

## 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, 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 elevated total phenolic compounds (48.76%), flavonoid compounds (54.85%), and restrained free radical DPPH (44.85%) compared to 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 showed an increase in the percentages of 1,8-cineole and methyl eugenol compared to control plants. The foliar application of putrescine resulted in a significant enhancement in the essential oil's key compounds in holy basil. In conclusion, foliar spray with putrescine emerges as a practical and straightforward approach to enhance both the quality and quantity of holy basil growth, particularly in semi-arid regions.

**Keywords:** Essential oil, Medicinal plants, Putrescine, Water deficiency.

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## 30 INTRODUCTION

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

40 Water, a pivotal element in sustainable development, emerges as a limiting factor for plant  
41 productivity, particularly in agricultural systems confronting regular and prolonged droughts,  
42 prevalent in semi-arid and arid regions globally. Drought stress induces a spectrum of  
43 morphological, physiological, and biochemical alterations in plants, including disruptions in water  
44 relations, suppression of cellular activities (Hatamian et al., 2017), and diminished chlorophyll and  
45 carotenoid content (Guo et al., 2016). The production of reactive oxygen species (ROS) during  
46 drought stress compromises plasma membrane integrity and protein function, resulting in  
47 metabolic dysfunction and substantial yield reduction (Gholami Zali and Ehsanzadeh, 2018). In  
48 response to drought stress, plants deploy various strategies, encompassing the accumulation of  
49 compatible solutes, regulation of photosynthetic parameters, synthesis of stress-related primary and  
50 secondary metabolites, activation of antioxidant enzymes, and alterations in gene expression  
51 (Morshedloo et al., 2017).

52 Prolonged drought conditions exacerbate soil degradation, compromising nutrient availability and  
53 intensifying plant stress responses. Under drought stress, the biosynthesis of phenolic and  
54 flavonoid compounds increases, contributing to antioxidant defense and stress tolerance. These  
55 compounds play a pivotal role in safeguarding cellular structures and maintaining overall plant  
56 health. Additionally, proline, a non-essential amino acid, accumulates in plant tissues during water  
57 deficit, acting as an osmo-protectant by stabilizing cell membranes and preventing dehydration-  
58 induced damage. Elevated proline levels correlate with improved drought resistance. Essential oils,  
59 rich in volatile compounds, find diverse applications in medicine, cosmetics, and aromatherapy.  
60 Drought stress significantly influences the composition and yield of essential oils in medicinal

61 plants. Some species increase oil production as a stress response, potentially enhancing their  
62 medicinal properties. Furthermore, chlorophyll and carotenoids, essential for photosynthesis, face  
63 alterations under drought stress. While chlorophyll content often decreases, affecting energy  
64 capture, balancing carotenoid levels becomes critical for maintaining photosynthetic efficiency  
65 (Rahman et al., 2023; Wagay et al., 2023).

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

81 Holy basil's aromatic properties, culinary uses, and essential oil content make it economically  
82 valuable both globally and in Iran. Its versatility and cultural significance contribute to its  
83 widespread cultivation and utilization. Notably, in the context of water scarcity and recurring  
84 droughts, holy basil's profound medicinal importance becomes even more pronounced (Singh and  
85 Chaudhuri, 2018). This study aims to explore the impact of putrescine spray on the quantity and  
86 quality of holy basil medicinal plant products under conditions of water scarcity. By elucidating  
87 the physiological and biochemical mechanisms underlying the response of holy basil to putrescine  
88 treatment under drought stress, this research endeavors to contribute to the development of  
89 sustainable agricultural practices. The findings of this study hold potential implications for  
90 enhancing crop resilience, optimizing medicinal plant production, and addressing the challenges  
91 posed by climate change-induced water deficiency. Furthermore, understanding the interplay

92 between polyamine regulation and water deficiency response in holy basil may unveil novel  
93 avenues for pharmacological applications, potentially enhancing the therapeutic efficacy of holy  
94 basil-based herbal remedies.

95

## 96 MATERIAL AND METHODS

### 97 Treatments and Experimental Design

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

109 **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 <sup>-1</sup> )	pH
Loamy-Sandy	273	7.6	0.07	34	39	27	0.42	1.61	7.2

110

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

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

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## 122 **Determination of Morphological Traits**

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

## 126 127 **Determination of RWC**

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

$$131 \text{RWC} = (\text{Fw} - \text{Dw}) / (\text{Tw} - \text{Dw}) \times 100 \quad [1]$$

132 Where, RWC is the relative water content, Dw is the dry weight of the leaf, Fw is the weight of the  
133 leaf after soaking, and Tw is the weight of the fully turgid leaf (Dehkordi et al., 2021).

## 134 135 **Determination of Photosynthetic Pigments**

136 Before harvesting, plant samples were prepared, and 0.25 g of young leaves were extracted in  
137 10 ml of 80% acetone. Chlorophyll a and b amounts were determined by measuring absorbance at  
138 663 and 645 nm wavelengths using a UNICO 2100 spectrophotometer. Calculations were  
139 performed based on milligrams per gram of fresh leaf (Lichtenthaler and Wellburn, 1985).

$$140 \text{Chlorophyll a} = (19.3 \times A_{663} - 0.86 \times A_{645}) V / 100W \quad [2]$$

$$141 \text{Chlorophyll b} = (19.3 \times A_{645} - 3.6 \times A_{663}) V / 100W \quad [3]$$

142 V= volume of the supernatant obtained from centrifugation A= light absorption at 663 and 645 nm  
143 wavelengths W= weight of the sample in grams.

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

## 146 147 **Determination of Total Phenolic (TPC) and Flavonoid Content (TFC)**

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

153

## 154 **Measurement of DPPH<sup>2</sup> Radicals Scavenging Capacity**

155 Antioxidant capacity was evaluated by measuring the ability to scavenge DPPH free radicals.

156 The percentage of DPPH radical inhibition was calculated using the formula (Valko et al., 2007):

$$157 \text{ Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

158 Where: Inhibition (%): percentage of DPPH free radical inhibition  $A_{\text{control}}$ : absorbance of control  
159 solution  $A_{\text{sample}}$ : absorbance of sample solution.

160

## 161 **Determination of Proline**

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

166

## 167 **Isolation and Analysis of Essential Oil**

168 Essential oil was extracted from dried holy basil using water distillation. Gas chromatography  
169 (GC) and gas chromatography-mass spectrometry (GC/MS) were employed for essential oil  
170 analysis, determining the relative percentage of each compound based on chromatogram spectrum  
171 area (Singh and Pandey, 2018).

172

## 173 **Statistical Analysis**

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

176

## 177 **RESULTS**

### 178 **Plant Height**

179 **Experimental factors significantly influenced plant height. Notably, there was a simple effect at**  
180 **a 1% probability level, and the interaction between Stress and Putrescine was significant at a 5%**  
181 **probability level (Table 2). The S0P2 and S0P1 treatments led to a 17% and nearly 12% increase**  
182 **in plant height, respectively, compared to untreated plants. Additionally, applying putrescine in the**  
183 **P1S2 and P2S2 treatments resulted in significant height increases (26% and 21%, respectively)**  
184 **compared to plants subjected to S2P0. These findings highlight the beneficial impact of foliar**

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<sup>2</sup> 2,2-diphenyl-1-picrylhydrazyl

185 putrescine application for enhancing plant growth under water-deficient conditions in holy basil

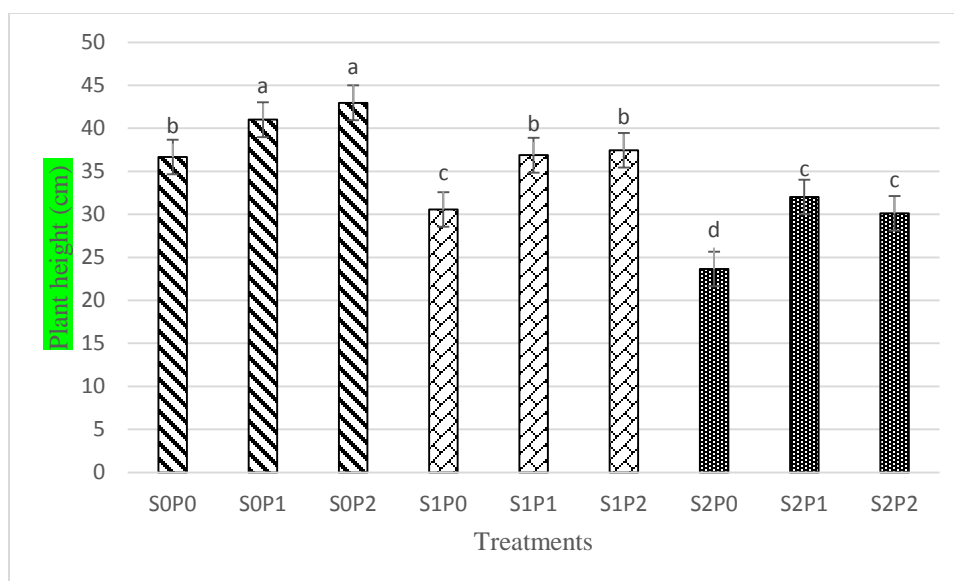
186 (Fig 1).

187 **Table 2.** Variance analysis of effects of foliar application of putrescine on morphophysiological and physiological traits  
 188 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	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

189 \*, \*\*, ns: Significantly difference at the 5 and 1 of probability level, and non-significantly difference, respectively.  
 190 WD: Water Deficiency, Put: Putrescine.

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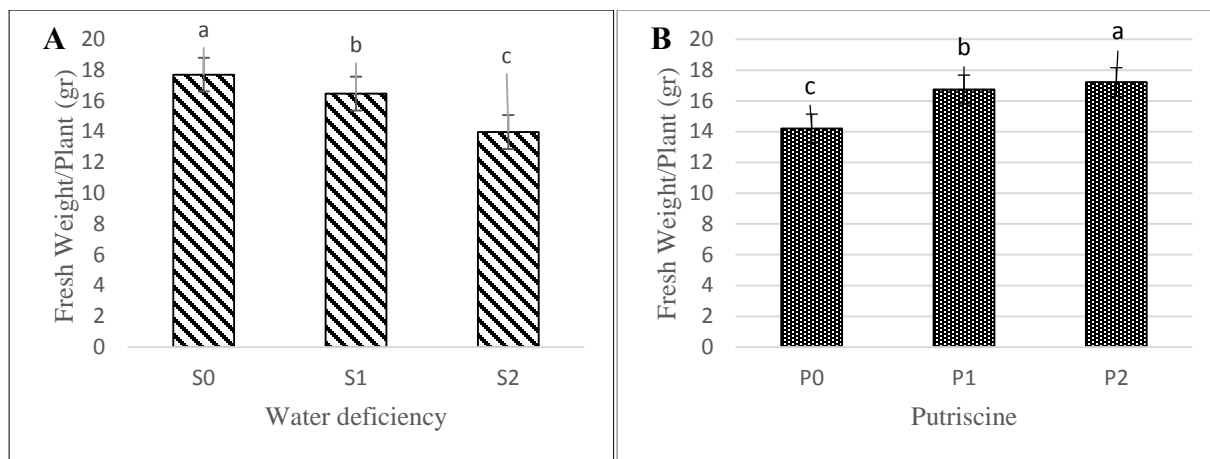


192 **Figure 1.** Impact of foliar application with varied putrescine levels and water deficiency on plant height. S0, S1, and  
 193 S2: irrigation levels at 100%, 75%, and 50% FC respectively. P0, P1, and P2: putrescine concentrations of 0, 0.1, and  
 194 0.2 mM respectively  
 195

196  
 197 **Plant Fresh Weight**

198 The main treatment effect on plant fresh weight showed statistical significance at the 1% level,  
 199 while the interaction effect did not reach significance (Table 2). The data indicates a more than  
 200 26% reduction in plant fresh weight with the S2 treatment compared to the control (Fig. 2A).  
 201 Conversely, an increase in putrescine concentration led to a significant rise in plant fresh weight  
 202 by over 21% compared to the control (Fig. 2B).

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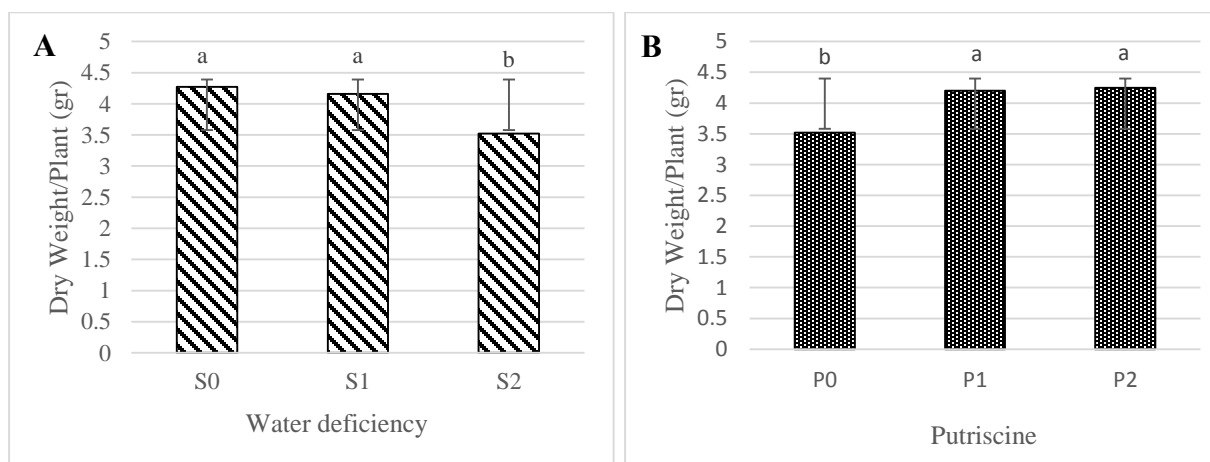


204  
205 **Figure 2.** Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on plant fresh weight.  
206 S0, S1, and S2: irrigation levels at 100%, 75%, and 50% FC respectively. P0, P1, and P2: putrescine concentrations of  
207 0, 0.1, and 0.2 mM respectively.

208

### 209 Plant Dry Weight

210 The impact of individual treatments on the dry weight of the plant exhibited statistical  
211 significance at the 1% level (Table 2). Mean data comparisons revealed the lowest dry weight under  
212 severe stress conditions (S2), indicating a 21% reduction in plant biomass under intensified drought  
213 stress compared to control (Fig. 3A). In contrast, a 20.73% and 19.31% increase in plant dry weight  
214 was observed under putrescine application conditions (P1 and P2, respectively) compared to the  
215 control (Fig. 3B).



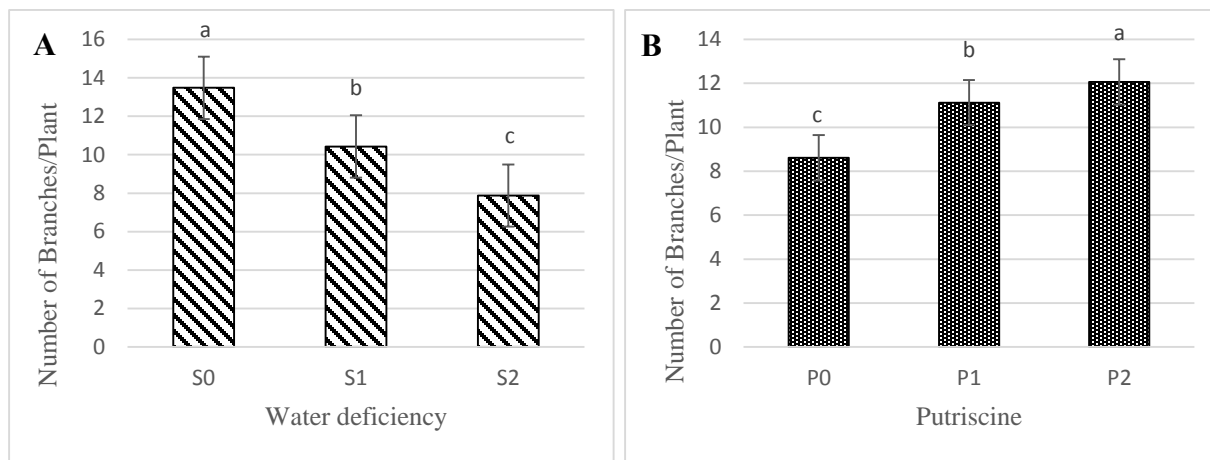
216  
217 **Figure 3.** Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on plant Dry weight.  
218 S0, S1, and S2 represent irrigation levels at 100%, 75%, and 50% FC respectively. P0, P1, and P2 denote putrescine  
219 concentrations of 0, 0.1, and 0.2 mM respectively.

### 220 Number of Branches

221 The influence of experimental treatments on the number of lateral branches exhibited statistical  
222 significance at the 1% level, while the interaction effect of treatments did not achieve statistical



223 significance (Table 2). Treatments P2 and S0 exhibited the highest number of branches per plant.  
 224 Our findings underscore a pronounced decline, approximately 75%, in the number of lateral  
 225 branches with escalating water deficiency (Fig. 4A). Conversely, an augmentation in putrescine  
 226 concentration led to a significant 40% increase in the number of lateral branches compared to the  
 227 control (Fig. 4B).



228 **Figure 4.** Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on the number of  
 229 branches. S0, S1, and S2 represent irrigation levels at 100%, 75%, and 50% FC respectively. P0, P1, and P2 denote  
 230 putrescine concentrations of 0, 0.1, and 0.2 mM respectively.  
 231  
 232

### 233 **Relative Water Content (RWC)**

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

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

Treatment	RWC (%)	Cha (mg g <sup>-1</sup> FW)	ChT (mg g <sup>-1</sup> FW)	TPC (mg GAEs g <sup>-1</sup> extract)	TFC (mg QEs g <sup>-1</sup> 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

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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 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.

### Photosynthetic Pigments

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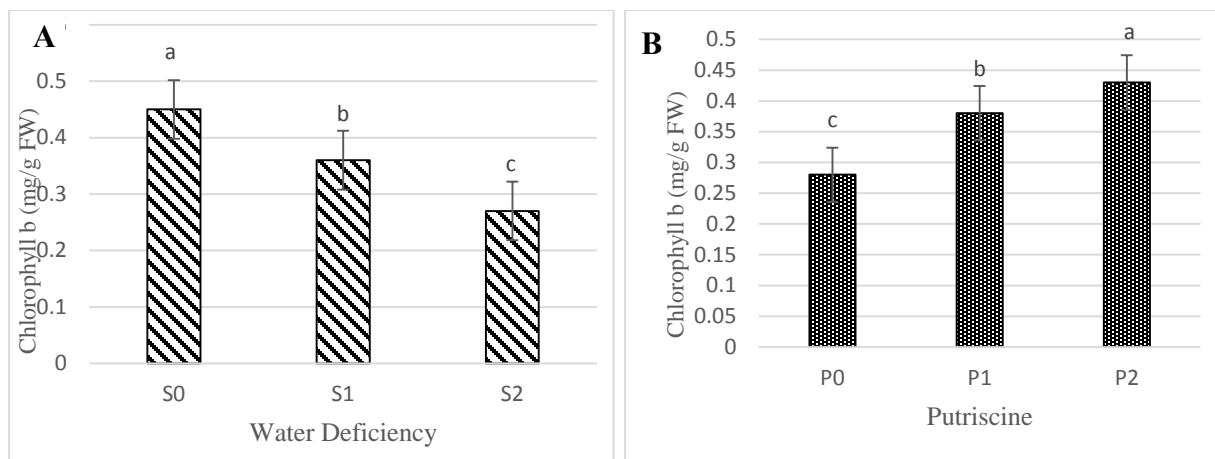
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The main effects of treatments involving Putrescine and water deficiency, as well as their interactions on chlorophyll a and total chlorophyll, were found to be significant (Table 2). Notably, plants treated with S0P2 displayed the highest concentrations 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 (Fig. 5).



269  
 270 **Figure 5.** Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on chlorophyll b. S0,  
 271 S1, and S2 represent irrigation levels at 100%, 75%, and 50% FC respectively. P0, P1, and P2 denote putrescine  
 272 concentrations of 0, 0.1, and 0.2 mM respectively.  
 273

274 **Total Phenolic and Flavonoid Contents (TPC and TFC)**

275 Total phenolic and flavonoid contents were significantly influenced by different irrigation  
 276 levels, putrescine application, and their interaction effects, as shown by statistical significance at  
 277 the 1% level (Table 4). Plants treated with S2P2 and S1P2 showed the highest phenolic content,  
 278 measuring 57.56 and 56.30 mg GAEs/g extract, respectively, while the lowest was recorded in  
 279 treatments S0P0, with 29.49 mg GAEs/g extract (Table 3). Similarly, both simple and interaction  
 280 effects of experimental factors significantly influenced flavonoid content at the 1% level (Table 4).  
 281 Flavonoid content increased significantly under severe stress conditions and with higher putrescine  
 282 concentration. Treatment S2P2 had the highest flavonoid content at 27.53 mg QEs/g extract, while  
 283 treatments S0P0 and S1P0 had the lowest, at 12.43 and 13.97 mg QEs/g extract, respectively (Table  
 284 3).

285 **Table 4.** Analysis of variance for the impact of foliar application with varying levels of putrescine and water deficiency  
 286 on biochemical traits of holy basil.

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

287 \*, \*\*, ns: Significant difference at the 5 and 1 probability levels, and non-significant difference, respectively.  
 288 WD: Water Deficiency, Put: Putrescine, TPC: Total Phenolic Content, TFC: Total Flavonoid Content, DPPH: DPPH  
 289 Radicals Scavenging Capacity, EO: Essential Oil  
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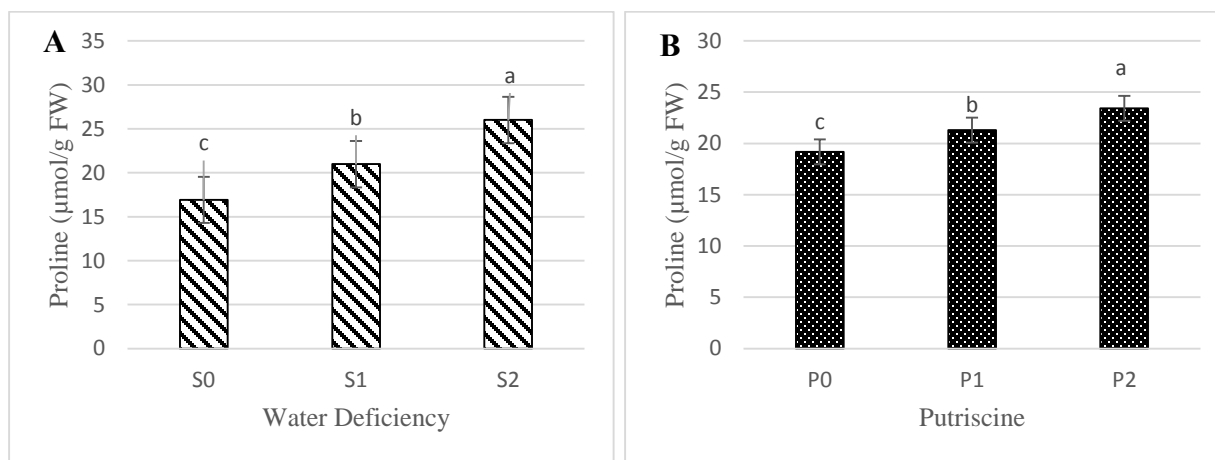
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## 292 **DPPH Radical Scavenging Effect**

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

## 300 **Proline Content**

302 Variance analysis results show significant effects of irrigation levels and putrescine on proline  
303 content at the 1% probability level. However, the interaction effect of these factors did not  
304 significantly impact this trait (Table 4). water deficiency notably increased proline levels in holy  
305 basil, and higher putrescine concentration corresponded to elevated proline levels in the plant (Fig  
306 6).



307 **Figure 6.** Impact of water deficiency (A) and foliar application with varied putrescine levels (B) on Proline. S0, S1,  
308 and S2 represent irrigation levels at 100%, 75%, and 50% FC respectively. P0, P1, and P2 denote putrescine  
309 concentrations of 0, 0.1, and 0.2 mM respectively.  
310

## 311 **Essential Oil Percentage**

313 The impact of water deficiency on the essential oil percentage in holy basil was not found to be  
314 statistically significant. However, foliar application of putrescine at the 1% probability level and  
315 the interaction effect of experimental factors at the 5% probability level demonstrated significant  
316 effects on this trait (Table 4). Comparison of mean data revealed a noteworthy increase in essential  
317 oil percentage with higher concentrations of putrescine. Examination of interaction effects

318 identified the most substantial concentrations of essential oils in plants subjected to treatments  
 319 S0P2, S2P2, and S1P2, with percentages of 0.3%, 0.29%, and 0.28%, respectively. Conversely, the  
 320 lowest concentrations were observed in plants treated with S0P0 and S1P0, registering values of  
 321 0.13% and 0.15%, respectively (Table 3).

322

### 323 **Essential Oil Constituents**

324 The GC-MS analysis of *Ocimum sanctum* identified 22 distinct compositions, as detailed in  
 325 Table 5. Focusing on compositions with the highest concentrations in the essential oil, our  
 326 discussion highlights their significance. Analysis of variance revealed notable effects of water  
 327 deficiency and foliar application of putrescine on 1,8-cineole and methyl eugenol compounds at a  
 328 significance level of 1%. Specifically, the application of putrescine significantly impacted eugenol  
 329 levels (see Table 4). Among the treatments, plants subjected to S0P1, S2P0, and S1P1 exhibited  
 330 the greatest quantities of 1,8-cineole, while levels in those treated with S2P2 and S1P1 were  
 331 statistically similar (refer to Table 3). The concentrations of methyl eugenol in treatments S2P2,  
 332 S2P1, S2P0, S0P1, and S1P2 were found to be statistically similar. The highest concentration,  
 333 observed in S2P2, was 23.60% (see Table 3). A notable increase in eugenol content was observed  
 334 with an escalation in putrescine concentration, with the highest level (27.54%) recorded in plants  
 335 treated with 0.2 millimolar putrescine (see Figures 7).

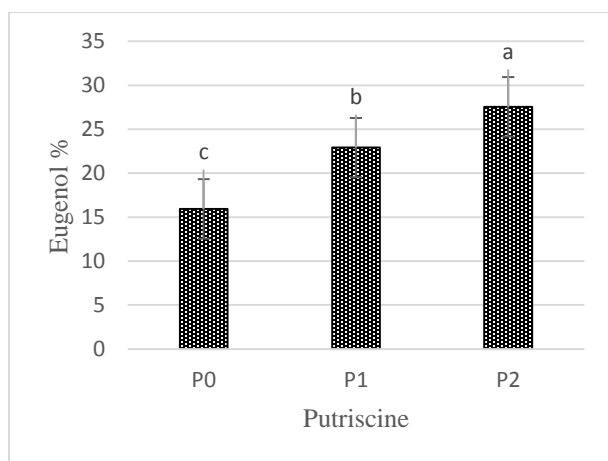
336

337

**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	$\alpha$ -Pinene	0.81
3	10.821	$\beta$ - 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	$\gamma$ -Terpinene	0.91
8	13.851	$\alpha$ -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	$\beta$ - Caryophyllene	5.87
17	24.861	$\alpha$ -Humulene	0.12
18	25.421	$\gamma$ - Elemene	0.38
19	25.715	Germacrene D	5.27
20	27.124	$\beta$ -Selinene	1.02
21	29.436	$\delta$ -Cadinene	0.02
22	34.813	Germacrene B	0.27

338



339  
 340 **Figure 7.** Impact of foliar application with varied putrescine levels on eugenol. P0, P1, and P2 denote putrescine  
 341 concentrations of 0, 0.1, and 0.2 mM respectively.

342  
 343 **DISCUSSION**

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

352 **Applying putrescine as a foliar spray led to increased growth parameters in holy basil, including**  
 353 **plant height, fresh and dry weights, and the number of branches per plant.** Several factors could  
 354 contribute to this enhancement in growth. Putrescine might have induced hormonal changes or  
 355 improved physiological processes such as photosynthesis, transpiration, and stomatal conductance,  
 356 thereby promoting vegetative growth. Additionally, foliar application of putrescine likely activated  
 357 biosynthetic enzymes, elongated internodes, and facilitated cell division, ultimately leading to  
 358 increased biomass production (Gonzales *et al.*, 2022). Moreover, putrescine's antioxidant properties  
 359 under normal conditions and its potential role in balancing cation-anion levels or serving as a  
 360 nitrogen source further support plant growth (Kundu *et al.*, 2022). Putrescine induces cytokinin  
 361 hormone, facilitating chlorophyll biosynthesis and chloroplast differentiation (Ahmed *et al.*, 2017).  
 362 This positive impact aligns with studies on *Thymus daenensis* (Shahroudi *et al.*, 2023) and *Thymus*  
 363 *vulgaris* L. (Abd-Elbar *et al.*, 2019). Numerous researchers have reported the beneficial effects of

364 foliar putrescine application on various plant growth parameters, possibly associated with  
365 increased endogenous levels of GA3 (gibberellic acid), IAA (auxin), CKs (cytokinin), and ABA  
366 (abscisic acid) (Yousefi *et al.*, 2021). Chlorophyll content, crucial for plant photosynthetic capacity,  
367 decreases with rising drought stress due to protein complex instability and increased chlorophyllase  
368 activity under dry conditions (Kalamartzis *et al.*, 2020). Putrescine application counteracts this  
369 decline, boosting photosynthetic pigments and mitigating drought stress's negative effects on holy  
370 basil chlorophylls. This corroborates previous findings showing increased basil chlorophyll content  
371 with external putrescine application (Hurtado *et al.*, 2023). Studies on *Lallemantia iberica* and  
372 *Calendula officinalis* also demonstrated heightened chlorophyll and carotenoid content with  
373 putrescine and spermine application (Ansari *et al.*, 2021; Danaee *et al.*, 2024). Putrescine plays a  
374 pivotal role in chloroplast membrane stability, indirectly safeguarding chlorophyll from  
375 degradation by protecting the thylakoid membrane. This protective mechanism significantly  
376 preserves plant photosynthesis (Nasiri *et al.*, 2021).

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

389 In our research, we observed a significant increase in proline content with the escalation of water  
390 deficiency, further augmented by higher concentrations of putrescine. Proline, a water-soluble  
391 amino acid, plays a crucial role in regulating cell osmotic pressure and protecting cells from  
392 dehydration. It functions under stress conditions by maintaining osmotic balance, protecting  
393 protein and cell membrane structures, stabilizing intracellular structures, and scavenging free  
394 radicals (Kamrava *et al.*, 2017). This suggests a potential synergistic effect between putrescine and

395 proline in enhancing the plant's ability to withstand water stress, emphasizing the intricate interplay  
396 between various stress-responsive molecules in plants.

397 Drought stress, along with putrescine application, significantly affects total phenolics, flavonoid  
398 content, and free radical scavenging capacity in holy basil. Putrescine effectively mitigates dry  
399 stress effects at specific concentrations by enhancing drought tolerance through interactions with  
400 osmolytes, nutrients, ROS signaling, antioxidant regulation, secondary metabolites, and plant  
401 hormones (Nasiri et al., 2021). The study also found that drought stress alone significantly  
402 increased total phenolic and flavonoid content in holy basil. Putrescine application further boosted  
403 this trend, peaking in plants subjected to severe dry stress and treated with a high concentration of  
404 putrescine. Consistent studies demonstrate increased phenolic and flavonoid production in plants  
405 as protective responses to dry stress (Dehghani Bidgoli, 2018; Osama *et al.*, 2019). Zeinali *et al.*  
406 (2023) noted a significant impact of putrescine on the total phenolic content, flavonoids, and  
407 antioxidant activity of *Salvia* plants.

408 Previous studies consistently highlight the positive role of putrescine in enhancing DPPH radical  
409 scavenging activity, consistent with our findings. This heightened activity can be attributed to  
410 increased phenolic compound presence. Research consistently demonstrates the substantial  
411 antioxidant activity of phenolic compounds, with *Silybum marianum* leaves showing a notable  
412 increase in antioxidant properties with rising phenolic compound levels (Estaji and Niknam, 2020).  
413 Consistent with the findings, there is a positive correlation between the concentration of holy basil  
414 essential oil and increasing putrescine concentration. Zahedi and Asadi (2023) reported that at 50  
415 mg/L, putrescine maximized dill essential oil content to 3.58%, while  $\alpha$ -phellandrene reached  
416 4.03%. Similarly, Karaman (2008) observed increased levels of linalool and 1,8-cineole in basil  
417 with application of spermine, spermidine, and putrescine. Mohammadi *et al.* (2018) documented a  
418 rise in thymol in Thyme plants following polyamine application. Dry stress was found to enhance  
419 1,8-cineole and methyl eugenol in essential oils without affecting eugenol quantity. Notably, high  
420 putrescine spray significantly increased eugenol in holy basil essential oil. Additionally, Zeinali *et al.*  
421 (2023), Nasiri *et al.* (2021), and Dehghani Bidgoli (2018) provide support for putrescine's direct  
422 and indirect roles in bioactive compound synthesis. These findings contribute to optimizing holy  
423 basil production, improving product quality, enhancing antioxidants, reducing oxidative damage,  
424 and serving as a natural preservative substitute, thus ensuring food product quality and safety.

425  
426



## 427 CONCLUSIONS

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

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### 558 افزایش تحمل ریحان مقدس (*Ocimum sanctum* L.) در برابر کمبود آب از طریق محلول پاشی 559 پوترسین

561 س. مفاخری، ب. اصغری، و ن. آزاد

#### 562 چکیده

563 کمبود آب چالش مهمی برای سیستم های کشاورزی جهانی ایجاد می کند و بر عملکرد محصول و کیفیت محصول تأثیر می  
564 گذارد. ترکیباتی مانند پوترسین پتانسیل افزایش انعطاف پذیری گیاه را در برابر تنش های محیطی نشان داده اند. این مطالعه  
565 گلدانی که در سال 1392 در دانشگاه بین المللی امام خمینی (ره) انجام شد، با هدف بررسی تأثیر سطوح مختلف آبیاری و  
566 محلول پاشی پوترسین بر صفات کمی و کیفی ریحان مقدس (*Ocimum sanctum* L.) در قالب طرح کاملاً تصادفی در سه  
567 تکرار انجام شد. کمبود آب در سه سطح (100%، 75% و 50% ظرفیت مزرعه) القا شد و محلول پاشی پوترسین در  
568 غلظت های 0، 0.1 و 0.2 میلی مولار استفاده شد. نتایج نشان داد که کمبود آب به طور قابل توجهی باعث کاهش شاخص های  
569 رشد گیاه، محتوای نسبی آب (RWC) و سطوح رنگدانه فتوسنتزی می شود. با این حال، محلول پاشی با پوترسین به طور  
570 موثر این اثرات نامطلوب را کاهش داد. علاوه بر این، ترکیب کمبود آب و استفاده از 0.2 میلی مولار پوترسین باعث افزایش  
571 کل ترکیبات فنلی (48.76%)، ترکیبات فلاونوئیدی (54.85%) و مهار رادیکال آزاد (DPPH (44.85%)) نسبت به شاهد شد.  
572 گیاهان تیمار شده با پوترسین نسبت به گروه شاهد افزایش قابل توجهی در درصد اسانس نشان دادند. علاوه بر این، با افزایش  
573 کمبود آب، ترکیب اسانس افزایش درصد 1،8-سیننول و متیل اوژنول را نسبت به گیاهان شاهد نشان داد. محلول پاشی پوترسین  
574 منجر به افزایش قابل توجهی در ترکیبات کلیدی اسانس در ریحان مقدس شد. در نتیجه، محلول پاشی با پوترسین به عنوان یک  
575 رویکرد عملی و ساده برای افزایش کیفیت و کمیت رشد ریحان مقدس، به ویژه در مناطق نیمه خشک ظاهر می شود.