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Effects of Stress Modifier Biostimulants on Vegetative Growth, Nutrients, and Antioxidants Contents of Garden Thyme (*Thymus vulgaris* L.) Under Water Deficit Conditions

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Abstract

The aim of this study is to investigate the effect of stress modulators on vegetative growth, antioxidants, and nutrient content of *Thymus vulgaris* L. under water deficit stress conditions. A factorial experiment was performed in the form of a randomized complete block design with 10 treatments and 3 replications in the 2019–2020 growing season. The factors were stress modulators at 5 levels (ZN: zinc nano-fertilizer, AA: amino acid, SW: seaweed, HA: humic acid and C: control) and irrigation regime at 2 levels [FIrr: full irrigation (100% field capacity) and DIrr: deficit irrigation (50% field capacity)]. The highest plant height, number of branches, and total dry weight of the garden thyme plant were observed in the foliar application of HA and SW under full irrigation conditions. Relative water content, chlorophyll a and b, and uptake of nutrients (N, P, and K) were reduced under water deficit stress, but the foliar application of stress modulators increased relative water content, chlorophyll content, and nutrient uptake of the garden thyme plant significantly compared with control. The water deficit increased proline content, total flavonoid, and phenol content in the garden thyme plant. So, the highest total flavonoid and phenol content was obtained from plants treated with HA, whereas proline content was higher in the control plants. Soluble sugars and essential oil increased significantly under water deficit stress conditions. The foliar application of HA compared to the control plant increased soluble sugars and essential oil in garden thymes. The activities of catalase, superoxide dismutase, and ascorbate peroxidase enzymes were improved in stress modulator treatments such as HA and SW compared to control plants under water deficit stress conditions. The plants of garden thymes showed a good response to stress modulator treatments under water stress conditions, and HA and SW treatments were found to be more effective.

Keywords Antioxidant · Humic acid · Seaweed · Irrigation · Sustainable agriculture

Introduction

Thyme species are herbaceous perennials and small shrubs of the Lamiaceae (Labiatae) family that are commercially grown on a large scale in many countries (Aebisher et al. 2021). Garden thyme (*T. vulgaris* L.) is an aromatic and medicinal herb native to the Western Mediterranean region's northern reaches (Aebisher et al. 2021). It is utilized as a medicinal herb because of its inflorescence and essential oil. Also, its infusions and decoctions are used as carminatives, digestives, antispasmodics, anti-inflammatory, and expectorants (Bistgani et al. 2019). Monoterpenes, sesquiterpenes, phenolic compounds, and flavonoids are found in *T. vulgaris* essential oil and extracts. Among these, thymol and carvacrol are the main components of essential oils (Bistgani et al. 2019).

One of the main effects of climate change is that environmental abiotic stresses such as water shortage stress and enhanced temperature are likely to increase in frequency and severity in many ecosystems as caused (Arpanahi et al. 2020; Nyawade et al. 2019). Water deficit stress is one of the main sources of limiting agricultural crop output in the world's arid and semiarid regions (Gitari

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et al. 2018; Amirnia et al. 2019). Water shortage stress causes a variety of physiological and metabolic responses, including reductions in plant growth, dry matter accumulation, stomatal conductance, gas exchange, chlorophyll content, leaf water potential, growth rate, and photosynthesis (Amirnia et al. 2019; Behdad et al. 2021; Raza et al. 2021; Seleiman et al. 2021). Several studies have reported the increased accumulation of reactive oxygen species (ROS) under drought stress (Babaei et al. 2021; Ngugi et al. 2021). Plants reduce the produced ROS via enzymatic and non-enzymatic antioxidant mechanisms (Babaei et al. 2021). ROS accumulation in cells impairs membrane lipids, proteins, and nucleic acids (Asghari et al. 2020) forcing them to employ enzymatic and non-enzymatic antioxidant mechanisms to cope with ROS-induced oxidative stress (Babaei et al. 2021).

One of the most effective practices in sustainable farming is the application of biological and organic fertilizers (Faridvand et al. 2021). Humic acid due to pseudo-hormonal compounds has favorable impacts on plant growth and development (Noroozisharaf et al. 2018). According to Aalipour et al. (2019), humic acid can significantly impact medicinal plants growth components such as cell membrane permeability, metabolic processes, cell respiration, photosynthetic efficiency, enzyme activation and cell elongation. Another potential advantage for humic acids is water deficit stress decline, as plants respond to humic matter by adjusting osmotic pressure via preserving water uptake and cellular swelling, thereby reducing the effects of water deficit stress (Khorasaninejad et al. 2018; Maitra et al. 2020). Seaweed extract used for various plants is essential because it contains a high amount of organic matter, vitamins, microelements, fatty acids as well as being rich in plant growth regulators such as auxins, gibberellins and cytokinin (Shafie et al. 2021). Seaweed has favorable impacts, in terms of increasing plant growth and development, as well as improving tolerance to environmental stress and enhancing antioxidant traits in plants (Mansori et al. 2016). Amino acids utilized in crops are achieved via enzymatic hydrolysis (Radkowski et al. 2021). Free amino acid content with a small molecular weight is extremely important since they are rapidly absorbed by plants (Shafie et al. 2021). They function as an organic carrier-a chelator, allowing for rapid and highly efficient nutrient delivery to plants (Shafie et al. 2021). Amino acids form very tiny, electrically neutral molecules with nutrients, which improves the uptake and transport of nutrient content inside plants under water deficit stress (Teixeira et al. 2019). Zinc is one of the most essential immobile micronutrients in plants, which has an important role in growth and development, tryptophan synthesis, carbohydrate metabolism, photosynthetic pigments and enzymatic properties (Heydarnejadiyan et al. 2020). Zinc nanoparticles by foliar application can be rapidly absorbed from the cytoplasmic membrane, decreasing zinc shortages in the soil and improving photosynthesis and plant growth and development under water deficit stress (Rossi et al. 2019).

According to our evaluation of the research, there is no information on the effects of stress modifiers such as zinc, amino acid, seaweed and humic acid on *T. vulgaris* under water deficit stress. In this regard, this study aims to evaluate the effects of stress modifiers such as zinc, amino acid, seaweed and humic acid on the growth, essential oil production and physiological responses of *T. vulgaris* under water deficit stress. The findings of this research can be used in the development of management strategies to enhance the widespread cultivation of *T. vulgaris*.

Materials and Methods

Experimental Design

The experiment was carried out at the research farm of the Medicinal Plants and Drugs Research Institute of the Urmia University of West Azerbaijan, Iran (45°10' E, 37°44' N, and 1338 m. above sea level). The experiment was performed in a factorial form based on a randomized complete block design with five stress modulators treatment levels (ZN: zinc nano-fertilizer, AA: amino acid, SW: seaweed, HA: humic acid and C: control) and irrigation regime at two levels [FIrr: full irrigation (100% field capacity) and DIrr: deficit irrigation (50% field capacity)] in three replicates during the 2019–2020 growing season.

Plant Material

The seeds of *T. vulgaris* L. needed for this experiment were supplied by the Pakan Bazr Seed Company. The seeds were initially planted into seedling trays containing a mixture of soil: perlite (2:1, v: v) in January 2019. After about three months, the seedlings were transferred to the experimental site on the farm. During the growing season, Time domain reflectometry was used to measure soil moisture content (θv) at depths of 0–30 cm (Kamali and Mahdian 2009). The information on the temperature and rainfall of the research site is provided in Fig. 1.

The foliar spray treatments included a foliar application of zinc nano-fertilizer (2 g l^{-1}), amino acid (2 ml l^{-1}), seaweed (2 ml l^{-1}) and humic acid (2 g l^{-1}) along with the control. Control treatment plants were also sprayed with water. The commercial seaweed extract of Ascophyllum Nodosum Simplex was prepared by Acadian AgriTech Co, Canada. The commercial product of amino acids Tecamin Max was also supplied by Agri Tecno Co, Spain. Humic acid was prepared from the Kimia Pars Shayankar Company, Tehran, Iran. The zinc nano-fertilizer was purchased

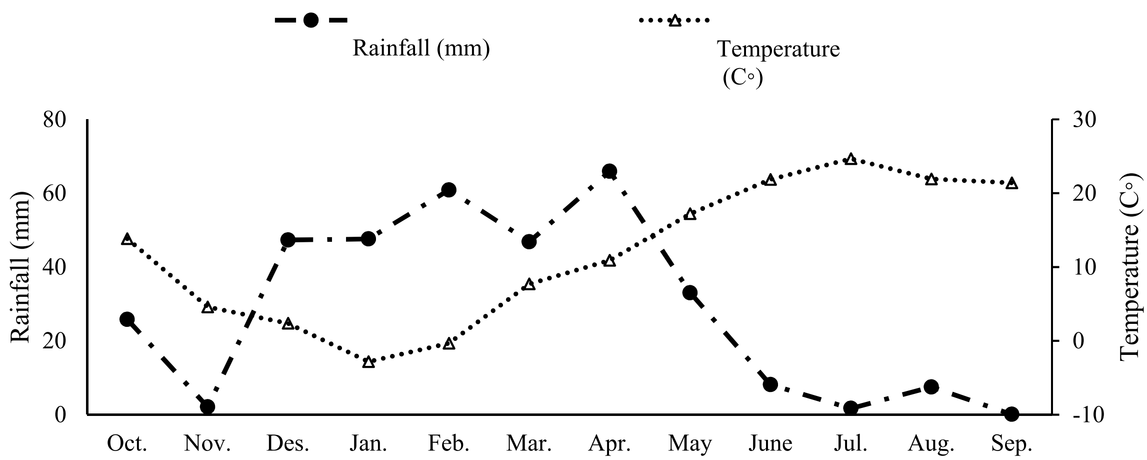

Fig. 1 Total rainfall and average monthly air temperature for the 2019–2020 growing seasons

from Biotechnology Company, Tehran, Iran. The results of soil analysis are presented in Table 1. Each plot was 3 × 4 m in size. The plant spacing between rows was 50 cm, while the interplant spacing was 30 cm. Foliar application was performed in the vegetative stage of garden thyme, simultaneously with water deficit stress in three stages with an interval of 10 days. Weeds were controlled by hand when needed.

Measurements

Growth Parameters

The measured growth parameters were plant height, number of branches per plant, and herbage dry weight per plant at the full flowering stage. The plant samples were oven-dried at 39 °C for 48 h to lose their moisture completely. After they dried, they were weighed on a scale.

Relative Water Content (RWC)

The relative water content of the leaf was determined according to the method described by Xu and Leskovar (2015) (Eq. 1).

Chlorophyll a and b Content (Chl a and b)

The content of chlorophyll a and b were determined using a spectrophotometer at wavelengths of 646.8 and 663.2 nm, respectively (Lichtenthaler and Wellburn 1983).

Total Phenol (TPC) and Flavonoid Content (TFC)

A methanolic extract was first provided from the samples to perform antioxidant experiments. Then, the total flavonoid and phenol content of the extracts was measured by the methods of Marinova et al. (2005) and Sakanaka et al. (2005).

Proline Content

Proline content was determined by the protocol of ninhydrin reagent. The absorption of the samples was measured at 515 nm with a spectrophotometer (Paquin and Lechasseur 1979).

$$\% RWC = \left[(fresh\ weight - dry\ weight) / (turgid\ weight - dry\ weight) \right] \times 100 \tag{1}$$

Table 1 Some properties of soil in the study site

EC(dS m^{-1})	pH	Texture	Clay	Silt	Sand	$CaCO_3$	
1.38	7.79	Clay loam	41%	36%	23%	15.71%	
N %	Organic carbon	Mn mg kg^{-1}	B	Zn	Fe	K	P
0.03	1.16	11.2	0.28	1.1	8.11	282	9.02

Total Soluble Sugars (TSS)

Total soluble sugars of leaves were estimated by the phenol–sulfuric acid method (Irigoyen et al. 1992). The absorbance was determined with a spectrophotometer at 625 nm.

Macronutrients

The nitrogen (N) was determined using the Kjeldahl method (Schuman Stanley and Knudsen 1973), whereas potassium (K) was determined by a flame photometer (Edward 1999). The determination of phosphorus (P) was performed at 660 nm (Kohler et al. 2007).

Enzyme Extractions and Assays

Fresh material (100 mg) was ground in 2 mL of 0.1 M potassium phosphate buffer (pH = 6) containing 5% polyvinylpyrrolidone (PVP). Then, the extracts were centrifuged at 15,000 rpm at 3 °C for 20 min, and the clear supernatant was used to estimate the activity of the enzymes by Tejera et al. (2004).

Catalase Activity (CAT)

Catalase activity was assessed based on the variation in hydrogen peroxide (H_2O_2) concentration at 240 nm. The reaction mixture contained 1.9 mL of 50 mM potassium phosphate buffer (pH = 7), 10 mM H_2O_2, and 0.2 mL of enzyme extract. Enzymatic activity was read based on absorption variations in 60 s per mg of protein (Aebi 1984).

Superoxide Dismutase Activity (SOD)

Superoxide dismutase activity was evaluated using the method outlined by Beyer and Fridovich (1987) to prevent nitroblue tetrazolium (NBT) photochemical decrease at 560 nm. One unit of SOD has been described as the amount of enzyme inhibiting a 50% decrease in NBT.

Ascorbate Peroxidase Activity (APX)

Ascorbate peroxidase activity was measured according to the method of Nakano and Asada (1987). The reaction mixture contained 1 mL 100 mM potassium phosphate buffer (pH 7), 1 mL ascorbic acid 0.5 mM, 0.1 mL H_2O_2 0.1 mM, and 100 μL enzyme extract. The absorption was read at 290 nm.

Essential Oil (EO) and Essential Oil Yield (EOY)

The essential oil was measured by water distillation using the Clevenger apparatus (Adams 2007). Essential oil yield was calculated using Eq. 2:

$$Essential\ oil\ yield = Essential\ oil\ percentage \times herbage\ dry\ weight\ per\ plant \quad (2)$$

Data Analysis

The data generated in this study were statistically analyzed using the SAS 9.1 software. Means were compared by LSD at the $p < 0.05$ level, and graphs were drawn in Excel.

Results

The plant height, the number of branches, total dry weight, total phenols and flavonoids, proline content, the activity of the CAT, SOD, and APX enzymes, and essential oil yield were influenced by the interaction between the irrigation regime and stress modifier biostimulants. RWC, chlorophyll content, total soluble sugar, nutrients of N, P, and K, and essential oil were only influenced by the simple effects of the irrigation regime and stress modifier biostimulants (Table 2).

Growth Traits

The plant height, the number of branches, and total dry weight were significantly increased by the stress modulators application in both irrigation regimes compared to the control plants (Fig. 2). The highest plant height (38.02 cm), the number of branches (28.66), and total dry weight (59.88 g plot^{-1}) were found to be related to full irrigation and the foliar application of HA, which did not differ significantly from SW treatment. The lowest plant height (19.86 cm), the number of branches (12.77), and total dry weight (19.10 g plot^{-1}) were obtained from plants with deficit irrigation and without the application of stress modulator (control) (Fig. 2).

Relative Water Content (RWC)

According to the results, the relative water content in full irrigation was higher than in deficit irrigation conditions (Fig. 3). The RWC of leaves in full irrigation and deficit irrigation conditions was 67.90 and 59.44%, respectively (Fig. 3). The mean comparison for the application of stress modulators revealed that the highest RWC of 73.13% was obtained from plants treated with HA, which did not differ significantly from SW treatment. The lowest one (51.41%) was obtained from treatment without the application of stress modulators (Fig. 3).

Table 2 Variance analysis of garden thyme traits as affected by irrigation regime and stress modifier biostimulants

Source of variation	Df	Plant height	Branches number	Dry weight	RWC	Chl a	Chl b	TPC	TFC	TSS	Proline	N	P	K	CAT	SOD	APX	EO	EOY
Reps	2	0.04	1.27	20.48	16.38	0.00002	0.009	0.1	0.0001	0.003	0.0001	0.005	0.00006	0.002	0.04	8.60	0.003	0.01	0.01
Irrigation (Irr)	1	902.11**	703.63**	4395.68**	537.71**	3.07**	1.72**	24.01**	0.93**	11.83**	10.49**	0.34**	0.01**	0.36**	17.10**	4366.44**	1.99**	2.88**	1.85**
Stress modifier (Str)	4	52.98**	49.46**	267.60**	519.16**	0.35**	0.18**	6.20**	0.04**	33.25**	0.95**	0.33**	0.007**	0.03**	1.04**	391.44**	0.17**	1.63**	0.85**
Irr × Str	4	13.96**	4.60*	26.85*	6.87^{ns}	0.00006^{ns}	0.002^{ns}	0.47**	0.01**	0.01 ns	0.22**	0.004^{ns}	0.000003^{ns}	0.001^{ns}	0.15**	26.21*	0.01*	0.002^{ns}	0.05**
Error	18	1.51	1.30	8.85	6.02	0.003	0.004	0.03	0.0003	0.08	0.006	0.003	0.0001	0.001	0.01	8.81	0.003	0.004	0.009
CV (%)		4.38	5.61	7.80	3.85	2.72	4.50	3.48	3.65	2.72	3.18	2.76	3.77	2.16	7.12	6.37	5.83	2.28	8.71

RWC: relative water content; *Chl a* and *b*: chlorophyll a and b content; *TPC*: total phenol content; *TFC*: total flavonoid content; *TSS*: total soluble sugars; *N*: nitrogen; *P*: phosphorus; *K*: potassium; *CAT*: catalase activity; *SOD*, superoxide dismutase activity; *APX, ascorbate peroxidase activity*; *EO, essential oil*; *EOY*, essential oil yield; *ns*, non-significant

*, **, and ns, significant at 5% and 1% levels of probability and non-significant, respectively

Chlorophyll Content

According to means comparison, the content of chlorophyll a and b in full irrigation conditions was greater than in deficit irrigation conditions (Fig. 4). The mean comparison indicated that the highest chlorophyll a and b were 2.47 and 1.68 mg g^{-1} FW, respectively (Fig. 4), which were observed by plants treated with HA, which did not differ significantly from SW treatment (Fig. 4).

Total Phenols and Flavonoids

The highest total phenols and flavonoids were 7.93 and 0.85 mg g^{-1} DW, respectively (Fig. 5), which were observed in plants treated with deficit irrigation and HA treatment. The lowest ones (3.42 and 0.29 mg g^{-1} DW) were obtained from plants with full irrigation and without the application of stress modulators (control) (Fig. 5). However, total phenols and flavonoids in deficit irrigation plants were significantly higher than in plants with full irrigation (Fig. 5).

Total Soluble Sugar and Proline Content

According to means comparison, the concentrations of total soluble sugar in deficit irrigation conditions were greater than in full irrigation conditions (Fig. 6). The mean comparison indicated that the highest concentrations of total soluble sugar (14.16 µmol g^{-1} FW) were in control. The lowest was 8.48 µmol g^{-1} FW obtained in plants sprayed with HA, which did not differ significantly from SW treatment (Fig. 6). The application of stress modulators resulted in a decrease in proline content in both irrigation regimes (Fig. 6). Accordingly, the highest proline content (3.80 µg g^{-1} FW) was obtained from control (deficit irrigation conditions without the application of stress modulators), which differed significantly from the other treatments. The lowest proline content (1.55 µg g^{-1} FW) was observed in plants treated with full irrigation and sprayed with HA (Fig. 6).

Nutrients of N, P and K

Based on the comparison of means, the highest percentage of nitrogen, phosphorus, and potassium were 2.29, 0.30, and 1.73% in full irrigation conditions, respectively, while the lowest ones (2.08, 0.25, and 1.52%) were obtained in deficit irrigation plants, respectively (Fig. 7). The percentage of N, P, and K were significantly higher in the full irrigation treatment than in deficit irrigation conditions (Fig. 7). The mean comparison for the effect of stress modulators indicated that the application of stress modulators increased the percentage of N, P, and K significantly versus control (Fig. 7). The highest percentage of N (2.44%), P (0.32%), and K (1.71%) were observed by plants treated with HA, which did not

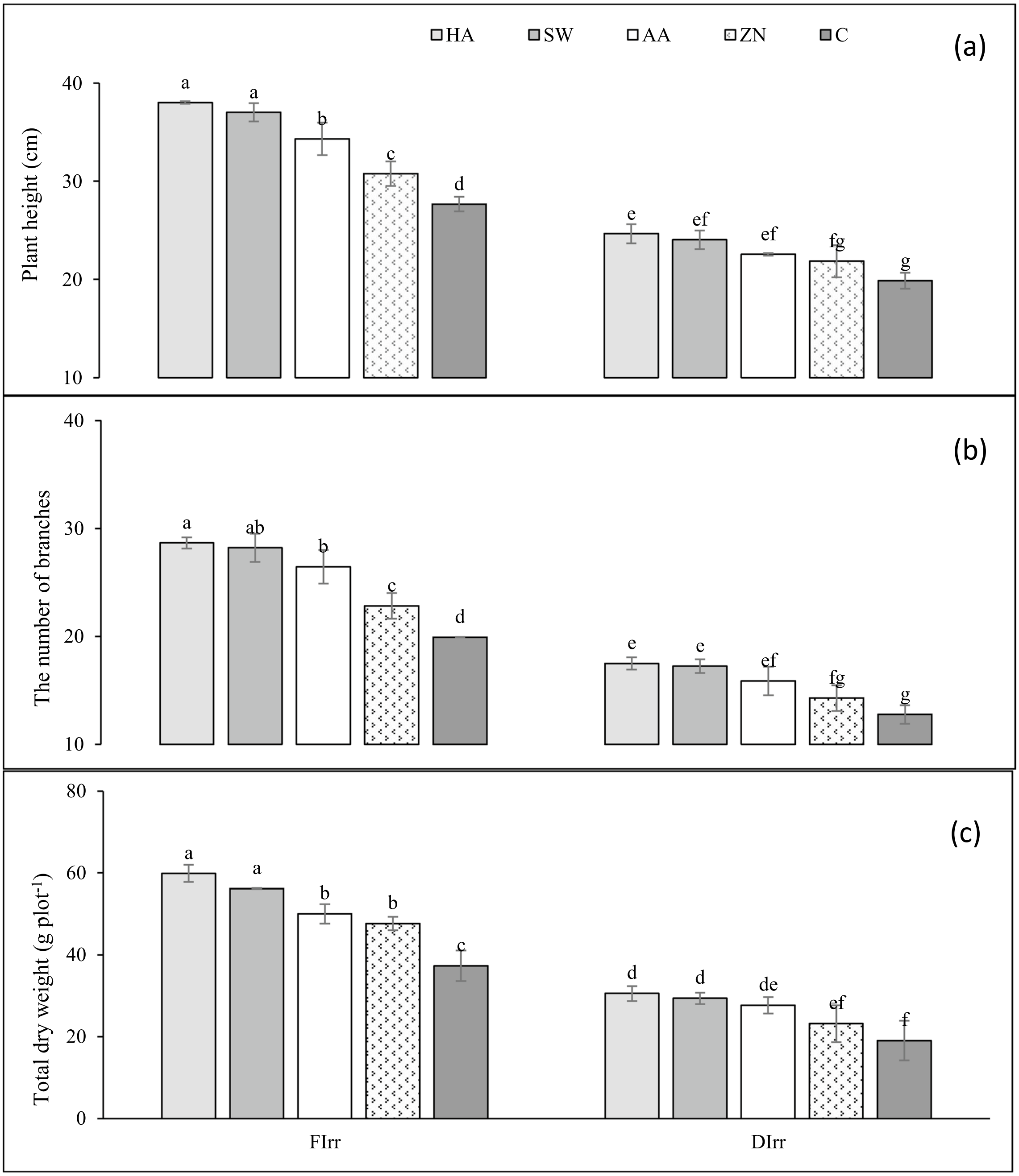

Fig. 2 Means comparison of the interactive effect of irrigation regime and stress modulators application on plant height (**a**), the number of branches (**b**), and total dry weight (**c**). The same letters show a non-significant difference at $P \leq 0.05$ by LSD test. ZN, zinc nano-fertilizer; AA, amino acid; SW, seaweed; HA, humic acid; C, control; FIrr, full irrigation; DIrr, deficit irrigation

differ significantly from SW treatment (Fig. 7). However, the lowest percentage of N (1.89%), P (0.23%), and K (1.51%) was obtained from control plants (without the application of fertilizer) (Fig. 7).

Antioxidant Enzymes Activity

The highest CAT, SOD, and APX were 3.31, 80.39, and 1.53 μmol g^{-1}, respectively (Fig. 8), which were observed in plants under deficit irrigation conditions and HA treatment. Also, there was no significant effect on CAT, and APX activity in deficit irrigation conditions for HA treatment compared to the SW. The lowest ones (0.79 and

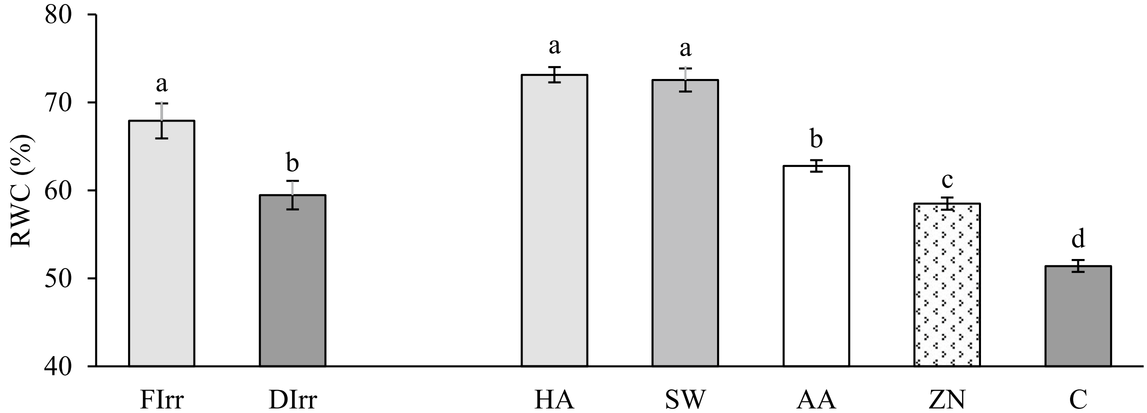

Fig. 3 Means comparison of the simple effect of irrigation regimes and stress modulators application on RWC. The same letters show a non-significant difference at P ≤ 0.05 by LSD test. ZN, zinc nano-fertilizer; AA, amino acid; SW, seaweed; HA, humic acid; C, control; FIrr, full irrigation; DIrr, deficit irrigation

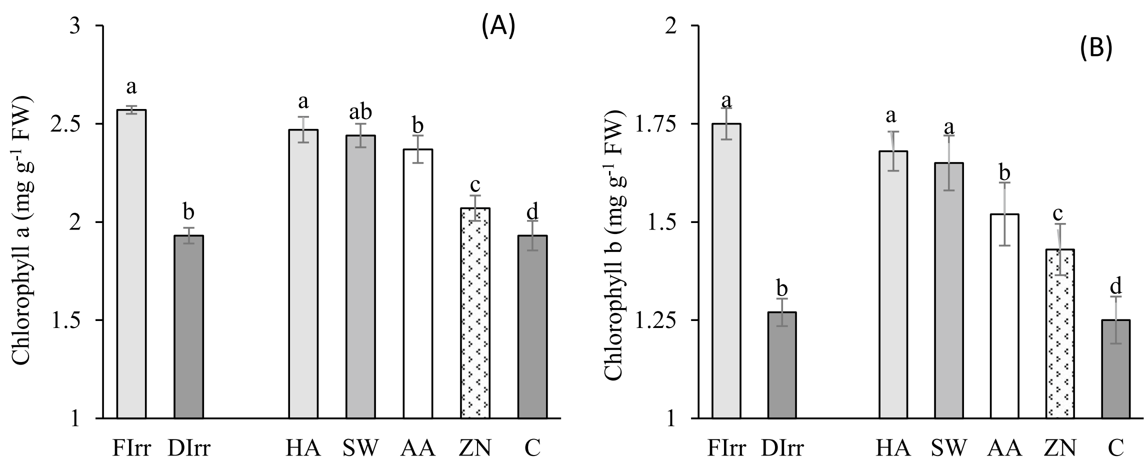

Fig. 4 Means comparison of the simple effect of irrigation regime and stress modulators application on chlorophyll a (**A**) and b (**B**) content. The same letters show a non-significant difference at P ≤ 0.05 by LSD test. ZN, zinc nano-fertilizer; AA, amino acid; SW, seaweed; HA, humic acid; C, control; FIrr, full irrigation; DIrr, deficit irrigation

33.14, and 0.58 μmol g^{-1}) were obtained from plants with full irrigation and without the application of stress modulators (control) (Fig. 8). Also, there was no significant effect on CAT, and APX activity in full irrigation conditions for HA treatment compared to the SW and AA. However, CAT, SOD, and APX activity in deficit irrigation plants were significantly higher than in plants in full irrigation conditions (Fig. 8).

Essential Oil and Essential Oil Yield

According to the results, the essential oil in deficit irrigation plants was higher than in full irrigation conditions (Fig. 9). The essential oil in deficit and full irrigation conditions was 2.65 and 3.27%, respectively (Fig. 9). Mean comparison for stress modulators revealed that the highest essential oil of 3.50% was obtained from plants treated with HA. The lowest one (2.25%) was obtained from the control treatment (Fig. 9). The highest essential oil yield of 1.90 g $plot^{-1}$ was obtained from plants that received full

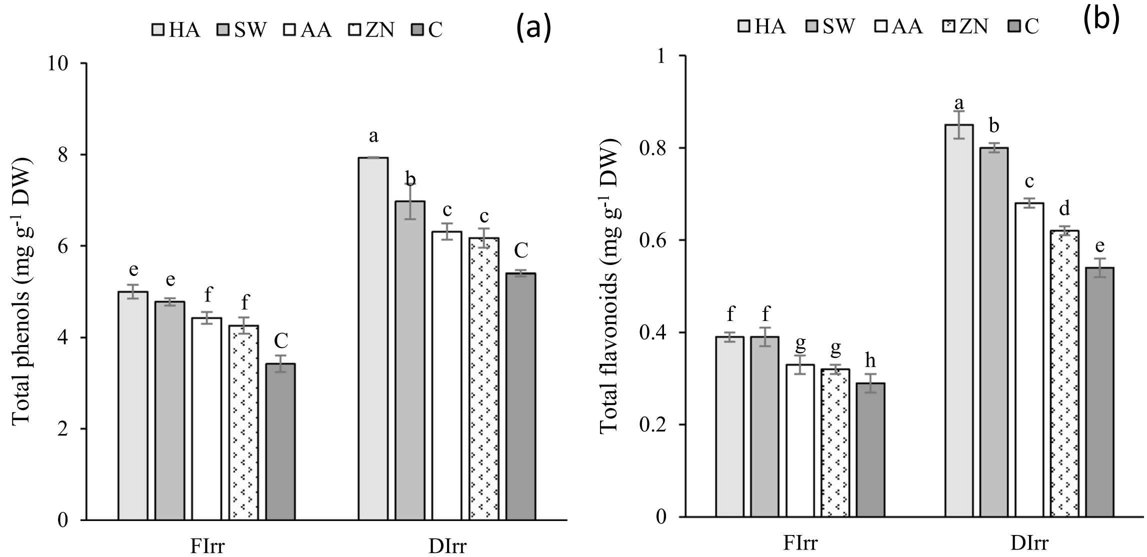

Fig. 5 Means comparison of the interactive effect of irrigation regime and stress modulators application on total phenols (**a**) and flavonoids (**b**). The same letters show a non-significant difference at P ≤ 0.05 by LSD test. ZN, zinc nano-fertilizer; AA, amino acid; SW, seaweed; HA, humic acid; C, control; FIrr, full irrigation; DIrr, deficit irrigation

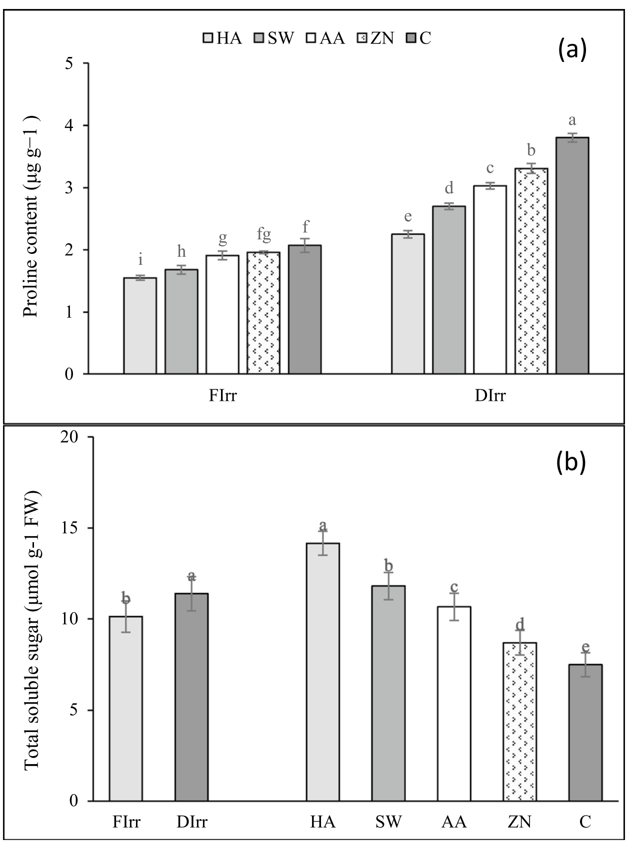

Fig. 6 Means comparison of the effect of irrigation regime and stress modulators application on proline content (**a**) and total soluble sugar (**b**). The same letters show a non-significant difference at P ≤ 0.05 by LSD test. ZN, zinc nano-fertilizer; AA, amino acid; SW, seaweed; HA, humic acid; C, control; FIrr, full irrigation; DIrr, deficit irrigation

irrigation and were treated with HA, whereas the lowest one (0.48 g plot^{-1}) was obtained from plants that received deficit irrigation and without the application of stress modulators (Fig. 9).

Discussion

Growth Traits

In the current research, the foliar application of HA, SW, AA, and ZN improved the growth traits of garden thyme under water deficit stress (Fig. 2). HA and SW were reported to improve crop growth and production in water shortage conditions by enhancing nutrient availability and uptake (Yildiztekin et al. 2018). Moreira et al. (2015) reported that foliar application of AA and ZN increased the efficiency of photosynthesis and improved the growth of alfalfa in tropical areas. The stimulatory effect of stress modulators such as SW and AA on plant growth, development, and yield is attributed to an increase in endogenous hormone content and function (Shafie et al. 2021).

Relative Water Content (RWC)

Relative water content is one of the most important characteristics of the plant's water balance. Relative water content plays an important role in regulating stomatal conductance and hence the photosynthetic rate of the plant (Amirnia et al. 2019). Reducing growth and root activity and increasing evapotranspiration from the plant community are known

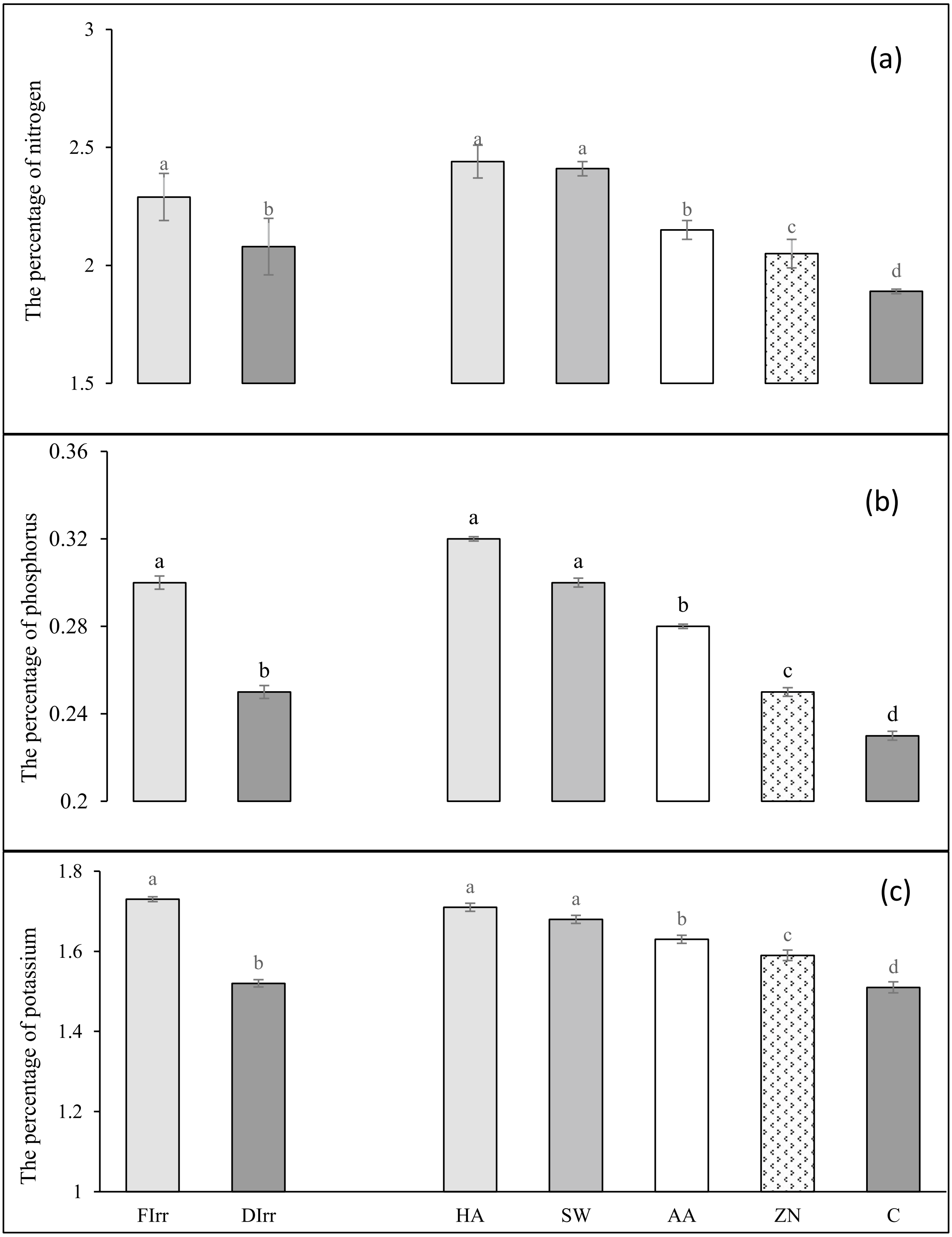

Fig. 7 Means comparison for the simple effect of irrigation regime and stress modulators application on the percentage of nitrogen (**a**), phosphorus (**b**), and potassium (a). The same letters show non-significant difference at P ≤ 0.05 by LSD test. ZN, zinc nano-fertilizer; AA, amino acid; SW, seaweed; HA, humic acid; C, control; FIrr, full irrigation; DIrr, deficit irrigation

to be factors contributing to RWC reduction (Khorasaninejad et al. 2018; Raza et al. 2021). Because HA, SW, and AA are effective in root development and therefore the ability to absorb water and nutrients, they can be expected to have a positive effect (Khorasaninejad et al. 2018; Shafie et al. 2021). Therefore, it seems that increasing the relative

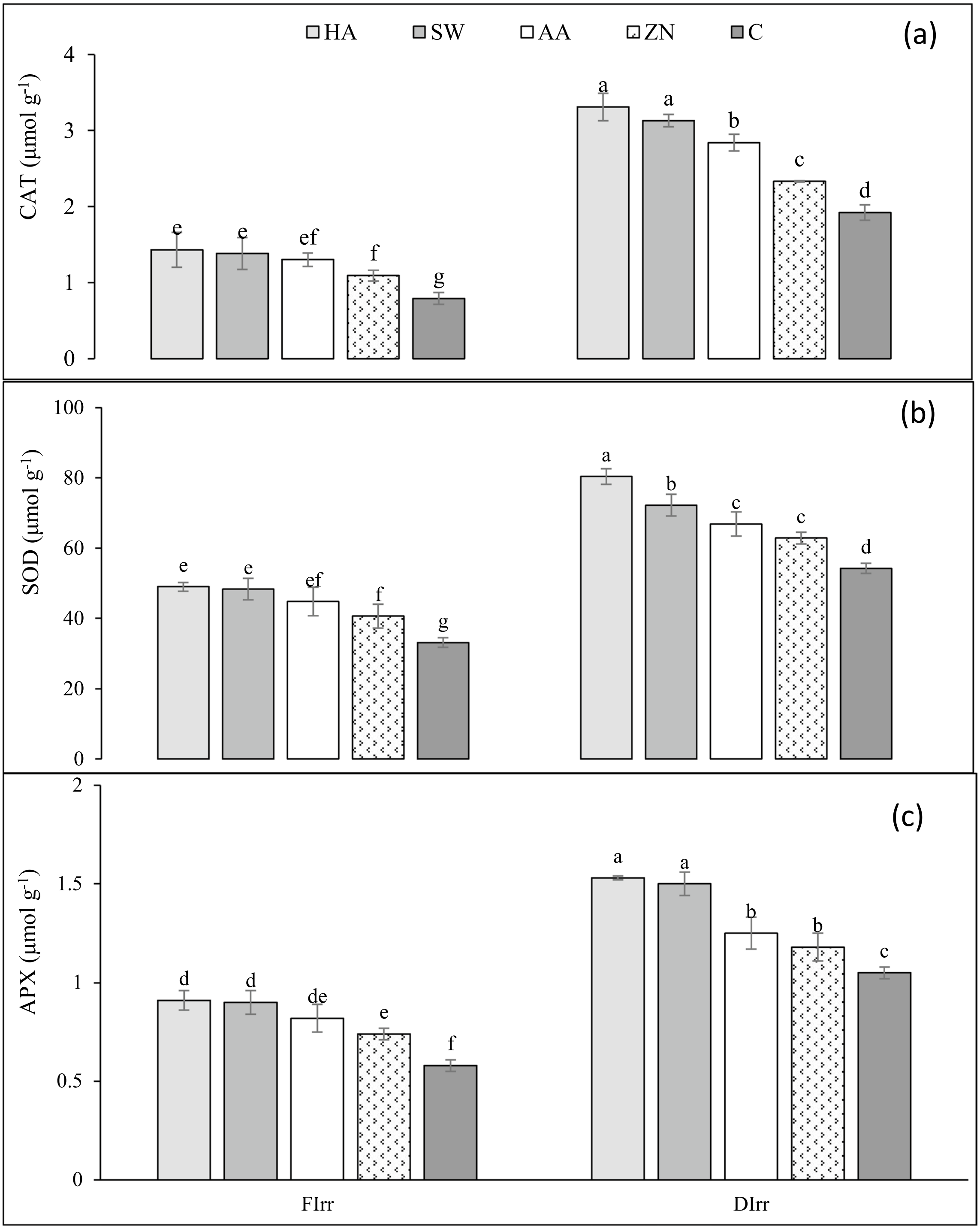

Fig. 8 Means comparison of the interactive effect of irrigation regime and stress modulators application on *CAT,* catalase activity (**a**); *SOD,* superoxide dismutase activity (**b**); and *APX,* ascorbate peroxidase activity (c). The same letters show non-significant difference at $P \leq 0.05$ by LSD test. ZN, zinc nano-fertilizer; AA, amino acid; SW, seaweed; HA, humic acid; C, control; FIrr, full irrigation; DIrr, deficit irrigation

water content of the leaf under drought stress is due to the improvement in the assimilation and photosynthesis and growth rate of plants as a result of the foliar application of SW, HA, AA, and ZN.

Chlorophyll Content

In this research, foliar application of SW, HA, AA, and ZN increased the garden thyme leaf chlorophyll content (Fig. 4).

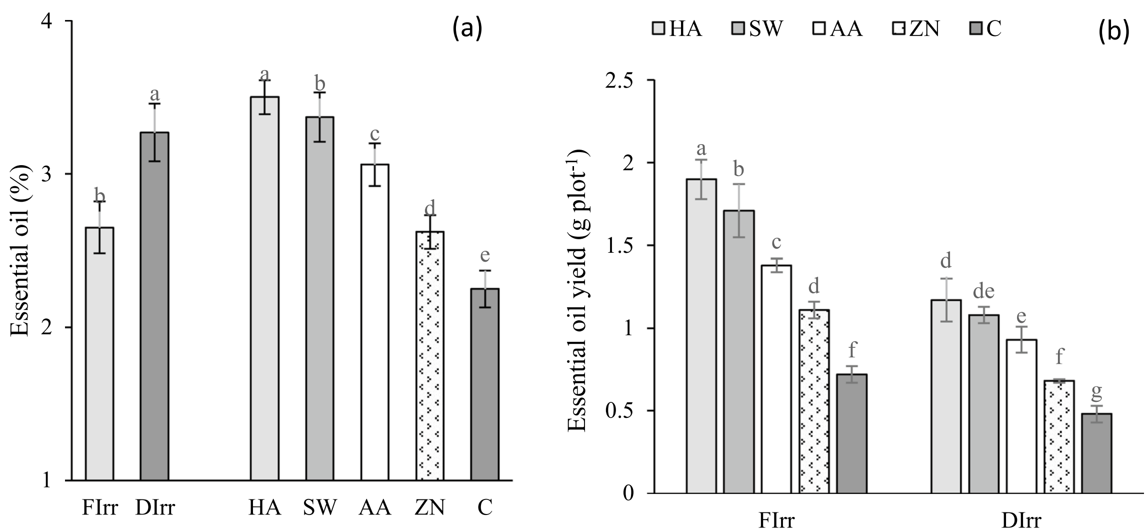

Fig. 9 Means comparison for the effect of irrigation regime and stress modulators application on essential oil (**a**) and essential oil yield (**b**). The same letters show non-significant difference at P ≤ 0.05 by LSD test. ZN, zinc nano-fertilizer; AA, amino acid; SW, seaweed; HA, humic acid; C, control; FIrr, full irrigation; DIrr, deficit irrigation

Because photosynthesizing pigments (chlorophyll) are always found attached to proteins (Amirnia et al. 2019), the application of stress modulators such as HA, SW, and AA provides the N requirements of photosynthesizing pigments and plant proteins, resulting in an increase in the amount of these pigments in the plants (Aalipour et al. 2019; Shafie et al. 2021). It is reported that the foliar application of SW and HA can enhance photosynthesis and increase tolerance to living and non-living stresses (Yildiztekin et al. 2018). Humic acid also increases photosynthetic activity in drought conditions by increasing the activity of the enzyme rubisco (Delfine et al. 2005). They found that chlorophyll is reduced significantly under severe drought stress conditions due to the low rate of synthesis or rapid decomposition of chlorophyll. The reason for chlorophyll loss in plants exposed to water deficit stress is the increased degradation of these pigments or the loss of their synthesis, as well as the disruption of enzyme activity responsible for the synthesis of photosynthesizing pigments, which reduces assimilation and thus entails yield losses (Amirnia et al. 2019; Nasar et al. 2021).

Total Phenols and Flavonoids

Based on the present results, the foliar application of SW, HA, AA, and ZN increased the total phenols and total flavonoids of the leaf of garden thyme in both irrigation regimes (Fig. 5). It is reported that the application of SW, HA, and AA enhanced the synthesis of phenolic compounds in plants under water shortage conditions (Akladious and Mohamed 2018). In this regard, Aminifard et al. (2012) reported that the foliar application of HA treatment increased the phenolic compounds of pepper plants compared to control. These increases may be attributed to the active role of HA in the improvement of antioxidants, which scavenge ROS that is associated with oxidative stress. Plant phenols and flavonoids are expected to play an important role in plants as defence compounds against the higher damage of free radicals, and other reactive oxygen species compounds as secondary natural metabolites (Rahimi et al. 2019). By increasing irrigation intervals, the phenol and flavonoids of thyme plants continued to accumulate in the highest amounts, and the application of stress modulators such as HA, SW, AA, and ZN diminished their accumulation.

Total Soluble Sugar and Proline Content

It is reported that soluble sugar has a role as an osmoprotectant, regulating osmotic adjustment, providing membrane protection, and scavenging toxic reactive oxygen species in water deficit stress conditions (Singh et al. 2015). Sakr et al. (2019) reported that increased photosynthesis and growth rate of red radish plants in field conditions with HA and SW treatments increased carbohydrate content rate. Proline performs as a cytosol enzyme protector (protection of carboxylase) and cell structure defender, so it is increased in cells under water shortage conditions (Amirnia et al. 2019). Among organic osmolytes, proline is the most prevalent and abundant suitable soluble substance that accumulates in water deficit stress conditions (Khorasaninejad et al. 2018). Sprayed plants with stress-modulating treatments such as HA and SW can usually utilize water and nutrients better to outperform non-sprayed plants under water shortage conditions (Yildiztekin et al. 2018). So, the proline content of those plants shows a lower increase as compared with non-sprayed plants.

Nutrients of N, P and K

In this research, foliar application of HA, SW, AA, and ZN increased the content of N, P, and K in the garden thyme leaf (Fig. 7). Enhanced nutrient absorption by SW and AA might be related to enhanced cell membrane permeability and hormone-like functions (Shafie et al. 2021). It has been observed that nutrient uptake and their accumulation in the thyme plant significantly decline in water shortage stress conditions (Mohammadzadeh and Pirzad 2021). Several explanations have been developed to describe this scientific finding. The decline in soil available water limits its uptake. In addition, as soil moisture is decreased, nutrient solubility is decreased (Amirnia et al. 2019; Mohammadzadeh and Pirzad 2021). A decline in water absorption leads to a reduction in photosynthesis and transpiration from a physiological point of view (Nasar et al. 2021). To save energy, the active translocation systems of the plants are also disrupted under these conditions. All these conditions together reduce the absorbing area of roots and nutrient uptake (Amirnia et al. 2019).

Antioxidant Enzymes Activity

Antioxidant enzyme activity augmentation is one of the defence strategies of the plant against many abiotic and biotic stresses (Ahmadian et al. 2021). When plants are subjected to drought stress, the concentration of ROS increases, which can harm proteins, lipids, carbohydrates, and nucleic acids. Plants for the purification and detoxification of ROS in cells have an enzymatic defence system that increases tolerance to drought stress (Ahmadian et al. 2021).

The SOD activity was enhanced by water shortage stress and showed an increase in plants treated with HA, SW, AA, and ZN compared to control plants (Fig. 8). By increasing the SOD enzyme activity, superoxide radicals are scavenged, and thus membrane damage and oxidative stress are reduced, increasing plants' oxidative stress tolerance (Ahmadian et al. 2021). Mansori et al. (2016) had similar findings and stated that an increase in SOD activity and a reduction in oxidative damage stress were directly correlated. Other key antioxidant enzymes that convert H_2O_2 to water, such as CAT and APX, are required for ROS detoxification under stressful conditions (Mansori et al. 2016).

Based on our findings, CAT and APX activities were increased in water deficit stress. Mansori et al. (2015) noticed increased CAT and APX activity in bean plants exposed to water stress and may have resulted in higher H_2O_2 scavenging. This finding supported the important effect of HA, SW, AA, and ZN on plant water deficit tolerance. The partnership of antioxidant enzymes is substantial for ROS scavenging in plant cells (Ahmadian et al. 2021). Many researchers have found that HA and SW increase APX activity, demonstrating HA and SW's powerful antioxidant effects, which have been related to bioactive compounds (Yildiztekin et al. 2018). It is reported that HA and SW improve water deficit stress tolerance by activating the antioxidative enzyme mechanism of superoxide dismutase, catalase, and ascorbate peroxidase activity, as well as by increasing the total phenolic compounds, which contribute to the defence of plants against oxidative damage to plant cells mediated by ROS (Latique et al. 2017; Khorasaninejad et al. 2018; Shafie et al. 2021). Our findings show that increased antioxidative enzyme activities due to SW, HA, AA, and ZN treatments may have ameliorated the physiological situation of garden thyme plants comprehensively.

Essential Oil and Essential Oil Yield

In response to water shortage stress, more metabolites and materials are produced in plants, and these substances prevent oxidation in the cells (Alavi-Samani et al. 2015). The beneficial roles of stress modulators such as HA, SW, AA, and ZN in the improvement of quantitative and qualitative yields of essential oils have been reported by previous studies (Najafivafa et al. 2015; Noroozisharaf and Kaviani 2018; Sakr et al. 2019). Essential oils are terpenoids that require acetyl-CoA, ATP and NADPH for synthesis (Pirzad and Mohammadzadeh 2018). Therefore, essential oil biosynthesis is reliant on the plant's inorganic nutrient supply (Mohammadzadeh and Pirzad 2021). In the current study, the application of stress modulators such as SW, HA, and AA might be due to an improvement in plant water relations and nutrient uptake compared to control plants, which in turn enhanced essential oil content. Under water shortage, essential oil yield decreases and/or increases caused by the interaction between the increased essential oil percentage and the reduced yield (Pirzad and Mohammadzadeh 2018). The application of stress modulators such as SW, HA, and AA indicated an eco-friendly strategy to enhance the sustainability of the production of bioactive molecules in the investigated thyme plant.

Conclusion

According to the results, plants of garden thyme declined under water deficit stress after treatment by stress modulators of HA and SW by reducing the impact of water deficit stress with an improvement in the vegetative growth traits, RWC, chlorophyll content, uptake of nutrients, and enzymatic antioxidant traits. The foliar application of HA and SW increased the plant's height, the number of branches, and the total dry weight of the plant in water shortage conditions. Also, we noticed the improvement of antioxidant system activity by the activation of enzymes (CAT, SOD, and APX) and increasing total phenols and flavonoids, which indicate the protection of plants against lipid peroxidation imposed by water deficit stress. Essential oil content was increased in water deficit stressed plants compared to full irrigated plants. The highest essential oil content was significantly observed in the treatment of HA. Overall, the results revealed that foliar applications of stress modulators could potentially mitigate the harmful effects of water deficits on garden thyme growth under such conditions. Therefore, our findings potentially suggest that the foliar application of HA and SW can alleviate the adverse effects of water stress on garden thyme, which could be an appropriate method for reducing the negative effects of water shortages, especially in arid and semiarid regions where water deficits are the main obstacle to plant growth.

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Declarations

Conflict of interest The co-authors have no competing interests to declare with respect to the current study.

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