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Enhancing Holy Basil (*Ocimum sanctum* L.) Tolerance to Water Deficiency through Putrescine Foliar Spray

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ABSTRACT

Water deficiency poses a significant challenge to global agricultural systems, impacting crop performance and product quality. Compounds like putrescine have demonstrated the potential to enhance plant resilience to environmental stresses. This pot study, conducted in 2023 at Imam Khomeini International University, Ghazvin, Iran, employed a factorial experiment based on a completely randomized design with three replications, aimed to assess the impact of varied irrigation levels and foliar application of putrescine on both quantitative and qualitative traits of holy basil (*Ocimum sanctum* L.). Water deficiency was induced at three levels (100, 75, and 50% of Field Capacity), and putrescine foliar spray was applied at concentrations of 0, 0.1, and 0.2 mM. Results indicated that water scarcity significantly reduced plant growth indices, Relative Water Content (RWC), and photosynthetic pigment levels. However, foliar spray with putrescine effectively mitigated these adverse effects. Furthermore, the combination of water deficiency and the application of 0.2 mM putrescine increased total phenolic compounds (48.76%), flavonoid compounds (54.85%), and restrained free radical DPPH (44.85%) compared to the control. Putrescine-treated plants exhibited a noteworthy increase in essential oil percentage compared to the control group. Furthermore, as water deficiency increased, the essential oil composition also increased the percentages of 1,8-cineole and methyl eugenol compared to the control plants. In conclusion, foliar application of putrescine resulted in a significant enhancement in the essential oil's key compounds in holy basil.

Keywords: Drought stress, Essential oil, Medicinal plants.

INTRODUCTION

Ocimum sanctum L., commonly referred to as holy basil, stands as a perennial herbaceous plant within the Lamiaceae family. Holy basil has earned recognition for its diverse medicinal attributes, including anti-diabetic, wound-healing, antioxidant, radiation-protective, immune-modulatory, anti-inflammatory, antimicrobial, anti-stress, and anti-cancer properties. Rich in essential oils, holy basil's key compounds encompass 1,8-cineole, eugenol, and methyl eugenol (Nguyen *et al*., 2022). Its historical and cultural significance spans centuries, with traditional medicinal practices in various cultures incorporating holy basil as a primary therapeutic agent. Moreover, the culinary realm values holy basil for its aromatic flavor, contributing to its widespread cultivation and consumption in diverse cuisines worldwide.

Water, a pivotal element in sustainable development, emerges as a limiting factor for plant productivity, particularly in agricultural systems confronting regular and prolonged droughts, prevalent in semi-arid and arid regions globally. Drought stress induces a spectrum of morphological, physiological, and biochemical alterations in plants, including disruptions in water relations, suppression of cellular activities

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(Hatamian *et al*., 2017), and diminished chlorophyll and carotenoid content (Guo *et al*., 2016). The production of Reactive Oxygen Species (ROS) during drought stress compromises plasma membrane integrity and protein function, resulting in metabolic dysfunction and substantial yield reduction (Gholami Zali and Ehsanzadeh, 2018). In response to drought stress, plants deploy various strategies, encompassing the accumulation of compatible solutes, regulation of photosynthetic parameters, synthesis of stress-related primary and secondary metabolites, activation of antioxidant enzymes, and alterations in gene expression (Morshedloo *et al*., 2017).

Prolonged drought conditions exacerbate soil degradation, compromising nutrient availability and intensifying plant stress responses. Under drought stress, the biosynthesis of phenolic and flavonoid compounds increases, contributing to antioxidant defense and stress tolerance. These compounds play a pivotal role in safeguarding cellular structures and maintaining overall plant health. Additionally, proline, a non-essential amino acid, accumulates in plant tissues during water deficit, acting as an osmo-protectant by stabilizing cell membranes and preventing dehydration-induced damage. Elevated proline levels correlate with improved drought resistance. Essential oils, rich in volatile compounds, find diverse applications in medicine, cosmetics, and aromatherapy. Drought stress significantly influences the composition and yield of essential oils in medicinal plants. Some species increase oil production as a stress response, potentially enhancing their medicinal properties. Furthermore, chlorophyll and carotenoids, essential for photosynthesis, face alterations under drought stress. While chlorophyll content often decreases, affecting energy capture, balancing carotenoid levels becomes critical for maintaining photosynthetic efficiency (Rahman *et al*., 2023; Wagay *et al*., 2023).

However, under prolonged drought stress, antioxidant defense systems may prove insufficient to mitigate the detrimental effects of ROS (Minhas *et al*., 2017). In this context, the utilization of osmotic active substances, such as polyamines, represents a promising approach to counteract environmental stress. Polyamines, including spermidine, spermine, and putrescine, function as plant-like hormone compounds extensively involved in diverse growth and physiological processes (Shi and Chan, 2014). They play a pivotal role in regulating gene expression in response to drought stress, contributing to the maintenance of cellular homeostasis, plasma membrane integrity, chlorophyll degradation inhibition, specific protein biosynthesis, and nitrogen-containing alkaloids (Kusano *et al*., 2015). Putrescine, a notable polyamine, emerges as a key player in plant responses to stress. Research indicates its regulatory role in physiological processes such as photosynthesis, stomatal behavior, and antioxidant activity (Tiburcio *et al*., 2014). Consequently, investigating the potential of putrescine to enhance drought tolerance in plants has gained significance. It is noteworthy that the effects of putrescine may vary based on concentration, and higher concentrations may not always yield beneficial results. Furthermore, different plant species may exhibit varied responses to putrescine treatments (Morshedloo *et al*., 2017).

Holy basil's aromatic properties, culinary uses, and essential oil content make it economically valuable both globally and in Iran. Its versatility and cultural significance contribute to its widespread cultivation and utilization. Notably, in the context of water scarcity and recurring droughts, holy basil's profound medicinal importance becomes even more pronounced (Singh and Chaudhuri, 2018), this study aims to explore the impact of putrescine spray on the quantity and quality of holy basil medicinal plant products under conditions of water scarcity. By elucidating the physiological and biochemical mechanisms underlying the response of holy basil to putrescine treatment under drought stress, this research endeavors to contribute to the development of sustainable agricultural practices. The findings of this study hold potential implications for enhancing crop resilience, optimizing medicinal plant production, and addressing the challenges posed by climate change-induced water deficiency. Furthermore, understanding the interplay between polyamine regulation and water deficiency response in holy basil may unveil novel avenues for pharmacological applications, potentially enhancing the therapeutic efficacy of holy basil-based herbal remedies.

MATERIAL AND METHODS

Treatments and Experimental Design

At Imam Khomeini International University's research greenhouse, a pot experiment was conducted following a factorial design with three replications. The study examined water deficiency levels (100, 75, and 50% field capacity denoted as S0, S1, and S2) and Putrescine spray concentrations (0, 0.1, and 0.2 mM denoted as P0, P1, and P2). Seeds, obtained from Bazran Seed Company, were sown in rows in plastic pots (24 cm diameter, 26 cm height). After thinning to retain five healthy and uniform plants per pot, consistent agricultural care was provided until treatments were applied at the six-leaf stage. Soil properties were thoroughly analyzed, and physical and chemical characteristics are summarized in Table 1. Due to inadequate nutrient levels in the experimental soil, before sowing the seeds, we applied 5 grams of NPK 20-20-20 fertilizer per kilogram of soil to each pot. Additionally, the mean daily greenhouse temperature and relative humidity were recorded as 29.5°C and 38.3%, respectively.

Putrescine treatment involved three foliar application stages. The first spray, at the six-leaf stage, occurred three days before irrigation treatments. Subsequent sprays were every 20 days. To enhance absorption, 0.5 mL of Tween 20 per liter was added as surfactant. Spraying ensured uniform wetting of all leaf surfaces (50 to 100 mL per pot at different growth stages). Control plants received distilled water.

The method for quantifying water deficiency entailed evaluating soil moisture levels and modulating water application rates through pot weighing, ensuring effective mitigation of water scarcity. Water deficiency treatments were maintained until the conclusion of the experiment.

Determination of Morphological Traits

At flowering stage, various traits such as plant height, lateral branches, and relative leaf water content were measured. Plants were harvested, immediately weighed, and then dried in shaded areas with ventilation. Dry weights were recorded for each plant.

Determination of RWC

Relative leaf water content was calculated by weighing the last developed leaf samples before and after 24-hour soaking in distilled water at 4°C. After oven drying at 70°C for 24 hours, dry weights were recorded, and RWC was determined using the following formula:

Table 1. Soil physical and chemical properties.

Soil texture	Available potassium (ppm)	Available phosphorus (ppm)	Total nitrogen (%)	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	Electrical conductivity (dS m^{-1})	pH
Loamy-Sandy	273	7.6	0.07	34	39	27	0.42	1.61	7.2

RWC= (Fw-Dw)/(Tw-Dw)×100 (1)

Where, RWC is the Relative Water Content, Dw is the Dry weight of the leaf, Fw is the weight of the leaf after soaking, and Tw is the weight of the fully Turgid leaf (Dehkordi *et al*., 2021).

Determination of Photosynthetic Pigments

Before harvesting, plant samples were prepared, and 0.25 g of young leaves were extracted in 10 mL of 80% acetone. Chlorophyll a and b amounts were determined by measuring absorbance at 663 and 645 nm wavelengths using a UNICO 2100 spectrophotometer. Calculations were performed based on milligrams per gram of fresh leaf (Lichtenthaler and Wellburn, 1985).

Chlorophyll a= (19.3×A663-0.86×A645) V/100W (2)

Chlorophyll b= (19.3×A645-3.6×A663) V/100W (3)

Where, V= Volume of the supernatant obtained from centrifugation, A= Light absorption at 663 and 645 nm wavelengths, W= Weight of the sample in grams.

The total chlorophyll content was quantified by summing the values of chlorophyll a and chlorophyll b.

Determination of Total Phenolic (TPC) and Flavonoid Content (TFC)

For TPC and TFC determination, 80% methanol was used for extraction. TPC was assessed by mixing 0.5 mL of the extract with Folin-Ciocalteu reagent and sodium carbonate solution, and absorbance was measured at 760 nm (Asghari *et al*., 2020). TFC was determined using the aluminum chloride colorimetric method. Results were expressed as milligrams of gallic acid and quercetin equivalents per gram of dry weight, respectively (Mafakheri and Asghari, 2018).

Measurement of DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radicals Scavenging Capacity

Antioxidant capacity was evaluated by measuring the ability to scavenge DPPH free radicals. The percentage of DPPH radical inhibition was calculated using the following formula (Valko *et al*., 2007):

Inhibition (%)= $[(A_{control}-A_{sample})/A_{control}]\times 100$ (4)

Where, Inhibition (%): Percentage of DPPH free radical inhibition; $A_{control}$: Absorbance of control solution, A_{sample}: Absorbance of sample solution.

Determination of Proline

We determined free proline content with adaptations to Bates *et al*.'s method (1973). Plant leaves samples were homogenized in 3% sulfosalicylic acid, centrifuged to collect supernatant. Next, the supernatant was mixed with acid-ninhydrin reagent, heated, and proline content was measured spectrophotometrically at 520 nm following toluene extraction.

Isolation and Analysis of Essential Oil

Essential oil was extracted from dried holy basil using water distillation. Gas Chromatography (GC) and Gas Xhromatography-Mass Spectrometry (GC/MS) were employed for essential oil analysis, determining the relative percentage of each compound based on chromatogram spectrum area (Singh and Pandey, 2018).

Statistical Analysis

Data analysis was performed using SPSS statistical software version 26. Mean values were compared using the Duncan multi-domain test at a 5% probability level.

RESULTS

Plant Height

Experimental factors significantly influenced plant height. Notably, there was a simple effect at a 1% probability level, and the interaction between stress and putrescine was significant at a 5% probability level (Table 2). The S0P2 and S0P1 treatments led to a 17% and nearly 12% increase in plant height, respectively, compared to the untreated plants. Additionally, applying putrescine in the P1S2 and P2S2 treatments resulted in significant height increases (26 and 21%, respectively) compared to plants subjected to S2P0. These findings highlight the beneficial impact of foliar putrescine application for enhancing plant growth under water-deficient conditions in holy basil (Figure 1).

Plant Fresh Weight

The main treatment effect on plant fresh weight showed statistical significance at the 1% level, while the interaction effect did not reach significance (Table 2). The data indicates more than 26% reduction in plant fresh weight with the S2 treatment compared to the control (Figure 2-A). Conversely, an increase in putrescine concentration led to a significant rise in plant fresh weight by over 21% compared to the control (Figure 2-B).

Plant Dry Weight

The impact of individual treatments on the dry weight of the plant exhibited statistical significance at the 1% level (Table 2). Mean data comparisons revealed the lowest dry weight under severe stress conditions (S2), indicating a 21% reduction in plant biomass under intensified drought stress compared to control (Figure 3-A). In contrast, a 20.73% and 19.31% increase in plant dry weight was observed under

Table 2. Variance analysis of the effects of foliar application of putrescine on morphophysiological and physiological traits of holy basil under water deficiency conditions.

Source of variation	df	Mean Square							
		Plant height	Fresh weight	Dry Weight	Number of branches per plant	RWC	Chl a	Chl b	Total Chl
WD	2	304.502 **	32.704 **	1.395 **	70.865 **	416.772 **	0.358 **	0.076 **	0.756 **
Put	2	124.576 **	23.690 **	1.494 **	28.723 **	182.543 **	0.265 **	0.055 **	0.561 **
WD×Put[a]	4	6.025 *	0.519 ns	0.086 ns	0.286 ns	10.552 *	0.031 **	0.001 ns	0.038 **
Error	18	4.049	0.203	0.082	0.276	1.161	.0011	.001	.001
Total	26								
CV (%)		21.07	8.74	12.20	9.72	21.3	7.7	6.36	7.1

[a] WD: Water Deficiency, Put: Putrescine; *, **, and ns: Significantly difference at the 5 and 1 of probability level, and non-significantly difference, respectively.

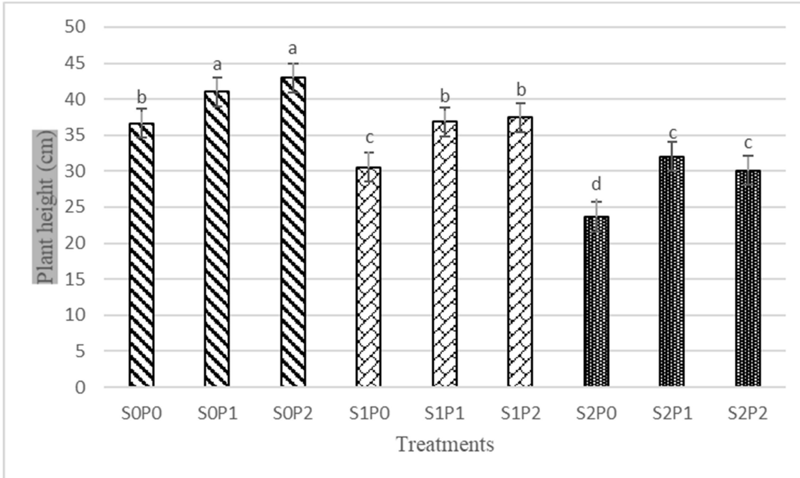
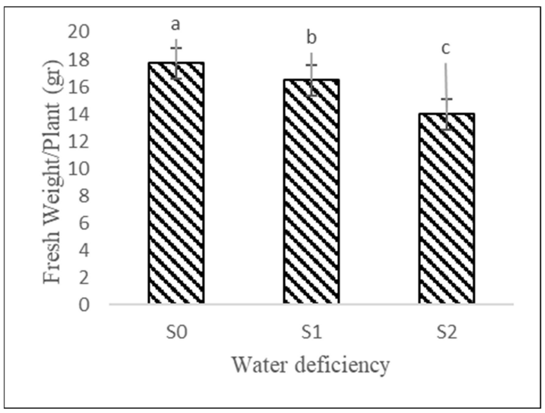


Figure 1. Impact of foliar application with varied putrescine levels and water deficiency on plant height. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC (Full Capacity), respectively. P0, P1, and P2: Putrescine concentrations of 0, 0.1, and 0.2 mM, respectively.



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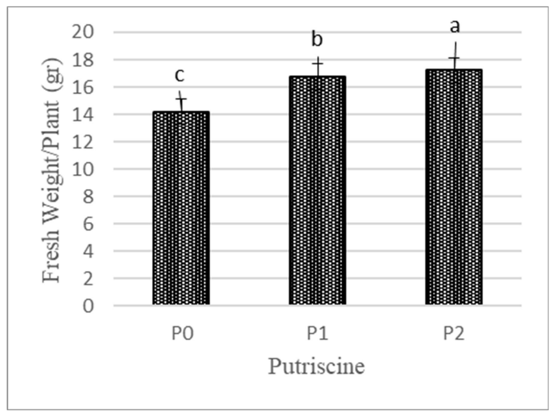

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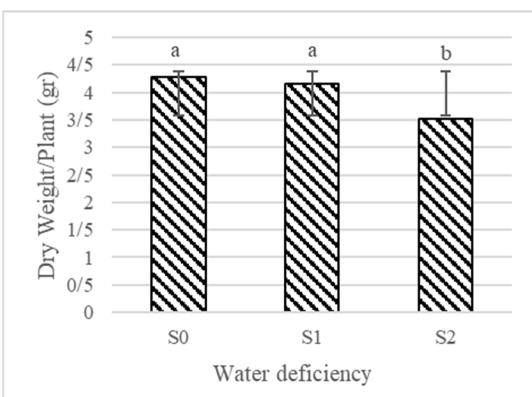
Figure 2. Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on plant fresh weight. S0, S1, and S2 and P0, P1, and P2 are defined in the text and Figure 1.



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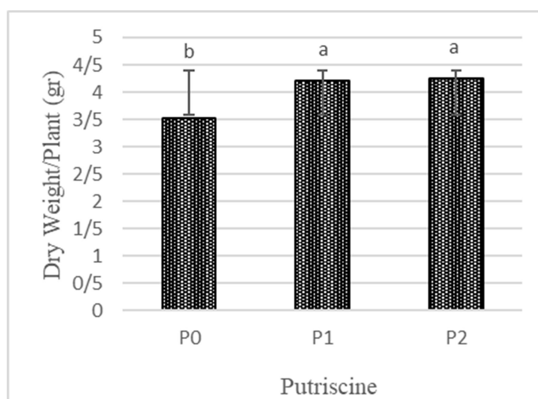

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Figure 3. Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on plant Dry weight. S0, S1, and S2 and P0, P1, and P2 are defined in the text and Figure 1.

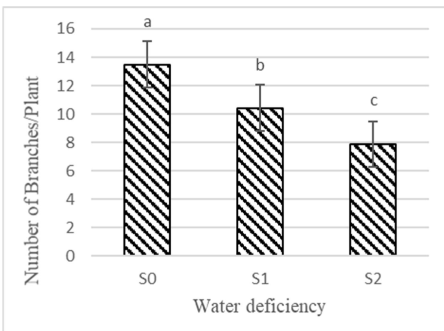
Putrescine application conditions (P1 and P2, respectively) compared to the control (Figure 3-B).

Number of Branches

The influence of experimental treatments on the number of lateral branches exhibited statistical significance at the 1% level, while the interaction effect of treatments did not achieve statistical significance (Table 2). Treatments P2 and S0 exhibited the highest number of branches per plant. Our findings underscore a pronounced decline, approximately 75%, in the number of lateral branches with escalating water deficiency (Figure 4A). Conversely, an augmentation in putrescine concentration led to a significant 40% increase in the number of lateral branches compared to the control (Figure 4B).

Relative Water Content (RWC)

The variance analysis underscores a significant influence of experimental treatments on RWC, at both 1 and 5% significance levels (Table 2). Notably, plants treated with S0P2 and S0P1 exhibited the highest RWC values, with enhancements ranging from 4.5 to 7% compared to the S0P0 treatment. Moreover, the augmentation of putrescine concentration appeared to mitigate the adverse effects of water deficiency on RWC. This observation is further exemplified in Table 3, where the P2S2 treatment manifests a notable increase of over 20% in RWC relative to the S2P0 treatment.



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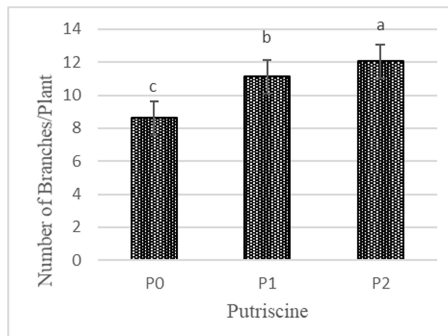


(B)

Figure 4. Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on the number of branches. S0, S1, and S2 and P0, P1, and P2 are defined in the text and Figure 1.

Table 3. Mean comparison between interaction effects of putrescine and water deficiency on holy basil.[a]

Treatment	RWC (%)	Cha (mg g^{-1}FW)	ChT (mg g^{-1} FW)	TPC (mg GAEs g^{-1} extract)	TFC (mg QEs g^{-1} extract)	DPPH (%)	E.O (%)	1-8-cineol (%)	Methyl eugenol (%)
S0P0	79.88 b	0.71 f	1.06 e	29.49 f	12.43 e	32.27 f	0.13 d	7.39 d	14.54 e
S0P1	83.44 a	1.12 b	1.61 b	50.03 c	19.40 d	47.68 c	0.24 b	11.00 a	21.75 abc
S0P2	86.09 a	1.25 a	1.78 a	55.23 b	24.25 b	52.06 b	0.30 a	8.56 cd	20.79 bc
S1P0	72.01 d	0.67 f	0.95 f	32.29 e	13.97 e	36.67 e	0.15 cd	8.42 cd	17.79 d
S1P1	77.57 bc	0.78 d	1.17 d	44.29 d	19.47 d	47.86 c	0.21 b	10.75 a	19.96 cd
S1P2	79.41 b	0.85 c	1.27 c	56.30 ab	24.72 b	52.28 b	0.28 a	8.84 bc	21.46 abc
S2P0	62.45 e	0.48 g	0.68 g	43.85 d	18.28 d	42.37 d	0.17 c	10.80 a	22.30 abc
S2P1	70.66 d	0.67 f	0.97 f	47.92 c	22.27 c	50.48 b	0.21 b	10.04 ab	22.75 ab
S2P2	75.47 c	0.74 de	1.08 e	57.56 a	27.53 a	58.52 a	0.29 a	10.06 ab	23.60 a

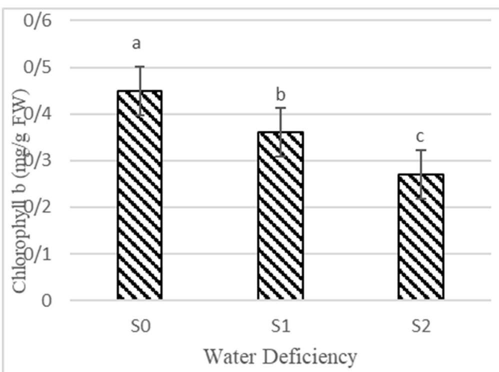
[a] Common letters in each column indicate the absence of a significant difference at a 5% probability level, as per the Duncan test. S0, S1, and S2 and P0, P1, and P2 are defined in the text and Figure 1.

Photosynthetic Pigments

The main effects of treatments involving putrescine and water deficiency, as well as their interactions on chlorophyll a and total chlorophyll, were found to be significant (Table 2). Notably, plants treated with S0P2 displayed the highest concentrations of chlorophyll a and total chlorophyll, measuring 1.25 and 1.78 mg per gram of fresh weight, respectively. Conversely, plants subjected to severe stress conditions without putrescine supplementation exhibited the lowest pigment levels. Additionally, it is noteworthy that treatments S1P1 and S1P2 demonstrated an increase in total chlorophyll of 18 and 33%, respectively, compared to S1P0. Furthermore, treatments S2P1 and S2P2 exhibited increases of 39 and 54% in total chlorophyll compared to S2P0, respectively (Table 3). Increasing drought severity resulted in a significant reduction in chlorophyll b levels; however, higher concentrations of putrescine notably increased chlorophyll b concentrations, with the highest levels observed in plants treated with P2 (Figure 5).

Total Phenolic and Flavonoid Contents (TPC and TFC)

Total phenolic and flavonoid contents were significantly influenced by different irrigation levels, putrescine application, and their interaction effects, as shown by statistical significance at the 1% level (Table 4). Plants treated with S2P2 and S1P2



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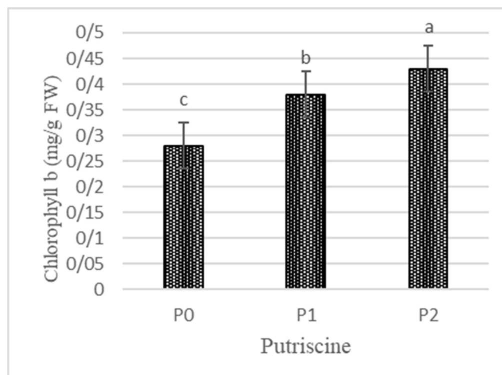

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Figure 5. Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on chlorophyll b. S0, S1, and S2 represent irrigation levels at 100, 75, and 50% FC respectively. P0, P1, and P2 denote Putrescine concentrations of 0, 0.1, and 0.2 mM respectively.

Table 4. Analysis of variance for the impact of foliar application of putrescine and water deficiency on biochemical traits of holy basil.[a]

Source of variation	df	MS							
		TPC	TFC	DPPH	Proline	EO	Cineol	Methyl eugenol	Eugenol
WD	2	81.161 **	41.122 **	96.422 **	186.647 **	0.0001 ns	4.174 **	37.981 **	4.263 ns
Put	2	1015.032 **	253.386 **	634.789 **	40.450 **	0.044 **	7.711 **	37.389 **	307.610 **
WD×Put	4	61.019 **	1.978 **	14.813 **	2.209 ns	0.001 *	3.842 **	10.043 **	5.279 ns
Error	18	1.540	1.063	1.345	0.952	0.000	0.488	1.624	2.204
Total	26								
CV (%)		24.84	16.33	21.18	15.28	12.0	4.89	10.69	16.37

[a] WD: Water Deficiency, Put: Putrescine, TPC: Total Phenolic Content, TFC: Total Flavonoid Content, DPPH: DPPH Radicals Scavenging Capacity, EO: Essential Oil; *, **, and ns: Significant difference at the 5 and 1 probability levels, and non-significant difference, respectively.

showed the highest phenolic content, measuring 57.56 and 56.30 mg GAEs g^{-1} extract, respectively, while the lowest was recorded in treatments S0P0, with 29.49 mg GAEs g^{-1} extract (Table 3). Similarly, both simple and interaction effects of experimental factors significantly influenced flavonoid content at the 1% probability level (Table 4). Flavonoid content increased significantly under severe stress conditions and with higher putrescine concentration. Treatment S2P2 had the highest flavonoid content at 27.53 mg QEs g^{-1} extract, while treatments S0P0 and S1P0 had the lowest, at 12.43 and 13.97 mg QEs g^{-1} extract, respectively (Table 3).

DPPH Radical Scavenging Effect

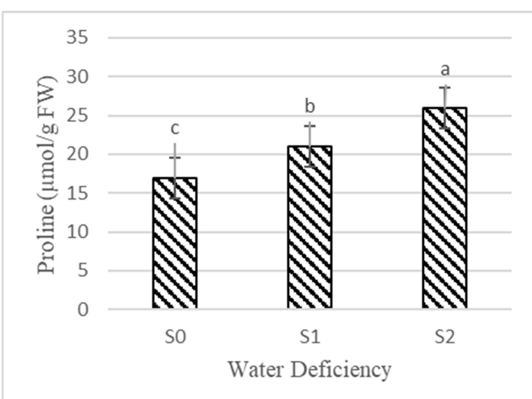
The impact of experimental factors on the free radical scavenging power of DPPH demonstrated statistical significance at the 1% probability level (Table 4). Table 3 illustrate that with increasing water deficiency and escalating putrescine concentration, the free radical scavenging power of DPPH increased. The interaction effect of experimental factors highlights that the most effective DPPH free radical scavenging power was observed in treatment S2P2, reaching 58.52%, while the lowest was recorded in treatment S0P0, with a value of 32.27%. This indicates an increase of more than 81%.

Proline Content

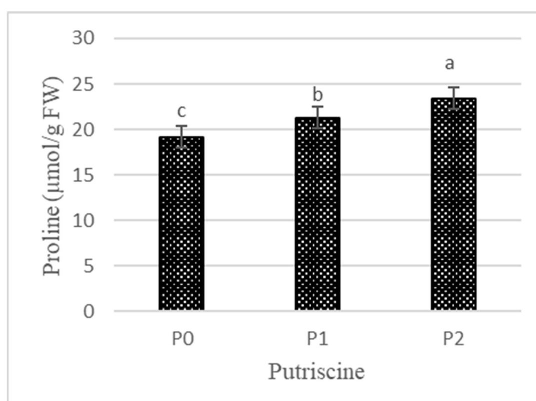
Variance analysis results show significant effects of irrigation levels and putrescine on proline content at the 1% probability level. However, the interaction effect of these factors did not significantly impact this trait (Table 4). Water deficiency notably increased proline levels in holy basil, and higher putrescine concentration corresponded to elevated proline levels in the plant (Figure 6).

Essential Oil Percentage

The impact of water deficiency on the essential oil percentage in holy basil was not significant. However, foliar application of putrescine at the 1% probability level and the interaction effect of experimental factors at the 5% probability level demonstrated significant effects (Table 4). Comparison of



(A)



(B)

Figure 6. Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on Proline. S0, S1, and S2 and P0, P1, and P2 are defined in the text and Figure 1, respectively.

mean data revealed a noteworthy increase in essential oil percentage with higher concentrations of putrescine. Examination of interaction effects identified the most substantial concentrations of essential oils in plants subjected to treatments S0P2, S2P2, and S1P2, with percentages of 0.3, 0.29, and 0.28%, respectively. Conversely, the lowest concentrations were observed in plants treated with S0P0 and S1P0, registering values of 0.13 and 0.15%, respectively (Table 3).

Essential Oil Constituents

The GC-MS analysis of *Ocimum sanctum* identified 22 distinct compositions, as detailed in Table 5. Focusing on compositions with the highest concentrations in the essential oil, our discussion highlights their significance. Analysis of variance revealed notable effects of water deficiency and foliar application of putrescine on 1,8-cineole and methyl eugenol compounds at a significance level

Table 5. GC-MS analysis of *Ocimum sanctum* essential oil.

No.	RT	Compounds	Percentage
1	7.186	3-Hexen-1-ol	0.02
2	9.140	α-Pinene	0.81
3	10.821	β- Pinene	0.09
4	11.482	Sabinene	0.03
5	11.963	1-8-cineole	10.06
6	12.021	p-Cymene	2.53
7	13.026	γ-Terpinene	0.91
8	13.851	α-Terpinolene	0.09
9	14.510	Linalool	0.32
10	16.012	Citronellal	0.24
11	17.234	Geranial	0.37
12	18.014	Thymol	2.03
13	18.581	Carvacrol	4.01
14	20.921	Eugenol	27.57
15	21.851	Methyl- eugenol	23.60
16	24.128	β- Caryophyllene	5.87
17	24.861	α-Humulene	0.12
18	25.421	γ- Elemene	0.38
19	25.715	Germacrene D	5.27
20	27.124	β-Selinene	1.02
21	29.436	δ-Cadinene	0.02
22	34.813	Germacrene B	0.27

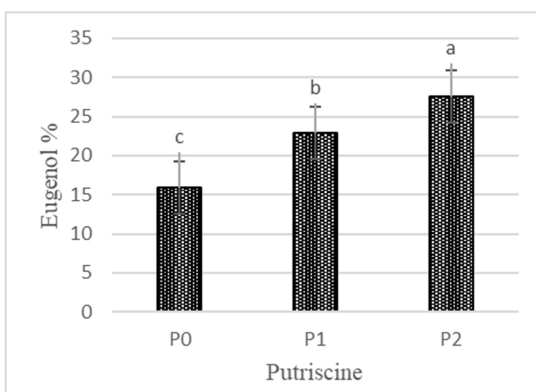


Figure 7. Impact of foliar application with varied putrescine levels on eugenol. P0, P1, and P2 denote putrescine concentrations of 0, 0.1, and 0.2 mM respectively.

of 1%. Specifically, application of putrescine significantly impacted eugenol levels (see Table 4). Among the treatments, plants subjected to S0P1, S2P0, and S1P1 exhibited the greatest quantities of 1,8-cineole, while levels in those treated with S2P2 and S1P1 were statistically similar (refer to Table 3). The concentrations of methyl eugenol in treatments S2P2, S2P1, S2P0, S0P1, and S1P2 were found to be statistically similar. The highest concentration, observed in S2P2, was 23.60% (Table 3). A notable increase in eugenol content was observed with an escalation in putrescine concentration, with the highest level (27.54%) recorded in plants treated with 0.2 mM putrescine (see Figures 7).

DISCUSSION

This study delved into the effects of water deficiency and foliar application of putrescine on the growth, development, and active ingredient content of holy basil. The findings revealed that water deficiency led to a reduction in various growth parameters of holy basil, including plant height, number of branches per plant, fresh weight, and dry weight. This decrease in growth can be attributed to the significant impact of water availability on vegetative growth processes such as cell division, elongation, and differentiation (Farhoudi, 2013). The decline in growth indices under dry conditions resulted from diminished chlorophyll levels, decreased photosynthesis, and subsequently, reduced cell division (Shahroudi *et al*., 2023).

Applying putrescine as a foliar spray led to increased growth parameters in holy basil, including plant height, fresh and dry weights, and the number of branches per plant. Several factors could contribute to this enhancement in growth. Putrescine might have induced hormonal changes or improved physiological processes such as photosynthesis, transpiration, and stomatal conductance, thereby promoting vegetative growth. Additionally, foliar application of putrescine likely activated biosynthetic enzymes, elongated internodes, and facilitated cell division, ultimately leading to increased biomass production (Gonzales *et al*., 2022). Moreover, putrescine's antioxidant properties under normal conditions and its potential role in balancing cation-anion levels, or serving as a nitrogen source, further support plant growth (Kundu *et al*., 2022). Putrescine induces cytokinin hormone, facilitating chlorophyll biosynthesis and chloroplast differentiation (Ahmed *et al*., 2017). This positive impact aligns with the studies on *Thymus daenensis* (Shahroudi *et al*., 2023) and *Thymus vulgaris* L. (Abd-Elbar *et al*., 2019). Numerous researchers have reported the beneficial effects of foliar putrescine application on various plant growth parameters, possibly associated with increased endogenous levels of GA3 (Gibberellic Acid), IAA (auxin), CKs (Cytokinin), and ABA (Abscisic Acid) (Yousefi *et al*., 2021). Chlorophyll content, crucial for plant photosynthetic capacity, decreases with rising drought stress due to protein complex instability and increased chlorophyllase activity under dry conditions (Kalamartzis *et al*., 2020). Putrescine application counteracts this decline, boosting photosynthetic pigments and mitigating drought stress's negative effects on holy basil chlorophylls. This corroborates previous findings showing increased basil chlorophyll content with external putrescine application (Hurtado *et al*., 2023). Studies on *Lallemantia iberica* and *Calendula officinalis* also demonstrated heightened chlorophyll and carotenoid content with putrescine and spermine application (Ansari *et al*., 2021; Danaee *et al*., 2024). Putrescine plays a pivotal role in chloroplast membrane stability, indirectly safeguarding chlorophyll from degradation by protecting the thylakoid membrane. This protective mechanism significantly preserves plant photosynthesis (Jahan *et al*., 2022).

RWC serves as a dependable indicator for assessing plant sensitivity to water deficiency. In this study, an escalation in drought stress resulted in a decrease in RWC values, aligning with findings reported by Damalas (2019) in basil plants. Under drought stress conditions, plants employ strategies to avert low water potential by regulating the balance between water uptake through roots and water loss through leaves. Typically, plants mitigate water loss by closing stomata, subsequently, reducing the rate of leaf transpiration (Damalas, 2019). The potential impact of the foliar application suggests that putrescine, in direct contact with the leaf surface, enhances the water status of epidermal and sub-epidermal cells. The role of putrescine in regulating osmotic pressure emerges as a mechanism for preserving RWC, thereby enhancing overall growth and productivity. Polyamines respond to adverse environmental conditions due to their ability to eliminate Reactive Oxygen Species (ROS) and regulate osmotic pressure (Shahroudi *et al*., 2023; Mohammadi Cheraghabadi *et al*., 2021).

In our research, we observed a significant increase in proline content with higher water deficiency, further augmented by higher concentrations of putrescine. Proline, a water-soluble amino acid, plays a crucial role in regulating cell osmotic pressure and protecting cells from dehydration. It functions under stress conditions by maintaining osmotic balance, protecting protein and cell membrane structures, stabilizing intracellular structures, and scavenging free radicals (Kamrava *et al*., 2017). This suggests a potential synergistic effect between putrescine and proline in enhancing the plant's ability to withstand water stress, emphasizing the intricate interplay between various stress-responsive molecules in plants.

Drought stress, along with putrescine application, significantly affects total phenolics, flavonoid content, and free radical scavenging capacity in holy basil. Putrescine effectively mitigates dry stress effects at specific concentrations by enhancing drought tolerance through interactions with osmolytes, nutrients, ROS signaling, antioxidant regulation, secondary metabolites, and plant hormones (Nasiri *et al*., 2021). The study also found that drought stress alone significantly increased total phenolic and flavonoid content in holy basil. Putrescine application further boosted this trend, peaking in plants subjected to severe dry stress and treated with a high concentration of putrescine. Consistent studies demonstrate increased phenolic and flavonoid production in plants as protective responses to dry stress (Dehghani Bidgoli, 2018; Osama *et al*., 2019). Zeinali *et al*. (2023) noted a significant impact of putrescine on the total phenolic content, flavonoids, and antioxidant activity of Salvia plants.

Previous studies consistently highlight the positive role of putrescine in enhancing DPPH radical scavenging activity, consistent with our findings. This heightened activity can be attributed to increased phenolic compound presence. Research consistently demonstrates the substantial antioxidant activity of phenolic compounds, with *Silybum marianum* leaves showing a notable increase in antioxidant properties with rising phenolic compound levels (Estaji and Niknam, 2020).

Consistent with the findings, there is a positive correlation between the concentration of holy basil essential oil and increasing putrescine concentration. Zahedi and Asadi (2024) reported that at 50 mg L^{-1} putrescine maximized dill essential oil content to 3.58%, while α-phellandrene reached 4.03%. Similarly, Karaman (2008) observed increased levels of linalool and 1,8-cineole in basil with application of spermine, spermidine, and putrescine. Mohammadi *et al.* (2018) documented a rise in thymol in Thyme plants following polyamine application. Dry stress was found to enhance 1,8-cineole and methyl eugenol in essential oils without affecting eugenol quantity. Notably, high putrescine spray significantly increased eugenol in holy basil essential oil. Additionally, Zeinali *et al*. (2023), Nasiri *et al*. (2021), and Dehghani Bidgoli (2018) provide support for putrescine's direct and indirect roles in bioactive compound synthesis. These findings contribute to optimizing holy basil production, improving product quality, enhancing antioxidants, reducing oxidative damage, and serving as a natural preservative substitute, thus ensuring food product quality and safety.

CONCLUSIONS

In conclusion, the application of putrescine demonstrates its efficacy in mitigating the adverse effects of water deficiency on holy basil, providing protection against dry conditions. As a crucial polyamine involved in nitrogen metabolism, putrescine promotes plant growth by supplying essential nitrogen, enhancing physiological processes such as increasing photosynthetic pigment content, and preserving water in plant tissues during water stress. These findings underscore the vital role of putrescine in holy basil growth, offering promising prospects even under limited water availability. Notably, foliar application of 0.2 mM putrescine solution emerges as a cost-effective strategy to enhance holy basil yield in dry conditions, eliciting both immediate defensive responses and long-lasting growth effects. This approach holds significant potential for sustainable agriculture, particularly in regions prone to water scarcity or drought stress. Moving forward, further investigation into the precise mechanisms underlying putrescine's effects and its potential applications in other crops is warranted to fully harness its benefits for sustainable agriculture. By deepening our understanding of putrescine's role and optimizing its application strategies, we can advance agricultural practices towards greater resilience and productivity in the face of environmental challenges.

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افزایش تحمل ریحان مقدس (*Ocimum sanctum* L.) در برابر کمبود آب از طریق محلول پاشی پوترسین

سودابه مفاخری، بهور اصغری، و نرگس آزاد

چکیده

کمبود آب چالش مهمی برای سیستم های کشاورزی جهانی ایجاد می کند و بر عملکرد محصول و کیفیت محصول تأثیر می گذارد. ترکیباتی مانند پوترسین پتانسیل افزایش انعطاف پذیری گیاه را در برابر تنش های محیطی نشان داده اند. این مطالعه گلدانی که در سال ۱۳۹۲ در دانشگاه بین‌المللی امام خمینی (ره) انجام شد، با هدف بررسی تأثیر سطوح مختلف آبیاری و محلول‌پاشی پوترسین بر صفات کمی و کیفی ریحان مقدس (*Ocimum sanctum* L.)در قالب طرح کاملاً تصادفی در سه تکرار انجام شد .کمبود آب در سه سطح (۱۰۰، ۷۵ و ۵۰% ظرفیت مزرعه) القا شد و محلول‌پاشی پوترسین در غلظت‌های ۰، ۰.۱ و ۰.۲ میلی‌مولار استفاده شد. نتایج نشان داد که کمبود آب به طور قابل توجهی باعث کاهش شاخص های رشد گیاه، محتوای نسبی آب (RWC) و سطوح رنگدانه فتوسنتزی می شود. با این حال، محلول پاشی با پوترسین به طور موثر این اثرات نامطلوب را کاهش داد. علاوه بر این، ترکیب کمبود آب و استفاده از ۰.۲ میلی مولار پوترسین باعث افزایش کل ترکیبات فنلی (۴۸.۷۶٪)، ترکیبات فلاونوئیدی (۵٤.۸۵٪) و مهار رادیکال آزاد DPPH (44.85٪) نسبت به شاهد شد. گیاهان تیمار شده با پوترسین نسبت به گروه شاهد افزایش قابل توجهی در درصد اسانس نشان دادند. علاوه بر این، با افزایش کمبود آب، ترکیب اسانس افزایش درصد ۱،۸-سینئول و متیل اوژنول را نسبت به گیاهان شاهد نشان داد. محلول پاشی پوترسین منجر به افزایش قابل توجهی در ترکیبات کلیدی اسانس در ریحان مقدس شد. در نتیجه، محلول پاشی با پوترسین به عنوان یک رویکرد عملی و ساده برای افزایش کیفیت و کمیت رشد ریحان مقدس، به ویژه در مناطق نیمه خشک ظاهر می شود.