and plant height. With a 13.93 cm, 3.070 mm, and 1.474g increase in plant height, stem diameter, and fresh weight, respectively. The results demonstrated that spraying with lemon juice at a concentration of 6 mL/L encouraged growth and produced the highest values, significant different from the other treatments. The effect of lemon juice on the shoot dry weight is an exception to these results. As no significant differences were observed between the treatments. Although foliar spraying with diluted lemon juice at a concentration of 2 mL/L gave the highest value. The interaction between the two study factors showed that plants irrigated three times a week. Treated with lemon juice at a concentration of 2 mL/L gave the highest values in plant height, fresh weight, and shoot dry weight (17.17 cm, 2.130 g, and 0.1715 g, respectively).

Leaf traits: The detrimental effects of water stress on the leaf indicators under investigation are evident in Table 2, which will subsequently damage photo-synthesis and leaf growth; the primary edible portion of the basil plant. We found that the shorter the periods of basil watering, the smaller the leaf area, relative water content, and chlorophyll content in the leaves. Plants irrigated three times a week recorded the highest values in these traits (8.094 cm², 78.87%, and 26.15 unit, respectively, compared to plants irrigated once a week). plants irrigated once a week recorded the lowest values (5.616 cm², 63.89%, and 23.67 unit, respectively). In terms of leaf area, there were no significant changes between plants treated with lemon juice and those that were not. But there was a noticeable increasing in the chlorophyll content in the leaves. The chlorophyll content in leaves of plants treated with lemon juice at all concentrations increased significantly. In contrast to the untreated group, which displayed the lowest significant value of 22.87 unit, the treatment with 4 mL/L produced the highest value of 26.71 unit. Comparing the 4 mL/L concentration with the control group, the 4 mL/L concentration resulted in the highest relative leaf moisture content, reaching 76.08%, significantly higher than the control group, which recorded the lowest relative leaf moisture content (64.72%). Regarding the interaction between the two study factors, the responses of the treatments to each factor varied. However, spraying two or three times per week yielded the best results. Plants treated with 6 mL/L lemon juice and irrigated three times weekly showed the highest leaf area (9.544 cm², compared to plants irrigated once daily and treated with 2 mL/L lemon juice). These plants recorded the lowest value. Plants irrigated three times weekly and not treated with lemon juice also exhibited highest relative water content in the leaves. They recorded the highest value of 93.70% with significant differences from the rest of the transactions, compared to plants irrigated once daily and not treated with lemon juice. These plants recorded the lowest value of 50.14%. Furthermore, irrigating the plants twice a week with spraying with 6 mL/L lemon juice resulted in the highest value for leaf chlorophyll content (28.37 unit, compared to plants irrigated once a week and not treated with lemon juice). They showed the lowest value for this trait at 19.83 unit.

Proline and enzymatic antioxidants (catalase and peroxidase): Water stress significantly increased the amount of proline and enzymatic antioxidants like catalase and peroxidase in the leaves, according to the measurements in Table 3. There were significant differences between plants that were irrigated at different irrigation intervals. Plants irrigated once a week had the highest levels of proline, catalase, and peroxidase (44.78 mg/100g, 41.05 unit/g, and 13.62 unit/g, respectively). While, plants irrigated three times a week had the lowest levels (34.14 mg/100g, 24.68 unit/g, and 9.65 unit/g, respectively). The leaves differed in their content of proline, catalase, and peroxidase after foliar spraying with lemon juice. Our results indicates that the external application of lemon juice enhanced the leaf content of these important compounds in the plant's tolerance to water stress. Spraying with lemon juice at a concentration of 2 mL/L caused significant differences in leaf proline content, yielding the highest value of 43.35 mg/100g. This value was not significantly different from the 4 mL/L treatment but differed significantly from the other treatments, especially the control group, which yielded the lowest value of 32.86 mg/100g. Similarly, spraying with lemon juice significantly increased leaf catalase enzyme content at both the 2 mL/L and 4 mL/L concentrations, recording the highest values of 40.12

unit/g and 34.20 unit/g, respectively, while there were no significant differences between these two concentrations, they differed from the 6 mL/L treatment and the control.

Table 1. The effect of lemon juice in the shoot traits in Basil plant growing in water stress

Water stress	Lemon	Plant Height	Stem diameter	Shoot fresh weight	Shoot dry weight
3 times	Control	11.42 de	1.13 c	1.553 bc	0.1120abcd
	2 mL/L	17.17 a	2.64 bc	2.130 a	0.1715 a
	4 mL/L	15.17 ab	3.37 ab	1.370 bcd	0.0068 e
	6 mL/L	14.33 bc	1.96 bc	1.583 bc	0.1099abcd
2 times	Control	12.93 bcd	1.26 c	0.957 def	0.0758 cd
	2 mL/L	8.67 f	1.30 c	0.840 ef	0.1040 bcd
	4 mL/L	11.42 de	2.81 bc	1.063 def	0.1545 ab
	6 mL/L	16.83 a	4.91 a	1.713 ab	0.1771 a
Once	Control	12.67 cd	1.35 bc	1.013 def	0.1018 bcd
	2 mL/L	9.70 ef	0.89 c	0.732 f	0.1011 bcd
	4 mL/L	9.73 ef	1.11 c	1.283 bcde	0.1395 abc
	6 mL/L	10.63 def	2.34 bc	1.127 cdef	0.0628 de
Effect of water stress	3 times	14.52 a	2.570 a	1.659 a	0.1014 a
	2 times	12.46 b	2.274 ab	1.143 b	0.1278 a
	Once	10.68 c	1.422 b	1.036 b	0.1013 a
	Control	12.34 b	1.244 c	1.174 b	0.0965 a
Effect of lemon juice	2 mL/L	12.11 b	1.610 bc	1.231 ab	0.1267 a
	4 mL/L	11.84 b	2.430 ab	1.239 ab	0.1009 a
	6 mL/L	13.93 a	3.070 a	1.474 a	0.1166 a

The rates followed by the same letter in each treatment don't differ significantly from each other according to Duncan's Multiple Range Test at the probability level 5%.

Table 2. the effect of lemon juice in the leaves traits in Basil plant growing in water stress

Water stress	Lemon	Leaves area	Relative Water contents	Chlorophyll
3 times	Control	5.423 de	93.70 a	24.70 abc
	2 mL/L	8.583 abc	67.86 cd	25.60 abc
	4 mL/L	8.913 ab	70.30 bcd	27.27 ab
	6 mL/L	9.455 a	83.63abc	27.03 ab
2 times	Control	6.917 abcd	50.24 e	24.07 ab
	2 mL/L	6.333 bcde	77.54 abcd	26.20 abc
	4 mL/L	5.747 cde	87.23 ab	25.47 abc
	6 mL/L	8.165 abcd	61.49 de	28.37 a
Once	Control	7.040 abcd	50.14 e	19.83 d
	2 mL/L	4.041 e	62.26 de	24.70 abc
	4 mL/L	5.927 cde	70.72 bcd	27.4 ab
	6 mL/L	5.457 de	72.46 bcd	22.77 cd
Effect of water stress	3 times	8.094 a	78.87 a	26.15 a
	2 times	6.791 b	69.12 b	26.02 a
	Once	5.616 b	63.89 b	23.67 b
	Control	7.849 a	64.72 b	22.87 b
Effect of lemon juice	2 mL/L	6.706 a	69.22 ab	25.50 a
	4 mL/L	6.460 a	76.08 a	26.71 a

6 mL/L 6.319 a 72.53 ab 26.06 a

The rates followed by the same letter in each treatment don't differ significantly from each other according to Duncan's Multiple Range Test at the probability level 5%.

Regarding peroxidase, the 4 mL/L concentration resulted in the highest leaf content of this enzyme and recorded 13.76 unit/g, significantly different from the other treatments, while the control group recorded the lowest value of 10.41 unit/g. According to the same table, plants treated with foliar spraying of lemon juice at a concentration of 2 and irrigated once a week had the highest leaf proline content, with a value of 50. This was also seen in the catalase enzyme results, where the greatest leaf catalase content of 68 was obtained with this treatment (foliar spraying of lemon juice at a concentration of 2 with weekly watering). On the other hand, plants that were not treated with lemon juice and were irrigated once a week had the highest leaf peroxidase content of 18.79 unit/g.

Table 3. The effect of lemon juice in leaves content of proline, catalase and peroxidase in basil plant growing in water stress

Water stress	Lemon	Proline	Catalase	Peroxidase
3 times	Lemon	32.94 de	51.61 b	8.47 de
	Control	36.15 cde	16.93 d	11.01 cd
	2 mL/L	38.00 bcde	18.56 d	10.50 d
	4 mL/L	29.47 e	11.62 d	8.62 de
	6 mL/L	20.18 f	16.12 d	3.97 f
2 times	Control	43.51 abc	34.88 c	8.91 de
	2 mL/L	42.86 abc	33.66 c	16.75 ab
	4 mL/L	39.48 bcd	30.60 c	17.82 a
	6 mL/L	45.45 ab	10.60 d	18.79 a
Once	Control	50.39 a	68.54 a	15.59 ab
	2 mL/L	42.70 abc	50.38 b	14.04 bc
	4 mL/L	40.59 bcd	34.68 c	6.05 ef
	6 mL/L	34.14 b	24.68 b	9.65 c
Effect of water stress	3 times	36.51 b	28.82 b	11.86 b
	2 times	44.78 a	41.05 a	13.62 a
	Once	32.86 b	26.11 b	10.41 b
Effect of lemon juice	Control	43.35 a	40.12 a	11.84 b
	2 mL/L	41.19 a	34.20 a	13.76 a
	4 mL/L	36.51 b	25.63 b	10.83 b

*The rates followed by the same letter in each treatment don't differ significantly from each other according to Duncan's Multiple Range Test at the probability level 5%.

Non-enzymatic antioxidants (Vitamin C, phenols and flavonoids): The effect of reducing irrigation intervals on the leaf content of vitamin C, phenols, and flavonoids is shown in Table 4. Water stress caused a significant increase in the leaf content of these important metabolic compounds produced by the plant in response to various stress conditions, compared to unstressed plants. Regarding vitamin C levels in the leaves, the table shows that the highest significant value for this antioxidant was recorded in plants irrigated once a week (185.8 mg/100g, significantly

higher than in plants irrigated two or three times a week). The latter of which gave the lowest value (85.6 mg/100g). The leaves' phenolic and flavonoid content produced outcomes that were comparable to those of vitamin C. For the antioxidant's phenolics and flavonoids, plants that were irrigated once a week had the highest values (1.191%, 0.573%, respectively). Whereas, plants that were irrigated three times a day had the lowest values (0.949%, 0.463%, respectively). Nonetheless, there was no discernible difference between the amounts of these chemicals and those of plants that received twice-weekly irrigation. The results of the same table show the effect of lemon juice on the content of the leaves of vitamin and phenols, but it did not achieve significant differences in the content of the leaves of flavonoids. The concentration of 6 mL/L recorded the highest significant value in the content of the leaves of vitamin C, recording 144.0 mg/100g, with a significant difference from the rest of the treatments, especially the control, which recorded the lowest value (101.2 mg/100g). In the same context, the plants treated with the concentration of 2 mL/L gave the highest significant value in the content of the leaves of phenols (1.200%, compared to the other treatments). As for flavonoids, there were no clear significant differences in the content of the leaves of this antioxidant, except that the plants treated with the concentration of 2 mL/L gave the highest value (0.579%).

Table 4. The effect of lemon juice in leaves content of vitamin C, phenols and flavonoids in basil plant growing in water stress

Water stress	Lemon	Vit. C	Phenols	Flavonoids
3 times	Lemon	68.0 d	0.928 e	0.426 b
	Control	60.7 d	1.001 de	0.473 b
	2 mL/L	100.5 c	0.949 e	0.411 b
	4 mL/L	113.2 bc	0.917 e	0.545 b
	6 mL/L	115.5 bc	1.303 ab	0.540 b
2 times	Control	68.9 d	1.388 a	0.556 ab
	2 mL/L	185.a	0.894 e	0.577 ab
	4 mL/L	123.6 b	1.167 bc	0.555 ab
	6 mL/L	120.0 bc	1.101 cd	0.543 b
Once	Control	188.5 a	1.212 bc	0.707 a
	2 mL/L	51.8 d	1.230 bc	0.541 b
	4 mL/L	195.1 a	1.220 bc	0.502 b
	6 mL/L	85.6 b	0.949 b	0.463 b
Effect of water stress	3 times	123.3 b	1.188 a	0.557 a
	2 times	185.8 a	1.191 a	0.573 a
	Once	101.2 b	1.111 b	0.503 a
Effect of lemon juice	Control	106.0 b	1.200 a	0.579 a
	2 mL/L	112.5 b	1.024 c	0.509 a
	4 mL/L	144.0 a	1.101 b	0.534 a

The data from this study demonstrate the detrimental effects of water stress on the growth and development of basil plants due to inadequate irrigation. Drought stress is regarded as one of the most harmful abiotic stresses on plant physiology and productivity worldwide. On the other hand, it is a more complex phenomenon since plants display a wide range of physiological and

biochemical reactions at the cellular and organismal levels. Stomatal closure, membrane damage, and altered activity of several enzymes, especially those involved in ATP synthesis, all contribute to a drop in the photosynthetic rate (Berthomieu et al. 2003). Water stress led to reduced plant growth with disruptions to water balance and water relations, as well as the accumulation of amino acids and enzymatic and non-enzymatic antioxidants (Ali et al., 2020). It is well known that the difference in plant growth resulting from reduced irrigation periods is due to the fact that water supplies play a pivotal role in transporting essential nutrients from the soil to the plant. And then to the photosynthetic sites in the leaves and other parts of the plant, leading to better growth by supplying the plant parts with enough of the elements necessary for metabolic processes. Our results indicate that reducing irrigation intervals resulted in a significant decrease in plant height, stem diameter, leaf chlorophyll content, relative water content, and fresh weight of the foliage. These results were supported by and are consistent with the findings of other researchers (Habib et al., 2020; Shemi et al., 2021; Shehzad et al., 2022). They reported that water scarcity causes a significant decrease in plant growth, amount of photosynthetic pigments, and relative water content of leaves. Shahid et al. (2019) showed that water stress reduces stem growth, length, and fresh weight of the plant. While, an increase in the rates of the aforementioned traits is observed in non-stressed plants. Kasim et al. (2020) confirmed that water stress levels significantly reduced the growth indicators under study, such as plant height, fresh weight of the vegetative mass, and the amount of chlorophyll in the leaves during the seedling and vegetative stages. Our results are also consistent with the results of Lu et al. (2025), who attributed this effect to the fact that drought-induced stress prompted plants to exhibit adaptive responses, including reduced growth, increased activity of antioxidant enzymes, osmotic regulation, and changes in endogenous hormone levels. The results showed that water scarcity significantly reduced plant height and stem diameter. This may be due to water stress negatively impacting plant length by inhibiting cell division, differentiation, and expansion, particularly in meristematic regions (apical meristem, which increases plant height, and lateral meristem, which increases stem diameter) and areas of high activity, such as the growing tips. These findings are consistent with those of Abdelaal et al. (2022), who also found that water scarcity significantly reduced stem length. Furthermore, insufficient irrigation reduced chlorophyll content in leaves, which may be attributed to the fact that leaves are a significant site of oxidative stress resulting from the production of high levels of ROS, primarily in the leaves (López-Serrano et al., 2019). In addition, the oxidative potential increases and stimulates the activity of the Chlorophyllase enzyme with the increase in water deficiency, which leads to the destruction of chlorophyll (Kalamartzis et al., 2020). Or the reason may be that the lack of water causes a decrease in the rate of photosynthesis by closing the stomata, which reduces the absorption of nutrients, including those involved in the formation of the chlorophyll molecule (López-Serrano et al., 2019). The results also showed a significant decrease in the shoot fresh weight with reduced irrigation intervals. This result is supported by Guo et al. (2018), who stated that water scarcity reduces the fresh weight of shoot. This may be due to the harmful effects of water stress on all plant metabolic processes, primarily ion and osmotic balance, membrane permeability to water and nutrients, and the synthesis of important molecules involved in membrane structure, such as proteins, and on all parts of the

plant (Guo et al., 2018). It also causes disturbances in the hormonal content and growth promoters such as auxins and gibberellins, and an increase in growth inhibitors such as ABA, resulting in inhibition and reduction of plant growth in general, which is reflected overall in the fresh and dry weight of the vegetative body (Al-Dhalimi and Al-Ajeel, 2020). From the results of our current study, we found that the matter is different with proline and enzymatic and non-enzymatic antioxidants. Reducing irrigation periods caused a clear increase in proline and enzymatic antioxidants such as peroxidase and catalase, and non-enzymatic antioxidants such as vitamin C, phenols, and flavonoids. These results support the findings of Shehzad et al. (2022). They reported that water deficiency causes an increase in both enzymatic and non-enzymatic antioxidants. The reason for the increase in the content of aerial parts of antioxidants may be due to the harmful effect caused by Reactive Oxygen Species (ROS), whose level rises in the case of various stresses as a kind of response of plant cells to those stresses. These ROS cause great damage to lipid membranes, proteins, and nucleic acid and can react with large molecules. Therefore, the plant deals with them through defensive means such as enzymatic and non-enzymatic antioxidants that rid the cells of reactive oxygen species (Hasanuzzaman et al., 2020). One of the main reasons for the synthesis and accumulation of antioxidants in plants is the natural tendency of plants to respond to various environmental stress conditions to enhance plant defenses to the maximum extent possible (Kasote et al., 2015). The biosynthesis processes of secondary metabolite compounds in response to stress factors are under genetic control, and their gene expression is regulated to encode the enzymes necessary to reduce stress caused by abnormal environmental conditions surrounding the plant (Kisa et al., 2021). In agriculture, sustainable alternatives to chemical inputs are becoming increasingly important. Among the most important of these alternatives are biostimulants, including lemon juice, which is the subject of our current study. Plants treated with lemon juice showed significantly better results than untreated plants in most of the studied indicators, such as plant height, stem diameter, fresh weight of the vegetative system, and leaf characteristics like chlorophyll content and relative water content, with the exception of leaf area, where the lemon juice-treated plants did not differ significantly from the untreated plants. This may be attributed to the presence of essential plant nutrients in lemon juice, including phosphorus, calcium, sodium, sulfur, boron, and other elements necessary for optimal stem and leaf vegetative growth. Lemon juice also contains magnesium, an essential element in chlorophyll formation in leaves (Klimek-Szczykutowicz et al., 2020), and is a source of vitamins such as B1, B5, B6, and vitamin C, as well as proteins and carbohydrates (Rafique et al., 2020). All of this significantly impacts the vegetative growth of the plant. Furthermore, lemon juice contributes to an increase in the fresh weight of vegetative parts and a marked increase in leaf water content. This is attributed to the presence of minerals and pectin in lemon juice, a component of plant cell walls, which helps these walls expand and increases their water potential (Al-Qudah et al., 2018). The bioactive compounds in lemon juice act as a natural growth stimulant for stressed crops and are also an important source of organic acids, sugars, vitamins, minerals, carbohydrates, vitamin C, malic acid, phenols, and flavonoids (Rady et al., 2023). Lemon juice is characterized by its high content of vitamin C and citrates, which act as organic substitutes and effectively improve photosynthesis, antioxidant capacity, and enzyme gene expression (Rady et

al., 2023). It also contains a range of secondary metabolites such as polyphenols, flavonoids, terpenes, and essential oils (Kačániová et al., 2022). In addition, the phenolic activity found in lemon juice, which is similar to the activity of the superoxide dismutase enzyme, gives it an important role in eliminating reactive oxygen species, thus reducing oxidative stress caused by water stress (Valcheva-Kuzmanova et al., 2007). Ewansiha et al. (2016) pointed out, as lemon juice contains a number of secondary metabolites such as tannins, phenols, flavonoids, alkaloids, and others. Finally, we corroborate our findings with those of Roque et al. (2024) who established that plant-derived biostimulants contain biologically active compounds with growth-promoting, antioxidant, and antibacterial properties. These biostimulants, from germination to fruiting, regulate mineral nutrition, primary and secondary metabolism, photosynthesis, oxidative metabolism, and cellular signaling activities.

Conclusion: Research in the biostimulants field is necessary to improve plant growth and development. Considering the negative effects caused by stimulants and chemicals used in the agricultural field. The current study was conducted using 4 levels of diluted lemon juice and using different irrigation periods. Results of our current study revealed that lemon juice which is one of biostimulants mitigated the harmful effects of water stress, improved vegetative growth, and enhanced both enzymatic and non-enzymatic antioxidant levels in stressed plants. A concentration of 6 mL/L yielded the best results for vegetative growth across most parameters. Conversely, a concentration of 2 mL/L resulted in the best results for leaf content of both enzymatic and non-enzymatic antioxidants.

Author contributions

Researcher Afrah Mahdi Al-Dhalimi, the sole author of this work, declares her responsibility for all aspects of this manuscript, beginning with establishing the hypothesis, developing the methodological framework, executing field survey, and laboratory experiments proceeding with statistical treatment of the raw data as well as its interpretation while retaining in consideration work of literature, language and scientific writing of data amending and correcting the data for final edition.

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Data availability

I affirm my commitment to the principle of scientific openness; all original data and results underlying the conclusions of this study are held by me and are available on request. Any researcher or member of the editorial board who wishes to have access to these data may do so by sending me a request that I consider justified for purposes of verifying the science herein.

Ethical considerations

The researcher would like to state categorically that this is research of pure academic ethics. The study did not involve clinical trials on human subjects, nor did it involve laboratory experimentation with animals. The biological specimens (plant/ microbial) were handled through biosafety measures pegged to internationally recognized standards and Good Laboratory practices (GLP).

Conflict of Interest

The research team, as the sole author, confirms that there are no conflicts of interest, either of a financial, personal, or academic nature, that may be construed to have influenced the objectivity of the results or conclusions of this research. No commercial affiliations had any role in the study design, interpretation of data, or the decision to submit the study for publication.

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تأثیر آب‌لیمو بر رشد، ویژگی‌های فیزیولوژیک، پرولین و آنتی‌اکسیدان‌های گیاه ریحان (*Ocimum basilicum*) در شرایط تنش آبی

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چکیده

هدف: ریحان (*Ocimum basilicum* L.) یک گیاه یک‌ساله رایج است که در مناطق مختلف جهان کشت می‌شود. تنش آبی یکی از مهم‌ترین چالش‌های محیطی است که رشد و توسعه گیاهان، از جمله ریحان، را تحت تأثیر قرار می‌دهد. هدف این مطالعه بررسی اثر آب‌لیمو در کاهش اثرات زیان‌بار تنش آبی بر گیاه ریحان بود.

مواد و روش‌ها: این مطالعه در فصل پاییز سال ۲۰۲۵ در یک نهالستان خصوصی در نجف، عراق انجام شد. بذرها در تاریخ ۲۰۲۵/۱۰/۲۷ در ظروف پلاستیکی و در قالب آزمایش فاکتوریل با دو عامل کشت شدند. عامل اول تنش آبی بود که شامل آبیاری گیاهان (یک، دو و سه بار در هفته) بود. عامل دوم غلظت‌های مختلف آب‌لیمو در چهار سطح (۰، ۲، ۴ و ۶ میلی‌لیتر در لیتر) بود. صفات مورفولوژیک و فیزیولوژیک اندازه‌گیری شدند. همچنین محتوای نسبی آب، مقدار کلروفیل، پرولین و آنتی‌اکسیدان‌های آنزیمی، غلظت پرولین، فعالیت آنزیم پراکسیداز، فعالیت آنزیم کاتالاز، اسید آسکوربیک، محتوای کل فنول‌ها (TPC) و فلاونوئیدها اندازه‌گیری شد. داده‌ها با استفاده از نرم‌افزار Genstat 2012 و بر اساس روش آنالیز واریانس (ANOVA) تجزیه و تحلیل شدند.

نتایج: نتایج نشان داد که کاهش دفعات آبیاری تأثیر منفی بر شاخص‌های رشد ریحان مانند ارتفاع گیاه، قطر ساقه، سطح برگ و برخی ویژگی‌های فیزیولوژیک مانند محتوای نسبی آب، کلروفیل و وزن تر اندام هوایی داشت. در مقابل، محتوای پرولین و آنتی‌اکسیدان‌های آنزیمی و غیرآنزیمی در برگ‌ها افزایش یافت. همچنین، نتایج نشان داد که غلظت‌های مختلف آب‌لیمو به عنوان یک محرک زیستی باعث بهبود شاخص‌های رشد، افزایش آنتی‌اکسیدان‌های آنزیمی و غیرآنزیمی و کاهش اثرات منفی تنش آبی شد.

نتیجه‌گیری: نتایج این مطالعه نشان داد که تنش آبی می‌تواند رشد رویشی ریحان را کاهش دهد، اما موجب افزایش ترکیبات دفاعی (پرولین، آنزیم‌ها و آنتی‌اکسیدان‌ها) می‌شود. آبیاری سه بار در هفته بهترین عملکرد رشد را نشان داد، در حالی که کاهش

آبیاری پاسخ‌های تنشی را افزایش داد. محلول‌پاشی آب‌لیمو، به‌ویژه در غلظت‌های ۲ تا ۶ میلی‌لیتر در لیتر، باعث بهبود رشد، ویژگی‌های فیزیولوژیک و افزایش تحمل به تنش شد. در مجموع، استفاده از آب‌لیمو می‌تواند راهکاری مؤثر برای کاهش اثرات منفی تنش آبی در گیاه ریحان باشد.

کلمات کلیدی: پراکسیداز، فلاونوئیدها، فنول‌ها، کاتالاز، ویتامین C

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