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Improving Effects of Salicylic Acid and Jasmonic Acid on Alleviation of Water-Deficit Stress on Thyme (*Thymus vulgaris* L.) Growth, Physiology, and Essential oil

Zahra Karimi, Mohammad Nasri*, Meysam Oveysi, Pourang Kasraei, Hamid Reza Larijani

Department of Agronomy, Faculty of Agricultural, Islamic Azad, Varamin-Pishva Branch, Tehran, Iran

*Corresponding author's email: DrMohammadNasri@yahoo.com

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ABSTRACT

Iran encompasses various climate zones, with approximately 85% of its agricultural land located in arid and semi-arid regions. To mitigate the effects of drought stress and enhance crop adaptation and yield, numerous advanced techniques, including hormone applications, have been extensively studied. This research aimed to investigate the effects of foliar application of different concentrations of salicylic acid and jasmonic acid on the morphological, physiological, and biochemical characteristics of garden thyme under water deficit stress conditions. The study was conducted using a split-factorial design within a randomized complete block framework, with three replications at the Varamin and Absard research fields in Iran during the 2019 growing season. The primary factor was irrigation, assessed at two levels: normal and water-deficit stress. Sub-factors were the foliar application of salicylic acid and jasmonic acid at various concentrations, and the treatment groups were in factorial arrangements. Salicylic acid was applied at concentrations of 0, 25, and 50 mg L^{-1}, while jasmonic acid was applied at 0, 1, and 2 mg L^{-1}. The results demonstrated that water deficit stress significantly reduced plant height, dry weight yield, and chlorophyll a, b, and total chlorophyll content, while it increased proline accumulation. Under water deficit stress, the maximum levels of dityrosine and 8-hydroxy-2'-deoxyguanosine (34.18 and 13.52 nmol mg^{-1} protein, respectively) were observed, which were 11% and 20% higher, respectively, than those under well-watered conditions. Furthermore, the interaction between foliar applications of different concentrations of salicylic acid and jasmonic acid mitigated the detrimental effects of water deficit stress, enhancing the tolerance of garden thyme. Overall, the findings suggest that under drought conditions, the application of salicylic acid and jasmonic acid can significantly improve the performance of garden thyme.

Introduction

The increasing global interest in medicinal plants and herbal medicines has drawn significant attention to these resources. Currently, the World Health Organization reports that 25% of common medicines are derived from plants (Keshavarz Mirzamohammadi et al., 2021a; Emami Bistgani et al., 2024). Garden thyme (*Thymus vulgaris* L.), a member of the mint family, is particularly valued for the medicinal properties found in its aerial parts, including leaves and flowers. Today, thyme is utilized in the production of medications for treating asthma, bronchitis, gastrointestinal disorders, and managing seizures and neuropathologies (Mohammadi et al., 2019).

Plants have developed various adaptive mechanisms to cope with environmental stresses, including physiological adjustments. Among these, the accumulation of compatible solutes through osmotic adjustment mechanisms has garnered significant attention (Lotfi et al., 2010). In thyme species, the primary phenolic compounds, thymol and carvacrol, are typically present in the essential oil as glucosides and lactosides (Rahimi et al., 2022). The high concentration of phenolic compounds in thyme is responsible for its potent antimicrobial, antifungal, antibacterial, and antiparasitic properties (Hosseini et al., 2023a).

The improvement of medicinal plants is an area of significant interest, with photosynthetic pigments and shoot length being key parameters associated with yield (shoot dry weight). These factors are particularly useful in developing high-yielding thyme plants with optimal levels of bioactive compounds (Kulak et al., 2021). Keshavarz Mirzamohammadi and colleagues (2021b) reported that water deficit stress reduced the dry weight of roots and shoots and the levels of total chlorophyll and carotenoids in peppermint (*Mentha piperita*) in Tehran, Iran, while increasing root length and proline content.

Extensive research has documented the physiological responses of plants to various abiotic stresses, highlighting the role of secondary metabolites in protecting plants and the interactions among sugars, abscisic acid, and gibberellin pathways in the plant's response to drought (Lotfi et al., 2010b). Water deficit stress can inhibit or halt physiological activities such as transpiration, photosynthesis, tissue and organ elongation, and enzyme activity (Li et al., 2019). Additionally, water deficit stress can alter the yield and composition of essential oils, driven by enzyme activity and metabolic adjustments (Li et al., 2019). Under drought, salinity, low temperatures, and other factors that reduce the water potential of cell sap, plants likely increase the concentration of osmolytes to maintain water absorption under stress conditions (Keshavarz and Khodabin, 2019).

Plant growth regulators play a crucial role in the adaptation to environmental stress (Howe et al., 2018). Among these, salicylic acid and jasmonic acid are particularly noteworthy. Jasmonates, including their derivative methyl jasmonate, act as internal growth regulators or inhibitors, playing key roles in growth, development, and stress response (Wasternack and Strnad, 2016). Additionally, jasmonates are vital signaling compounds in the induction process leading to the accumulation of secondary metabolites (Chen et al., 2019). Jasmonic acid has been reported to enhance tolerance to water deficit stress by promoting chlorophyll accumulation and activating antioxidant enzyme systems such as ascorbate peroxidase, glutathione reductase, superoxide dismutase, peroxidase, and catalase. It also reduces lipid peroxidation, as indicated by decreased levels of malondialdehyde and hydrogen peroxide (da Graca et al., 2016).

Salicylic acid has been shown to increase fresh and dry weight as well as metabolite content in mustard (*Brassica juncea*) (Gharibiyan et al., 2023). It also inhibits auxin oxidation, thereby influencing growth (Li et al., 2019). Moreover, salicylic acid has been reported to improve grain yield and harvest index in basil and marjoram (Popova et al., 2009). Given that much of Iran's soil is located in arid and semi-arid regions, various advanced techniques, including hormone applications, have been explored to mitigate the effects of drought stress. We hypothesize that irrigation disruption has a heritable effect on yield and essential oil composition. Therefore, this research was conducted to investigate the effects of foliar application of different concentrations of salicylic acid and jasmonic acid on several quantitative and qualitative parameters of garden thyme under water deficit conditions in the Absard and Varamin regions of Iran.

Material and Methods

Experimental site

This research was conducted using a split-factorial design within a randomized complete block framework, with three replications at two locations: Varamin research field and Absard during the 2019 crop year. The study area is characterized by a semi-arid Mediterranean climate, with moderate to cool mountainous conditions. The region experiences an average annual precipitation of 210 mm and has an average annual temperature of 26°C (Fig. 1).

Treatments

The main factors in this study consisted of two irrigation treatments: irrigation at 75% (normal irrigation) and 60% (water deficit stress) of the depletion of available soil water (Yousefzadeh et al., 2023). The subfactors included the foliar application of salicylic acid at concentrations of 0, 25, and 50 mg L^{-1}, and jasmonic acid at concentrations of 0, 1, and 2 mg L^{-1}, arranged in a factorial design (Li et al., 2019). Foliar applications were performed on all plants early in the morning, before sunrise, at the mid-vegetative growth stage and the onset of flowering. Both salicylic acid and jasmonic acid were purchased from Sigma Chemical Co. (St. Louis, USA).

The foliar applications were conducted using a pressurized backpack sprayer with a 12-L capacity, calibrated to deliver 1000 L ha-1 of spray solution. The sprayer was equipped with a spiral solid cone spray nozzle. The control treatment involved the application of sterile distilled water. Each replication included 18 treatments, with each treatment comprising six planting lines, each 4 m in length. The rows were spaced 50 cm apart, with 15 cm between plants within a row. The distance between treatments was 0.5 m, and the distance between replications was 1 m (Portu et al., 2018).

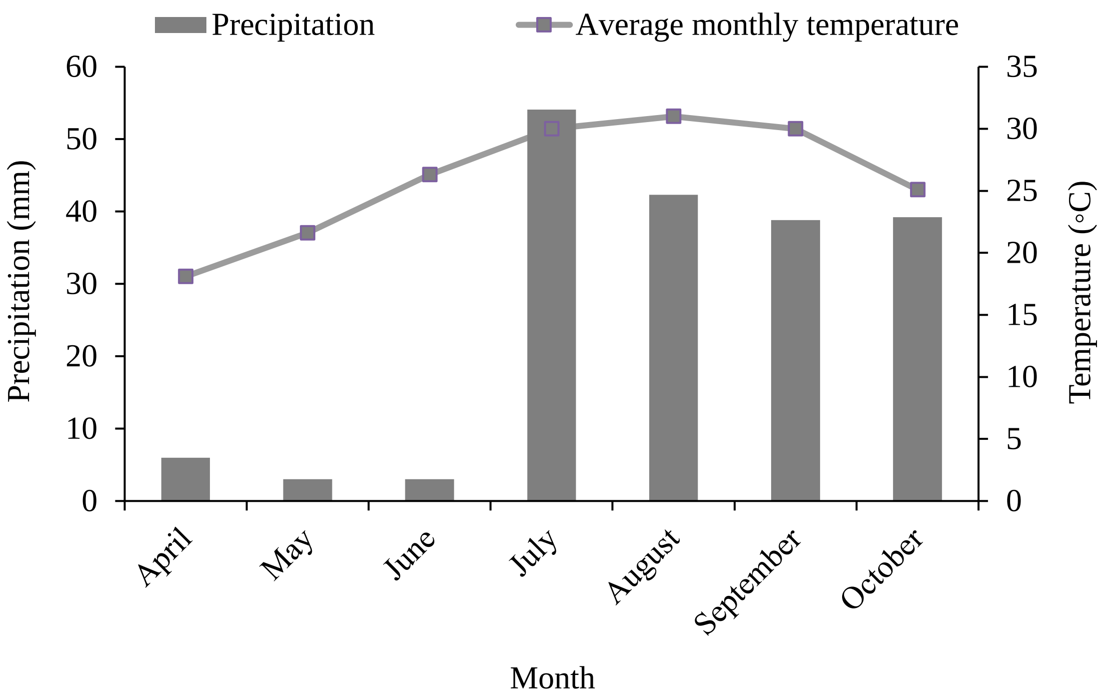

Fig. 1. Average monthly air temperature and precipitation during the period of April-October in 2019.

Data collection

After 100 d (just before flowering), the total chlorophyll content (mg g^{-1} FW) was obtained using acetone 80% and the content of total chlorophyll content were calculated in mg g^{-1} fresh weight (FW) of the sample (Equation 1) (Arnon, 1949) by a spectrophotometer (Varian Cary Win UV 6000i, Australia):

$$Total\ chlorophyll:\ (12.7 \times A_{663}) - (2.69 \times A_{645})$$

The proline content was measured using the method of Bates et al. (1973), based on the acidic ninhydrin reaction, with absorbance readings taken at 520 nm. Malondialdehyde (MDA) content in plant leaves was assessed using the method of Keshavarz and Khodabin (2019), with an extinction coefficient of 155 mM^{-1} cm^{-1}. The analysis of dityrosine and 8-hydroxy-2'-deoxyguanosine (D-OH-DG) was conducted following the protocols recommended by Bogdanov and Bical (1999) and Steven and Josef (1978).

For the measurement of antioxidant status, 0.2 g of freeze-dried leaf tissues were ground in 50 mM potassium phosphate buffer, and 0.2 mL of the centrifuged solution was used to determine crude enzyme activity (Variam Cary Win UV 6000i, Australia). Superoxide dismutase (SOD) activity was estimated using the method described by Giannopolitis and Ries (1977), with absorbance measured at 560 nm. Guaiacol peroxidase (GPX) enzyme activity was measured following the method of Ghanati et al. (2002), by assessing the absorbance of a reaction solution containing ascorbic acid and H_2O_2 at 290 nm using a spectrophotometer. Catalase (CAT) activity was determined using the method described by Cakmak and Horst (1991), which monitors the rate of reduction of H_2O_2 at 240 nm in the reaction

mixture. All antioxidant activities were expressed as units per mg of protein.
The traits under study were measured from the sampling lines, excluding marginal effects, at the mid-flowering stage. Plant height was recorded in centimeters from the fourth and fifth sampling lines at the onset of flowering. Total dry weight was determined by drying the samples in an oven at 60°C for 24 hours, followed by weighing. The essential oil content was determined using hydrodistillation of a 15 g sample of dry leaves with a Clevenger-type apparatus. The oils were dried over sodium sulfate, evaluated as a percentage by weight, and stored in a freezer until analysis (Hosseini et al., 2023a). Essential oil composition was analyzed using gas chromatography-mass spectrometry (GC-MS), with thermal programming following the method of Adams (1995).

Statistical analysis
Data were subjected to statistical analysis using the SAS software (version 9.2). To determine significant differences between treatments, the comparison of mean values was carried out via the LSD test ($P \leq 0.05$). XLSTAT software (2018 version) was used in generating the results of principal component analysis.

Results
Principal component analysis (PCA) and cluster analysis (CA)
The results of the principal component analysis (PCA) (Fig. 2) revealed that the first and second components had eigenvalues greater than one and were therefore selected as significant components. The first component accounted for 82.59% of the total variance, while the second component explained an additional 9.83%, together accounting for 92.42% of the total variance.
The biplot generated from the first and second components indicated that several treatment combinations clustered together based on their high affinity and correlation with specific traits. Specifically, the treatments with 0 mg L^{-1} jasmonic acid and 0 mg L^{-1} salicylic acid under water deficit conditions, 1 mg L-1 jasmonic acid and 0 mg L^{-1} salicylic acid under water deficit conditions, 0 mg L^{-1} jasmonic acid and 50 mg L^{-1} salicylic acid under water deficit stress, 1 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under water deficit stress, and 0 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under water deficit stress formed a distinct group. This group showed a strong correlation with proline content and glutathione peroxidase activity.
Additionally, another cluster was identified, comprising treatments with 1 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under water deficit stress, 2 mg L^{-1} jasmonic acid and 0 mg L^{-1} salicylic acid under water deficit stress, 3 mg L^{-1} jasmonic acid and 50 mg L^{-1} salicylic acid under water deficit stress, and 3 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under water deficit stress. This group was strongly associated with traits such as D-OH-DG, thymol content, superoxide dismutase activity, dityrosine levels, malondialdehyde content, and essential oil percentage.

Under well-watered conditions, the PCA results showed that the treatments with 2 mg L^{-1} jasmonic acid and 50 mg L^{-1} salicylic acid, 3 mg L^{-1} jasmonic acid and 0 mg L^{-1} salicylic acid, 3 mg L^{-1} jasmonic acid and 50 mg L^{-1} salicylic acid, and 3 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid were grouped together based on their high affinity with the measured traits. These treatments were highly correlated with plant height, catalase (CAT) activity, total chlorophyll content, and total dry weight.
The dendrogram derived from the cluster analysis (Fig. 3) revealed that the treatments in the current experiment were divided into three distinct groups based on all the measured traits. The separation of different treatments into three distinct clusters indicates a significant level of diversity among the investigated treatments.
In the first cluster, five treatments grouped together due to their high similarity: 0 mg L^{-1} jasmonic acid and 0 mg L^{-1} salicylic acid under well-watered conditions, 1 mg L^{-1} jasmonic acid and 0 mg L^{-1} salicylic acid under well-watered conditions, 3 mg L^{-1} jasmonic acid and 50 mg L^{-1} salicylic acid under well-watered conditions, 3 mg L^{-1} jasmonic acid and 0 mg L^{-1} salicylic acid under well-watered conditions, and 3 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under well-watered conditions.
The second cluster contained the largest number of treatments, comprising eight in total, which were grouped together based on their close proximity. These treatments included: 2 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under water deficit conditions, 2 mg L^{-1} jasmonic acid and 50 mg L^{-1} salicylic acid under water deficit conditions, 3 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under well-watered conditions, 0 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under well-watered conditions, 2 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under well-watered conditions, 0 mg L^{-1} jasmonic acid and 50 mg L^{-1} salicylic acid under well-watered conditions, 3 mg L^{-1} jasmonic acid and 25 mg L^{-1}

salicylic acid under water deficit conditions, and 3 mg L^{-1} jasmonic acid and 50 mg L^{-1} salicylic acid under water deficit conditions.

The third cluster, based on high relatedness, consisted of five treatments: 0 mg L^{-1} jasmonic acid and 50 mg L^{-1} salicylic acid under water deficit conditions, 0 mg L^{-1} jasmonic acid and 25 mg L^{-1} salicylic acid under water deficit conditions, 3 mg L^{-1} jasmonic acid and 0 mg L^{-1} salicylic acid under water deficit conditions, 0 mg L^{-1} jasmonic acid and 0 mg L^{-1} salicylic acid under water deficit conditions, and 2 mg L^{-1} jasmonic acid and 0 mg L^{-1} salicylic acid under water deficit conditions.

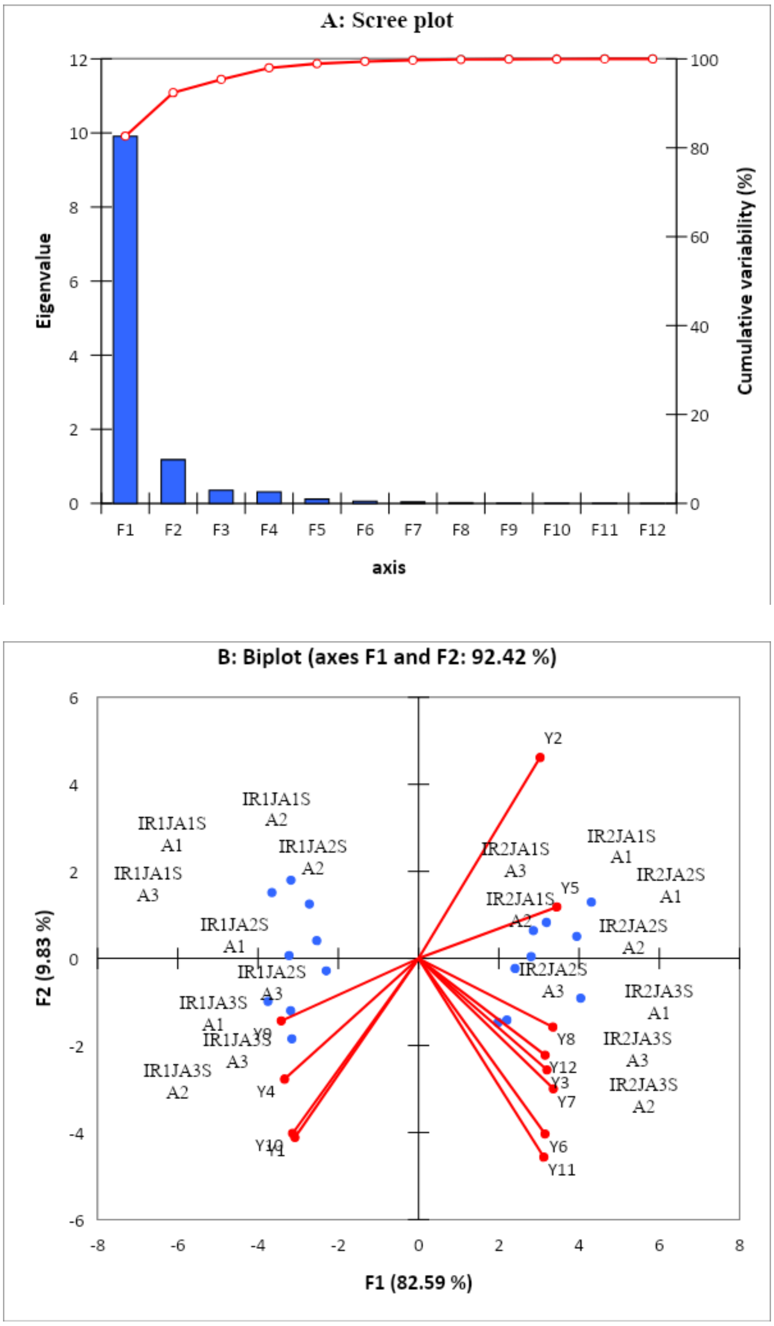

Fig. 2. Screen plot obtained from principal component analysis (A), Biplot dived from first and second component based on all studied traits (B). Total chlorophyll content (Y1), proline (Y2), superoxide dismutase (Y3), catalase (Y4), glutathione peroxidase (Y5), malondialdehyde (Y6), dityrosine (Y7), D-OH-DG (Y8), plant height (Y9), total dry weight (Y10), essential oil percentage (Y11) and thymol (Y12), IR1: well-watered condition, IR2: water deficit stress, JA1: Jasmonic acid 0 (mg L^{-1}), JA2: Jasmonic acid 1 (mg L^{-1}), JA3: Jasmonic acid 2 (mg L^{-1}), SA1: Salicylic acid 0 (mg L^{-1}), SA2: Salicylic acid 25 (mg L^{-1}) and SA3: Salicylic acid 50 (mg L^{-1})).

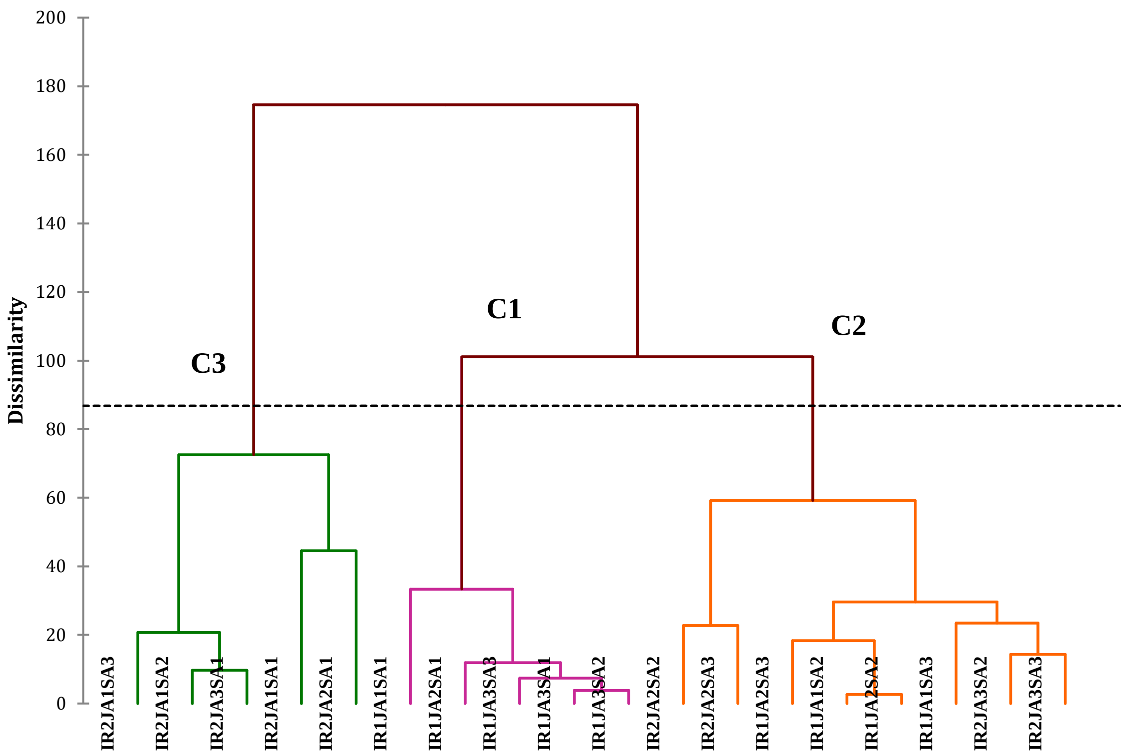

Fig. 3. Cluster analysis based on all studied traits in garden thyme plant. (IR1: well-watered condition, IR2: water deficit stress, JA1: Jasmonic acid 0 (mg L^{-1}), JA2: Jasmonic acid 1 (mg L^{-1}), JA3: Jasmonic acid 2 (mg L^{-1}), SA1: Salicylic acid 0 (mg L^{-1}), SA2: Salicylic acid 25 (mg L^{-1}) and SA3: Salicylic acid 50 (mg L^{-1})).

Physiological traits

The analysis of variance (Table 1) revealed that total chlorophyll (TChl) content was significantly influenced by irrigation, salicylic acid, and jasmonic acid treatments (P≤0.01). Additionally, the interaction between irrigation and salicylic acid was also significant for TChl content (P≤0.01). The results indicated that increasing the concentration of jasmonic acid led to an increase in TChl content, with the highest and lowest TChl levels observed in the 2 mg L^{-1} jasmonic acid treatment (4.93 mg g^{-1} fresh weight) and the treatment without jasmonic acid application (4.39 mg g^{-1} fresh weight), respectively (Fig. 4). Specifically, the application of 2 mg L^{-1} jasmonic acid resulted in a 12% increase in TChl content compared to the control. The interaction between irrigation and salicylic acid showed that under well-watered conditions, TChl content increased with higher concentrations of salicylic acid. The application of 50 mg L^{-1} salicylic acid resulted in a 26% increase in TChl content compared to the treatment without salicylic acid (Table 2). Conversely, under water deficit stress conditions, the application of 25 mg L^{-1} salicylic acid led to a 13% decrease in TChl content compared to the control, while the application of 50 mg L^{-1} salicylic acid increased TChl content by 20% relative to the control. Overall, water deficit stress reduced TChl content across all salicylic acid concentrations compared to well-watered conditions.

Proline content was significantly influenced by irrigation, salicylic acid, and the interaction between irrigation and salicylic acid (P≤0.01) (Table 1). The interaction between irrigation and salicylic acid (Table 2) revealed that the highest proline content was observed in the treatment without salicylic acid application under water deficit stress (43.95 mg g^{-1} fresh weight). Under water deficit stress, there was no significant difference in proline content between the treatments with no salicylic acid application and the application of 25 mg L^{-1} salicylic acid (43.28 mg g^{-1} fresh weight). Conversely, the lowest proline content was recorded in the treatment with 50 mg L^{-1} salicylic acid under well-watered conditions (38.19 mg g^{-1} fresh weight). In both well-watered and water deficit conditions, the application of salicylic acid resulted in a reduction in proline content compared to the treatment without salicylic acid application.

Table 1. Combined analysis of variance on some physiological traits, yield and essential oil profile of garden thyme (*Thymus vulgaris* L.) as affected by irrigation regimes and foliar application of jasmonic acid and salicylic acid.

SOV	Df	TChl	Proline	SOD	CAT	GPX	MDA	DT	D-OH-DG	PH	TDW	OP	Thymol
Place	1	2.34^{ns}	8.2^{ns}	1.57^{ns}	29.4^{ns}	33.05^{ns}	14.2^{ns}	3.23^{ns}	11.8^{ns}	9.94^{ns}	41730^{ns}	0.0008^{ns}	328.3^{ns}
Block (place)	4	2.23	6.8	0.35	4.15	12.05	16.3	1.64	5.01	6.23	10861	0.0001	186.5
Irrigation (IR)	1	86.2**	289.1**	32.32**	320.02**	536.8**	955.9**	383.7**	205.9*	1765**	674843**	0.017**	1253.2^{ns}
Place × IR	1	0.58^{ns}	4.6^{ns}	0.12^{ns}	0.009^{ns}	1.55^{ns}	2.14^{ns}	1.5^{ns}	8.01^{ns}	14.2^{ns}	65359^{ns}	0.00006^{ns}	437.2^{ns}
IR × Block (place)	4	1.03	1.53	0.27	4.47	1.91	8.38	1.007	17.8	10.2	9266	0.00014	240.02
Salicylic acid (SA)	2	13.1**	46.3**	0.82**	27.34**	12.6**	77.7**	11.2**	0.48**	34.4**	86144**	0.0018**	37.1^{ns}
Jasmonic acid (JA)	2	2.63**	5.45^{ns}	0.058^{ns}	0.13^{ns}	0.01^{ns}	7.34*	0.46^{ns}	2.93^{ns}	42.2**	17751**	0.00003^{ns}	7.32^{ns}
IR × SA	2	1.29**	12.06**	3.02**	1.82*	5.21**	15.6**	0.24^{ns}	1.73^{ns}	40.09**	3268^{ns}	0.0002**	12.7^{ns}
IR × JA	2	0.37^{ns}	0.56^{ns}	0.054^{ns}	5.75**	37.33**	10.02**	2.33^{ns}	0.022^{ns}	17.8**	82498**	0.00003^{ns}	201.3**
SA × JA	4	0.27^{ns}	0.11^{ns}	0.0024^{ns}	0.096^{ns}	0.03^{ns}	0.19^{ns}	0.61^{ns}	0.20^{ns}	0.64^{ns}	4520^{ns}	0.00000009^{ns}	4.29^{ns}
IR × SA × JA	4	0.1^{ns}	0.05^{ns}	0.001^{ns}	0.08^{ns}	0.12^{ns}	0.68^{ns}	1.24^{ns}	0.082^{ns}	1.94^{ns}	1112^{ns}	0.000001^{ns}	2.04^{ns}
SA × Place	2	0.4^{ns}	6.45^{ns}	0.001^{ns}	0.91^{ns}	0.011^{ns}	1.23^{ns}	0.55^{ns}	0.27^{ns}	1.97^{ns}	1337^{ns}	0.000001^{ns}	3.7^{ns}
JA × Place	2	0.1^{ns}	0.36^{ns}	0.0008^{ns}	0.078^{ns}	0.016^{ns}	0.018^{ns}	0.51^{ns}	1.18^{ns}	1.75^{ns}	614^{ns}	0.0000004^{ns}	9.21^{ns}
IR × SA × Place	2	0.01^{ns}	2.99^{ns}	0.003^{ns}	0.26^{ns}	0.013^{ns}	0.65^{ns}	0.98^{ns}	0.11^{ns}	5.32^{ns}	1418^{ns}	0.0000005^{ns}	38.8^{ns}
IR × JA × Place	2	0.11^{ns}	0.004^{ns}	0.001^{ns}	0.042^{ns}	0.22^{ns}	2.29^{ns}	0.18^{ns}	0.093^{ns}	0.72^{ns}	101^{ns}	0.0000009^{ns}	14.62^{ns}
SA × JA × Place	4	0.07^{ns}	0.036^{ns}	0.002^{ns}	0.081^{ns}	0.011^{ns}	1.26^{ns}	0.08^{ns}	0.25^{ns}	1.59^{ns}	1119^{ns}	0.0000002^{ns}	44.8^{ns}
IR × SA × JA × Place	4	0.15^{ns}	0.073^{ns}	0.001^{ns}	0.06^{ns}	0.008^{ns}	1.28	0.1^{ns}	0.41^{ns}	2.14^{ns}	887^{ns}	0.000001^{ns}	10.8^{ns}
Error	64	0.14	2.09	0.033	0.42	0.29	1.65	1.002	2.38	3.16	2375	0.00001	32.7
CV (%)		8.1	3.4	2.29	4.07	3.54	9.27	3.09	12.7	10.53	5.95	2.29	12.09

TChl: total chlorophyll content; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase; MDA: malondialdehyde; DT: dityrosine; D-OH-DG: 8-hydroxy-2'-deoxyguanosine; PH: plant height; TDW: total dry weight; OP: essential oil percentage. **, * and ns indicate no significance and significance at 0.01 and 0.05, respectively.

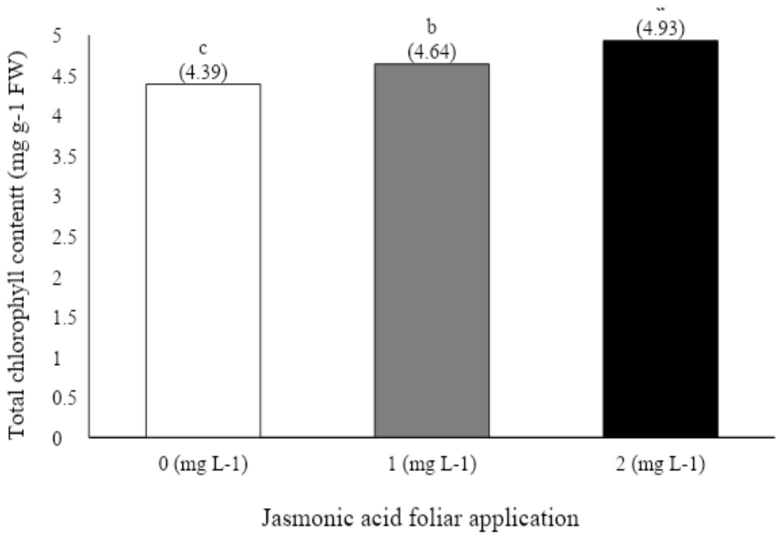

Fig. 4. Effect of jasmonic acid foliar application on total chlorophyll content of garden thyme (*Thymus vulgaris* L.).

Table 2. Interaction of irrigation regime × salicylic acid foliar application on the studied traits of garden thyme (*Thymus vulgaris* L.).

irrigation regime	Salicylic acid (mg L^{-1})	TChl (mg g^{-1} Fw)	Proline (mg g^{-1} Fw)	SOD (U mg protein min^{-1})	CAT (U mg protein min^{-1})	GPX (U mg protein min^{-1})	MDA (ηmol mg^{-1} FW)	PH (cm)	OP (%)
Well watered conditions	0	5.02^{c}	41.51^{c}	6.97^{d}	16.9^{c}	12.9^{e}	9.83^{e}	21.01^{a}	0.162^{d}
	25	5.32^{b}	40.50^{d}	7.64^{c}	17.7^{b}	13.4^{d}	11.1^{d}	20.9^{a}	0.178^{c}
	50	6.32^{a}	38.19^{e}	7.75^{c}	18.2^{a}	12.5^{f}	11.7^{d}	20.8^{a}	0.180^{c}
Water deficit stress	0	3.66^{e}	43.95^{a}	8.69^{a}	12.9^{e}	18.3^{a}	15.2^{c}	11.0^{d}	0.194^{b}
	25	3.24^{f}	43.28^{ab}	8.60^{a}	14.6^{d}	17.5^{b}	16.1^{b}	12.5^{c}	0.200^{a}
	50	4.39^{d}	42.79^{b}	8.34^{b}	14.9^{d}	16.5^{c}	19.1^{a}	15.03^{b}	0.203^{a}

TChl: total chlorophyll content; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase; MDA: malondialdehyde; PH: plant height; OP: essential oil percentage. Significant differences (P≤0.05) are indicated with different letters according to the least significant difference (LSD) test.

Superoxide dismutase (SOD) enzyme activity was significantly affected by irrigation, salicylic acid, and their interaction (P≤0.01) (Table 1). The interaction between irrigation and salicylic acid showed that in the absence of water stress, the application of 25 mg L^{-1} and 50 mg L^{-1} salicylic acid increased SOD enzyme activity by 10% and 11%, respectively, compared to the treatment without salicylic acid (Table 2). However, under water deficit conditions, the application of 50 mg L^{-1} salicylic acid led to a 4% decrease in SOD activity compared to the control, although there was no significant difference between the application of 25 mg L^{-1} salicylic acid and the control. Overall, SOD activity was higher under water deficit conditions than in well-watered conditions across all salicylic acid concentrations.

Catalase (CAT) enzyme activity was significantly influenced by irrigation (P≤0.01), salicylic acid (P≤0.01), the interaction between irrigation and salicylic acid (P≤0.05), and the interaction between irrigation and jasmonic acid (P≤0.01) (Table 1). The interaction between irrigation and salicylic acid indicated that the highest CAT enzyme activity was observed in the treatment with 50 mg L^{-1} salicylic acid under well-watered conditions (18.2 U mg^{-1} protein min^{-1}), which was 8% higher than the treatment without salicylic acid (Table 2). Conversely, the lowest CAT enzyme activity was found in the treatment without salicylic acid under water deficit conditions (12.9 U mg^{-1} protein min^{-1}). In general, CAT enzyme activity was lower under water deficit conditions compared to well-watered conditions at all levels of salicylic acid application.

Regarding the interaction between irrigation and jasmonic acid, it was found that the highest CAT activity occurred in the treatment without jasmonic acid under well-watered conditions (18.04 U mg^{-1} protein min^{-1}), while the lowest CAT activity was observed in the treatment without jasmonic acid under water deficit conditions (Table 3). Under well-watered conditions, the application of 1 mg L^{-1} and 2 mg L^{-1} jasmonic acid reduced CAT activity by 3% and 4%, respectively, compared to the control. In contrast, under water deficit conditions, the application of 1 mg L^{-1} and 2 mg L^{-1} jasmonic acid increased CAT activity by 4% and 6%, respectively, compared to the control.

Table 3. Interaction of irrigation regime × jasmonic acid foliar application on the studied traits of garden thyme (*Thymus vulgaris* L.).

Irrigation regime	Jasmonic acid (mg L^{-1})	CAT (U mg protein min^{-1})	GPX (U mg protein min^{-1})	MDA (ηmol mg^{-1} FW)	PH (cm)	TDW (Kg ha^{-1})	Thymol (%)
Well-watered conditions	0	18.04^{a}	11.8^{f}	10.8^{cd}	20.64^{a}	926^{a}	40.96^{d}
	1	17.50^{b}	13.2^{e}	10.3^{d}	20.57^{a}	883^{b}	44.64^{cd}
	2	17.4^{b}	13.8^{d}	11.4^{c}	21.60^{a}	880^{b}	46.25^{bc}
Water deficit stress	0	13.7^{d}	18.5^{a}	17.7^{a}	10.92^{c}	659^{d}	53.13^{a}
	1	14.3^{c}	17.1^{b}	16.4^{b}	13.33^{b}	766^{c}	49.66^{ab}
	2	14.5^{c}	16.6^{c}	16.2^{b}	14.29^{b}	790^{c}	49.50^{ab}

CAT: catalase; GPX: glutathione peroxidase; MDA: malondialdehyde; PH: plant height; TDW: total dry weight. Significant differences ($P \le 0.05$) are indicated with different letters according to the least significant difference (LSD) test.

Glutathione peroxidase (GPX) enzyme activity was significantly ($P \le 0.01$) affected by irrigation, salicylic acid, irrigation × salicylic acid, and irrigation × jasmonic acid (Table 1). The interaction between irrigation and salicylic acid showed that the highest amount of GPX enzyme activity was related to the treatment with no application of salicylic acid under water deficit stress conditions (18.3 U mg protein min^{-1}), whereas the lowest amount of GPX enzyme activity was observed in the treatment with 50 mg L^{-1} of salicylic acid under well-watered conditions (12.5 U mg protein min^{-1}). In general, GPX enzyme activity was higher under water deficit stress conditions than under well-watered conditions at all concentrations of salicylic acid. The interaction between irrigation and jasmonic acid showed that, in the absence of water stress, GPX enzyme activity was higher at all concentrations of jasmonic acid compared to water deficit conditions (Table 3). The highest amount of GPX enzyme activity was observed in the treatment with no application of jasmonic acid under water deficit stress conditions (18.5 U mg protein min^{-1}), while the lowest amount of GPX enzyme activity was observed in the treatment with no application of jasmonic acid under well-watered conditions (Table 3). According to the results, malondialdehyde (MDA) was significantly affected by irrigation ($P \le 0.01$), salicylic acid ($P \le 0.01$), jasmonic acid ($P \le 0.05$), irrigation × salicylic acid ($P \le 0.01$), and irrigation × jasmonic acid ($P \le 0.01$) (Table 1). Based on the results of the irrigation × salicylic acid interaction, it was observed that the application of salicylic acid under water deficit stress conditions and well-watered conditions increased MDA compared to the treatment without salicylic acid (Table 2). In the absence of water stress, although the application of salicylic acid increased MDA compared to the treatment without salicylic acid, there was no significant difference between the application of 25 and 50 mg L^{-1} of salicylic acid in terms of MDA. Under water deficit stress conditions, the application of 50 mg L^{-1} of salicylic acid caused a 26% increase in MDA compared to the treatment without salicylic acid application. The interaction between irrigation and jasmonic acid showed that the highest amount of MDA was observed in the treatment with no application of jasmonic acid under water deficit conditions (17.7 nmol mg^{-1} FW) (Table 3). On the other hand, the lowest amount of MDA was observed in the treatments with no application of jasmonic acid and the application of 1 mg L^{-1} jasmonic acid under well-watered conditions, at 10.8 and 10.3 nmol mg^{-1} FW, respectively, with no statistical difference between them. In general, the amount of MDA under water deficit stress was higher than under well-watered conditions at all concentrations of jasmonic acid. Dityrosine was significantly ($P \le 0.01$) affected by irrigation and salicylic acid (Table 1). The value of dityrosine under water deficit conditions was 34.18 nmol mg^{-1} protein, showing a 12% increase compared to well-watered conditions (Table 4). The results showed that with the application of salicylic acid, the amount of dityrosine increased compared to the treatment without salicylic acid, but there was no significant difference between the concentrations of 25 and 50 mg L^{-1} of salicylic acid (Table 5).

Based on the results, D-OH-DG was significantly ($P \le 0.01$) affected by irrigation and salicylic acid (Table 1). The amount of D-OH-DG under water deficit stress conditions was 13.52 nmol mg^{-1}

protein, which showed a 26% increase compared to well-watered conditions (Table 4). The results showed that the application of 50 mg L^{-1} of salicylic acid caused a 2% increase in D-OH-DG compared to the treatment with no application of salicylic acid, and there was no significant difference between the no application of salicylic acid and the application of 25 mg L^{-1} of salicylic acid (Table 5).

Table 4. Effect of irrigation regimes on DT and D-OH-DG of garden thyme (*Thymus vulgaris* L.).

Irrigation regimes	DT (ηmol mg^{-1} protein)	D-OH-DG (ηmol mg^{-1} protein)
Well-watered	30.41^{b}	10.75^{b}
Water deficit stress	34.18^{a}	13.52^{a}

DT: dityrosine; D-OH-DG: 8-hydroxy-2'-deoxyguanosine. Significant differences (P≤0.05) are indicated with different letters according to the least significant difference (LSD) test.

Table 5. Effect of salicylic acid foliar application on DT, D-OH-DG and total dry weight of garden thyme (*Thymus vulgaris* L.).

Salicylic acid (mg L^{-1})	DT (ηmol mg^{-1} protein)	D-OH-DG (ηmol mg^{-1} protein)	TDW (Kg ha^{-1})
0	31.7^{b}	12.05^{b}	771^{b}
25	32.3^{ab}	12.09^{b}	813^{ab}
50	32.8^{a}	12.27^{a}	868^{a}

DT: dityrosine; D-OH-DG: 8-hydroxy-2'-deoxyguanosine; TDW: total dry weight. Significant differences at $P \leq 0.05$ have been indicated with different letters according to least significant difference (LSD) test.

Agronomical traits

Plant height was significantly affected by irrigation, salicylic acid, jasmonic acid, as well as the interactions between irrigation × salicylic acid and irrigation × jasmonic acid (P≤0.01) (Table 1). Interaction analysis between irrigation and salicylic acid revealed that there was no significant difference in plant height among the various concentrations of salicylic acid under well-watered conditions. However, under water deficit stress conditions, the application of 25 and 50 mg L^{-1} of salicylic acid resulted in an increase in plant height by 14% and 37%, respectively, compared to the treatment without salicylic acid (Table 2). Overall, plant height was greater under well-watered conditions than under water deficit stress conditions across all concentrations of salicylic acid. Regarding the interaction between irrigation and jasmonic acid, there was no significant difference in plant height among the different concentrations of jasmonic acid under well-watered conditions (Table 3). Conversely, in water deficit conditions, the application of 1 and 2 mg L^{-1} of jasmonic acid led to increases in plant height by 22% and 31%, respectively, compared to the treatment without jasmonic acid.

Total dry weight was significantly affected by irrigation, salicylic acid, jasmonic acid, and the interaction between irrigation and jasmonic acid (P≤0.01) (Table 1). The interaction between irrigation and jasmonic acid demonstrated that the application of jasmonic acid resulted in a decrease in total dry weight under both water deficit stress and well-watered conditions. There was no significant statistical difference between treatments using 1 and 2 mg L^{-1} of jasmonic acid (Table 3). The highest total dry weight was observed in the treatment without jasmonic acid under well-watered conditions, measuring 926 kg ha^{-1}, while the lowest total dry weight was recorded in the treatment without jasmonic acid under water deficit stress conditions, measuring 659 kg ha^{-1} (Table 3).

Furthermore, the results indicated that the highest total dry weight was achieved with the application of 50 mg L^{-1} of salicylic acid (868 kg ha^{-1}), which represented a 13% increase compared to the treatment without salicylic acid. There was no significant statistical difference between the treatments with 25 and 50 mg L^{-1} of salicylic acid (Table 5).

Essential oil percentage and thymol content

The essential oil percentage was significantly (P≤0.01) affected by irrigation, salicylic acid, and the interaction between irrigation and salicylic acid (Table 1). Based on the results of the interaction between irrigation and salicylic acid, in both well-watered and water-deficit conditions, the application of salicylic acid increased the essential oil percentage compared to the treatment with no application of salicylic acid (Table 2). No significant difference was observed between the use of 25 and 50 mg L^{-1}

salicylic acid in terms of essential oil percentage. The highest essential oil percentages were observed in the treatment with 25 and 50 mg L^{-1} of salicylic acid under water-deficit stress conditions, at 0.200% and 0.203%, respectively, while the lowest essential oil percentage was observed in the treatment with no application of salicylic acid under well-watered conditions (0.162%). In general, the essential oil percentage under water-deficit stress was higher than under well-watered conditions for all concentrations of salicylic acid. The results of the analysis of variance showed that thymol was only affected by the interaction between irrigation and jasmonic acid ($P \leq 0.01$) (Table 1). In the absence of water stress, the application of 2 mg L^{-1} of jasmonic acid increased thymol by 13% compared to the treatment without jasmonic acid (Table 3). Although thymol content under water-deficit conditions was higher at all concentrations of jasmonic acid compared to well-watered conditions, there was no significant difference between the different concentrations of jasmonic acid application under water-deficit stress conditions.

Discussion

Garden thyme is a significant species due to its medicinal compounds (Rahimi et al., 2022). Environmental stresses, particularly water stress, have substantial impacts on the physiological and phytochemical properties of medicinal plants (Hosseini et al., 2023a). In the present study, water deficit stress resulted in a reduction in total dry weight. However, the application of jasmonic acid at concentrations of 1 and 2 mg L^{-1} led to an increase in total dry weight compared to the untreated control. It appears that water deficit stress impairs the functionality of the photosynthetic apparatus, thereby disrupting carbon fixation (Keshavarz et al., 2021). Additionally, water deficit stress interferes with the absorption of micro and macro elements, negatively affecting other physiological traits (Chandrasekaran, 2022). These disturbances in nutrient absorption, reduced carbon fixation, and other adverse effects due to water deficit stress collectively contribute to a decline in plant growth and consequently, a decrease in total dry weight (Keshavarz-Mirzamohammadi et al., 2021b).

A promising approach to ameliorate plant growth under environmental stress is the application of plant growth regulators such as salicylic acid and jasmonic acid. Jasmonic acid confers protection against environmental stresses by modulating the expression of various genes (Li et al., 2019) and serves as a cellular regulator involved in multiple growth stages, including seed development, root growth, fertility, fruit ripening, and senescence (Hosseini et al., 2023a). In response to water deficit stress, jasmonic acid activates various genes to mitigate the detrimental effects of stress (Wang et al., 2021). In this study, the application of jasmonic acid increased total dry weight under water deficit stress conditions. Moreover, our results demonstrated that jasmonic acid enhanced the total chlorophyll content in garden thyme. Similarly, the application of salicylic acid increased total chlorophyll content under both water deficit and well-watered conditions. This suggests that both salicylic acid and jasmonic acid improve photosynthesis by enhancing chlorophyll content, thereby increasing carbon fixation and ultimately leading to higher total dry weight.

Li et al. (2019) reported that water stress reduced the growth and yield of dry matter in peppermint plants in China, and that jasmonic acid application alleviated the negative effects of stress, which aligns with our findings. In the present study, the application of salicylic acid and jasmonic acid also resulted in increased plant height under water deficit stress. This suggests that under stressful conditions, these growth regulators positively influence chlorophyll synthesis, photosynthetic activity, nutrient absorption, and cell division, contributing to increased plant height. A study conducted in Sari, Iran, showed that increased plant height enhances leaf and stem development, leading to a rise in total dry weight (Hosseini et al., 2023a).

In the context of environmental stress physiology, it is generally accepted that the accumulation of compatible solutes plays a crucial role in maintaining cellular osmotic balance. For instance, proline accumulation in plants has been associated with increased tolerance to water stress (Hosseini et al., 2023a). An Iranian study reported that proline accumulation in plant tissues improved resistance to water stress (Keshavarz et al., 2018). In the present experiment, proline levels increased under water stress, but the application of salicylic acid reduced proline content. It is likely that salicylic acid enhanced physiological processes such as photosynthesis, respiration, and nutrient absorption, thereby mitigating the effects of water deficit stress in garden thyme. Consequently, the reduced stress intensity from salicylic acid application appears to logically correspond with decreased proline synthesis. The findings on the effects of water deficit stress and salicylic acid on proline content are consistent with the results of Mohammadi et al. (2019) on *Thymus vulgaris* in Iran.

In this study, catalase (CAT) activity decreased under water deficit stress, while salicylic acid application increased CAT activity under both stress and well-watered conditions. The reduction in CAT activity under water deficit stress is likely due to the inactivation of the enzyme caused by excessive production of reactive oxygen species (ROS), leading to enzyme degradation (Keshavarz, 2020; Hosseini et al., 2023b). It appears that salicylic acid application enhanced the production of this enzyme by upregulating the expression of genes related to CAT synthesis (Li et al., 2019).

In the present study, water deficit stress resulted in increased activities of GPX and SOD enzymes. However, the application of salicylic acid exhibited a contradictory effect; under non-stress conditions, salicylic acid increased SOD activity, while under water deficit stress conditions, it led to a decrease in both GPX and SOD activities. The SOD enzyme plays a critical role as the first line of defense in plants by mitigating the toxic effects of reactive oxygen species (ROS) (Hosseini et al., 2023a). Specifically, SOD catalyzes the dismutation of the superoxide radical into hydrogen peroxide (Keshavarz and Khodabin, 2019). An increase in SOD activity subsequently leads to higher production of H2O2, which is then reduced by peroxidase and catalase (CAT) enzymes, thereby decreasing the overall H_2O_2 content (Keshavarz, 2020).

Salicylic acid has been shown to protect proteins and cellular structures from water stress-induced damage by inducing the expression of antioxidant enzymes (Urmi et al., 2023). GPX enzymes, which possess peroxidase activity, play a crucial biological role in safeguarding organisms against oxidative damage. The primary biochemical function of GPX is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Kulak et al., 2021).

The production of ROS in plants is a typical response to water stress; however, excessive ROS accumulation and its direct interaction with various macromolecules can cause oxidative damage, leading to reduced plant growth and performance (Mohammadi et al., 2019). ROS are unavoidable byproducts of aerobic metabolism, generated during electron transport chain reactions within mitochondria, chloroplasts, and peroxisomes (Li et al., 2019). Under stress conditions, ROS can damage proteins, biological membranes, and DNA, ultimately leading to cell death and lipid peroxidation.

An experiment conducted in Sari, Iran, demonstrated that salicylic acid enhances the antioxidant capacity by acting on H_2O_2, thereby protecting the plant from oxidative stress (Hosseini et al., 2023b). In the current study, water deficit stress was found to elevate the levels of biomarkers such as dityrosine, 8-OH-dG, and MDA. These molecular biomarkers serve as indicators of the severity of cellular damage under environmental stress (Yousefzadeh et al., 2023).

According to Hosseini et al. (2023b), water shortage stress in a greenhouse experiment conducted in Sari, Iran, led to the degradation of cell membranes and increased MDA production in mint (Mentha spp.) leaves. MDA, a peroxidation product of unsaturated fatty acids in phospholipids, is widely used as a marker to assess the extent of membrane damage under water stress conditions (da Graca et al., 2016). Water deficit stress induces oxidative stress in cells, disrupting their physiological processes. The presence of free radicals within the cell causes damage to membrane lipids and fatty acids, leading to the production of lipid and hydroperoxyl radicals. These newly formed radicals can accelerate lipid oxidation reactions (Hosseini et al., 2023b).

Interestingly, the application of salicylic acid in this study led to an increase in MDA levels, contrary to the expected outcome, as salicylic acid is typically known to reduce MDA (Hosseini et al., 2023a). To elucidate the underlying mechanism of this unexpected increase in MDA, future studies should focus on measuring the content of free radicals present in the plant.

Salicylic acid is a phenolic hormone that not only regulates the internal processes of plants but also serves as a plant growth regulator, playing a crucial role in modulating a range of physiological processes. These include photosynthesis, stomatal closure, transpiration, chlorophyll synthesis, protein synthesis, inhibition of ethylene biosynthesis, and the absorption and transport of essential elements (Shemi et al., 2021). The application of growth regulators and inhibitors in medicinal plants may stimulate not only growth but also the production of secondary metabolites (Hosseini et al., 2023a).

In our experiments, the application of jasmonic acid under non-stress conditions resulted in an increase in thymol synthesis. However, under water stress conditions, jasmonic acid did not have a significant impact on thymol synthesis. Furthermore, both water deficit stress and the application of salicylic acid led to an increase in essential oil percentage, a finding that aligns with the results of Kulak et al. (2021) in basil plants studied in India. Environmental stresses, particularly water deficit stress, have been shown to enhance the concentration of certain phytochemical compounds in medicinal plants

(Hosseini et al., 2023b). This observation is consistent with the findings of the present study, where we report an increase in both essential oil percentage and thymol content in garden thyme.

The absence of a significant effect of jasmonic acid on thymol synthesis under water stress conditions could be attributed to jasmonic acid's role in mitigating stress intensity and promoting plant growth, thereby allowing the plant to allocate less energy toward the synthesis of secondary metabolites like thymol. Conversely, in the absence of water stress, jasmonic acid appears to enhance thymol synthesis, possibly by influencing pathways involved in thymol production, such as gene expression and the activity of various enzymes. Salicylic acid similarly increases essential oil percentage by modulating the expression of genes and enzymes that are pivotal in essential oil biosynthesis (Choudhary et al., 2021).

Conclusions

The results of this study demonstrated that the application of salicylic acid at a concentration of 50 mg L^{-1} effectively increased the total dry weight of garden thyme plants. Additionally, salicylic acid at concentrations of 25 and 50 mg L^{-1} enhanced the essential oil percentage under water deficit conditions. Jasmonic acid also positively influenced thymol synthesis, with a significant increase observed at a concentration of 2 mg L^{-1}. Furthermore, jasmonic acid at this concentration promoted the synthesis of total chlorophyll (TChl). In several instances, the application of both salicylic acid and jasmonic acid positively affected the activity of antioxidant enzymes. Molecular biomarkers, including 8-OH-dG and dityrosine, increased in response to water deficit stress, indicating substantial damage to the cellular structure of garden thyme plants due to the stress. Based on these findings, it is recommended to use salicylic acid and jasmonic acid as growth regulators to enhance the synthesis of secondary metabolites and improve physiological research on garden thyme plants when exposed to water deficit stress.

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Author contributions

ZK designed and performed the experiment. MN wrote the manuscript. MO and PK helped in revising the manuscript and improved the language. H-RL coordinated the research project. All authors have read and approved the final manuscript and agreed to the published version of the manuscript.

Conflict of Interest

The authors indicate no conflict of interest in this work.

References

Adams RP. 1995. Identification of Essential Oil Components by Gas Chromatography /Mass Spectroscopy. Allured Publishing Co. IL.

Arnon DI. 1949. Copper enzymes in isolated chloroplasts, polyphennoloxidase in *Beta vulgaris*. Plant Physiology 24, 1–150.

Bates LS, Waldern RP, Teave ID. 1973. Rapid determination of free proline for water stress studies. Plant and Soil 39, 205-207.

Bogdanov MF, Bical MB. 1999. Acarbon column based LCEG approch to routin 8-OH-dG measurments in biological matrices. Free Radical Biology & Medicine 27, 647-666.

Cakmak I, Horst W. 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glycine max*). Plant Physiology 83, 463-468.

Chandrasekaran M. 2022. Arbuscular mycorrhizal fungi mediated enhanced biomass, root morphological traits and nutrient uptake under drought stress: A meta-analysis. Journal of Fungi 8(7), 660.

Chen XY, Wang DD, Fang X, Chen XY, Mao YB. 2019. Plant specialized metabolism regulated by jasmonate signaling. Plant and Cell Physiology 60, 2638–2647. https://doi.org/10.1093/pcp/pcz161.

Choudhary S, Zehra A, Mukarram M, Wani KI, Naeem M, Khan MMA, Aftab T. 2021. Salicylic acid-mediated alleviation of soil boron toxicity in *Mentha arvensis* and *Cymbopogon flexuosus*: Growth, antioxidant responses, essential oil contents and components. Chemosphere 276, 130153.

da Graca JP, Ueda TE, Janegitz T, Vieira SS, Salvador MC, de Oliveira MCN, Zingaretti SM, Powers SJ, Pickett JA, Birkett MA, Hoffmann-Campo CB. 2016. The natural plant stress elicitor cis-jasmone causes cultivar-dependent reduction in growth of the stink bug, *Euschistus heros* and associated changes in flavonoid concentrations in soybean, Glycine max. Phytochemistry 131, 84-91. https://doi.org/10.1016/j.phytochem.2016.08.013

Emami Bistgani Z, Barker AV, Hashemi M. 2024. Physiology of medicinal and aromatic plants under drought stress. The Crop Journal. Available online. https://doi.org/10.1016/j.cj.2023.12.003

Ghanati F, Morita A, Yokota H. 2002. Induction of suberin and increase of lignin content by excess boron in tabacco cell. Soil Science and Plant Nutrient 48(3), 357-364

Gharibiyan P, Roozban MR, Rahemi M, Vahdati K. 2023. Exogenous salicylic acid improves growth and physiological status of two pistacia species under salinity stress. Erwerbs-Obstbau 65, 1441-1452. https://doi.org/10.1007/s10341-023-00841-8

Giannopolitis C, Ries S. 1977. Superoxid desmutase. I. Occurence in higher plant. Plant Physiology 59, 309-314.

Hosseini SJ, Tahmasebi-Sarvestani Z, Mokhtassi-Bidgoli A, Keshavarz H, Kazemi Sh, Khalvandi M, Pirdashti H, Hashemi-Petroudi SH, Nicola S. 2023a. Functional quality, antioxidant capacity and essential oil percentage in different mint species affected by salinity stress. Chemistry and Biodiversity 20(4), e202200247. https://doi.org/10.1002/cbdv.202200247

Hosseini SJ, Tahmasebi-Sarvestani Z, Mokhtassi-Bidgoli A, Keshavarz H, Kazemi Sh, Khalvandi M, Pirdashti H, Bovand F, Abassian A. 2023b. Do various levels of salinity change chlorophyll fluorescence, nutrient uptake, and physiological characteristics of Mentha ecotypes. Industrial Crops and products 203, 117199. https://doi.org/10.1016/j.indcrop.2023.117199

Howe GA, Major IT, Koo AJ. 2018. Modularity in jasmonate signaling for multistress resilience. Annual Review of Plant Biology 69: 387-415. https://doi.org/10.1146/annurev-arplant-042817-040047

Keshavarz H, Hosseini SJ, Sedibe MM, Achilonu MC. 2021. Arbuscular mycorrhizal fungi used to support Iranian barley (*Hordeum vulgare* L.) cultivated on cadmium contaminated soils. Applied Ecology and Environmental Research 20(1), 43-53. http://dx.doi.org/10.15666/aeer/2001_043053

Keshavarz H, Khodabin G. 2019. The role of uniconazole in improving physiological and biochemical attributes of bean (*Phaseolus vulgaris* L.) subjected to drought stress. Journal of Crop Science and Biotechnology 22(2), 161-168. https://doi.org/10.1007/s12892-019-0050-0

Keshavarz H, Modarres-Sanavy SAM, Mahdipour Afra M. 2018. Organic and chemical fertilizer affected yield and essential oil of two mint species. Journal of Essential Oil-Bearing Plants 21(6), 1674-1681. https://doi.org/10.1080/0972060X.2018.1497545

Keshavarz H. 2020. Study of water deficit conditions and beneficial microbes on the oil quality and agronomic traits of canola (*Brassica napus* L.). Grasas Y Aceites 71(3), e373. https://doi.org/10.3989/gya.0572191

Keshavarz Mirzamohammadi H, Modarres-Sanavy SAM, Sefidkon F, Mokhtassi-Bidgoli A, Mirjalili MH. 2021a. Irrigation and fertilizer treatments affecting rosmarinic acid accumulation, total phenolic content, antioxidant potential and correlation between them in peppermint (*Mentha piperita* L.). Irrigation Science 39, 671-683. https://doi.org/10.1007/s00271-021-00729-z

Keshavarz Mirzamohammadi H, Tohidi-Moghadam HR, Hosseini SJ. 2021b. Is there any relationship between agronomic traits, soil properties and essential oil profile of peppermint (*Mentha piperita* L.) treated by fertiliser treatments and irrigation regimes? Annals of Applied Biology 179(3), 331-344. https://doi.org/10.1111/aab.12707

Kulak M, Jorrín-Novo JV, Romero-Rodriguez MC, Yildirim ED, Gul F, Karaman S. 2021. Seed priming with salicylic acid on plant growth and essential oil composition in basil (*Ocimum basilicum* L.) plants grown under water stress conditions. Industrial Crops and Products 161, 113235. https://doi.org/10.1016/j.indcrop.2020.113235

Li N, Han X, Feng D, Yuan D, Huang LJ. 2019. Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: do we understand what they are whispering? International Journal of Molecular Sciences 20(3), 671.

Lotfi N, Vahdati K, Amiri R, Kholdebarin B. 2010b. Drought-induced accumulation of sugars and proline in radicle and plumule of tolerant walnut varieties during germination phase. Acta Hortic 861, 289-296. https://doi.org/10.17660/ActaHortic.2010.861.39

Lotfi N, Vahdati K, Hassani D, Kholdebarin B, Amiri R. 2010a. Peroxidase, guaiacol peroxidase and ascorbate peroxidase activity accumulation in leaves and roots of walnut trees in response to drought stress. Acta Horticulturae 861, 309-316. https://doi.org/10.17660/ActaHortic.2010.861.42

Mohammadi H, Amirikia F, Ghorbanpour M, Fatehi F, Hashempour H. 2019. Salicylic acid induced changes in physiological traits and essential oil constituents in different ecotypes of *Thymus kotschyanus* and *Thymus vulgaris* under well-watered and water stress conditions. Industrial Crops and Products 129, 561-574.

Popova LP, Maslenkova LT, Yordanova, RY, Ivanova AP, Krantev AP, Szalai G, Janda T. 2009. Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. Plant Physiology and Biochemistry 47: 224-231.

Portu J, López R, Santamaría P, Garde-Cerdán T. 2018. Methyl jasmonate treatment to increase grape and wine phenolic content in Tempranillo and Graciano varieties during two growing seasons. Scientia Horticulturae 240, 378-386. https://doi.org/10.1016/j.scienta.2018.06.019

Rahimi A, Mohammadi MM, Siavash Moghaddam S, Heydarzadeh S, Gitari H. 2022. Effects of stress modifier biostimulants on vegetative growth, nutrients, and antioxidants contents of garden thyme (*Thymus vulgaris* L.) under water deficit conditions. Journal of Plant Growth Regulation 41, 2059-2072. https://doi.org/10.1007/s00344-022-10604-6

Shemi R, Wang R, Gheith EMS, Hussain HA, Hussain S, Irfan M, Cholidah L, Zhang K, Zhang S, Wang L. 2021. Effects of salicylic acid, zinc and glycine betaine on morpho-physiological growth and yield of maize under drought stress. Scientific Reports 11(1), 3195.

https://doi.org/10.1038/s41598-021-82264-7.

Steven AK, Joseph MH. 1978. Lipid peroxidase in sample as measured by liquid choromatography separation. Elin Chemistry 32, 217-220.

Urmi TA, Islam MM, Zumur KN, Abedin MA, Haque MM, Siddiqui MH, Murata Y, Hoque MA. 2023. Combined effect of salicylic acid and proline mitigates drought stress in rice (*Oryza sativa* L.) through the modulation of physiological attributes and antioxidant enzymes. Antioxidants. 12(7), 1438.

Wang X, Li Q, Xie J, Huang M, Cai J, Zhou Q, Dai T, Jiang D. 2021. Abscisic acid and jasmonic acid are involved in drought priming-induced tolerance to drought in wheat. The Crop Journal 9(1), 120-132.

Wasternack C, Strnad M. 2016. Jasmonate signaling in plant stress responses and development – active and inactive compounds. New Biotechnology 33(5B), 604-613. https://doi.org/10.1016/j.nbt.2015.11.001

Yousefzadeh S, Keshavarz H, Modares-Sanavy SAM. 2023. Effects of ecological agriculture approaches on dragonhead (*Dracocephalum moldavica* L.) productivity and oil yield. Arabian Journal of Geoscience 16, 373. https://doi.org/10.1007/s12517-023-11461-7