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Evaluation of Kadostim and Humiforte Effects on Some of the Physiological and Biochemical Characteristics of Summer Savory (*Satureja hortensis*) under Drought Stress

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ABSTRACT

The use of plant growth stimulants can contribute to improving plant yield and resistance to abiotic stresses such as drought stress. In order to evaluate the effect of two biostimulants on some physiological and biochemical characteristics of summer savory under drought stress, this experiment was conducted in the Research, Education and Natural Resources Center of Kermanshah Province as a factorial experiment based on the completely randomized design with 3 replications in 2024. Three factors, including irrigation levels (100, 75 and 50% Evp), Kadostim (0 and 1 in 1000) and Humiforte (0 and 1 in 1000) were used. The results indicated that the drought stress decreased the total chlorophyll and relative water content. In contrast, the drought stress led to significant enhancement of essential oil, total phenol, proline, total soluble sugar, malondialdehyde and electrolyte leakage. Biostimulants treatment caused improvement in all evaluated characteristics. Therefore, the highest total chlorophyll (2.88 mg. g^{-1} FW) was obtained in 100% Evp with Kadostim and Humiforte. Also, the highest relative water content (86.83%) was in 100% Evp with Kadostim. Although the highest essential oil was obtained in 50% Evp without Kadostim and Humiforte, the highest total phenol, proline and total soluble sugar (12.22 nmol. g^{-1} FW, 29.67 mg. g^{-1} FW and 3.92 mg. g^{-1} FW, respectively) were observed in 50% Evp with Humiforte. Also, Kadostim in 50% Evp irrigation level showed the highest proline (29.25 mg. g^{-1} FW), total soluble sugar (3.87 mg. g^{-1} FW) and malondialdehyde (2.9 nmol. g^{-1} FW). The use of Kadostim and Humiforte could reduce electrolyte leakage by 42.22% and 42.44%, respectively. In conclusion, the application of biostimulants, mainly Kadostim, contributes to improving some biochemical and physiological characteristics of summer savory exposed to drought stress.

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1. Introduction

Summer savory (*Satureja hortensis*) belongs to the Lamiaceae family, which is an aromatic annual plant (Ejaz *et al*., 2023). The increased interest in savory is ascribed to its chemical compositions and profound biological activity (Popvici *et al*., 2019). The primary identified compounds in this plant are volatile, phenolic acids, flavonoids and other compounds (Tepe and Cilkiz, 2016). It has been proven that its essential oil is an antimicrobial agent (Markovic *et al*., 2011). The leaf extract of it used for the treatment of toothache and bronchitis (Adiguzel *et al*., 2007). Abiotic stresses have influences on chemical compositions of medicinal plants. Drought stress is one of the main effective environmental stresses that affect the growth, yield, essential oil and metabolic activities in medicinal plants (Ghasemi Pirbalouti *et al*., 2014). Proline accumulation is related to enhancement of stress tolerance and in the study of Alizadeh et al. (2020), the proline level was increased in *S. hortensis* L. in response to drought stress (Bistgani *et al*., 2017). In stressful conditions, phenolic compounds are increased that are responsible for scavenging the reactive oxygen species (ROS) (Selmar and Kleinwächter, 2013). Davazdahemami et

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al. (2014) showed that by increasing drought stress, the essential oil yield of *Satureja* decreased.

In recent years, the use of plant growth stimulants has increased to improve plant yield and resistance to abiotic stresses (Du Jardin, 2015). One of these plant growth stimulants is amino acids (Nardi *et al*., 2016). The amino acids play various roles in plants, including osmotic adjustment, ion transmission, gene expression by protein synthesis, optimizing the oxidation and reduction process and opening and closing of stomata. Due to these roles, amino acids inhibit growth and stop in drought conditions (Patterson *et al*., 2009). Kadostim, as a plant growth stimulant, contains several amino acids that provide nitrogen and potassium for plants (Abou Dahab and Abd El-Aziz, 2006). Humiforte has 6% total nitrogen, 2% organic matter and several amino acids (Azarpira *et al*., 2020). Humiforte as a biological substance, triggers metabolism and metabolic processes to improve efficiency in plants. Also, it has the acid amine base formula and can stimulate improved qualitative and quantitative yield of plants (Du Jardin, 2015). The improvement of growth parameters due to Humiforte use is related to nitrogen supply (Seyedi *et al*., 2024).

It was reported that the application of Kadostim and humiforte improved essential oil yield and total flavonoid of German chamomile (*Matricaria recutita* L.) (Golzadeh *et al*., 2012). Sani (2011) evaluated the impact of amino acids and irrigation regimes on flixweed (*Descurainia Sophia* L.) and indicated that Kadostim and humiforte had significant effects on morphological and physiological characteristics of flixweed and the highest essential oil was produced in Kadostim treatment in seed filling stage. The effect of biostimulants, including Kadostim and Humiforte, on seed yield as well as yield components of psyllium (*Plantago psyllium* L.) was evaluated and the results showed that the highest thousand seed weight was achieved by Kadostim treatment. Biostimulants usage can adequately alleviate the application of chemical manures (Shekari *et al*., 2014). Also, the positive effect of biostimulants on proline adjustment during drought stress in chicory (*Cichorium intibus*) was been reported (Ramroodi *et al*., 2017).

According to the above findings, the application of amino acids can be used as an appropriate material to face drought. There are limited reports available concerning the influence of biostimulants like Kadostim and Humiforte on improving drought tolerance in medicinal plants especially *S. hortensis*. To the best of our knowledge, there is no study on the interaction effects of biostimulants × irrigation regimes on essential oil and the biochemical composition of *S. hortensis*. So, the purpose of this study is to evaluate these biostimulants' ability to mitigate the detrimental effects of water stress in summer savory.

2. Materials and methods

2.1. Experimental site and treatments

A factorial experiment based on the completely randomized design with 3 replications was carried out in the Research, Education and Natural Resources Center of Kermanshah Province (47˚, 4΄ E and 34˚, 19΄ N and 1200m above sea level) in 2024. Three factors, including irrigation level [100 (control), 75 and 50% Evp (evapotranspiration)], Kadostim [0 (control) and 1 in 1000] and Humiforte [0 (control) and 1 in 1000], were used (treatments) and the integration of them. At first, polyethylene pots (10 Lit) were filled from a soil mixture (farm soil: washed sand: Hummus with equal ratios). The physical and chemical characteristics of the experimental soil of the project are presented in Table 1.

Table 1. Physical and chemical characteristics of the experimental soil (Kermanshah Laboratory of Soil Science, Agricultural Research and Education Center)

EC (µS.cm)	TSS* (mg. L^{-1})	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{+2} / Mg^{+2}	Na^+	pH
		(meq.L^{-1})						
870	557	0	6.8	21	0.08	8.1	0.98	7.5

*TSS: Total soluble solids

The summer savory seeds were provided by Esfahan Pakan Bazr Company and planted in March 2024. After the primary establishment of plants, the pots were moved outdoors. Irrigation levels [100 (control), 75 and 50% Evp (evapotranspiration)] were calculated according to the Lysimeteric method and applied. Evapotranspiration is defined as the amount of water used by control seedlings that are always irrigated at the normal level. The calculation of irrigation amount according to weight method during each period was inevitable. In this sense, the complete irrigated seedlings were continuously weighed and then their evapotranspiration was calculated based on the method of Doorenbos and Pruitt (1997). The physical and chemical characteristics of irrigation water are presented in Table 2. Then, in two steps (6-leaf and 2 weeks later), the plants were treated with the biostimulants foliar application. These biostimulants were purchased from a distribution agency of agricultural institutions, Dam Kesht Company, Sarpole-Zahab City. At the end of plant growth, after harvesting, some of the physiological and biochemical characteristics of summer savory leaves were measured.

Table 2. Characteristics of water for Irrigation (Kermanshah Laboratory of Soil Science, Agricultural Research and Education Center)

Silt (%)	Sand (%)	Absorbable K^+ (ppm)	Absorbable P^+ (ppm)	Organic carbon (%)	pH
31.7	48	2510	5.9	3.45	7.12

2.2. Chlorophyll (Chl) and essential oil

To measure Chl content in savory leaves, initially, Chl was been extracted from 0.1 g fresh leaf tissue by homogenizing it with 5 mL acetone 80% acetone in a mortar. After centrifugation of earned supernatant (3000 rpm for 10 min), the light absorbance of the supernatant was read with a spectrophotometer (Varian Cary 100 UV, USA), at 663 nm (the maximum absorbance for Chl a) and 645 nm (the maximum absorbance for Chl b). Ultimately, the Chl content was calculated using Equation 1 (Strain and Svec, 1966).

(1) $$\text{Total Chl} = (20.21 \times A645) + (8.02 \times A663)$$

To measure of essential oil percent, the plants were dried in the shade for 2 weeks. From every replication, 100 g of leaves was used to measure of essential oil. The samples were completely crushed and the essential oil extraction was conducted for 3 h with Clevenger. The essential oil for every sample was calculated and reported based on 100 g Dry weight.

2.3. Total phenol, proline and total soluble sugar

To measure total phenol, Folin reagent was used (Singleton and Rossi, 1965). Phenol was extracted from a 100 mg leaf sample using 3 mL of methanol 85% methanol in a mortar and the light absorbance was read at 765 nm with a spectrophotometer using a gallic acid standard curve. The extraction and measurement of proline was carried out with Bates et al. (1973) method. Initially, 0.5 g of plant leaf was pounded in mortar and then 10 mL sulfosalicylic acid 3% was added. Afterward, the absorbance was read with a spectrophotometer at 520 nm using a standard curve.

To measure the total soluble sugar, initially, 300 mg of sample tissue was homogenized using 5 mL ethanol (95%). Then, the centrifugation was conducted for 10 min (3500 rpm). The obtained supernatant was mixed with anthrone reagent, and the light absorbance was subsequently read at 625 nm, as described by Buysse and Merckx (1993) with minor modifications.

2.4. Relative water content (RWC)

The relative water content (RWC) was determined following the method described by Gucci et al. (1997) (Equation 2). Leaf samples were initially weighed (fresh weight), then submerged in distilled water for 24 hours. After soaking, surface moisture was gently removed, and the leaves were weighed again to obtain the saturated weight. Finally, the leaves were oven-dried at 75ºC for 48 hours and weighed to determine the dry weight.

(2) $$\text{RWC} = (\text{FW-DW}) / (\text{TW-DW}) \times 100$$

Where FW, DW, and TW represent the fresh weight, dry weight, and saturated weight of the samples, respectively.

2.5. Malondialdehyde (MDA) and electrolyte leakage (EL)

To measure MDA content, the method of Stewart and Bewley (1980) was used. At first, 0.5 g of the leaf was crushed with liquid nitrogen and 5 mL phosphate buffer 50 mM was added to the obtained powder and centrifuged at 14000 rpm and the absorbance was read in 600 nm by spectrophotometer (Varian Cary 100 UV, USA). The EL was measured with the method of Korkmaz et al. (2007). Leaf discs were washed with distilled water 3 times after elimination of surface contaminations and then by adding 10 mL distilled water to every disc, they were shaken for 24 h and EC was read with EC meter (EC1). Afterward, the samples were placed in an autoclave for 20 min at 121ºC and after cooling, EC was read again (EC2). The EL was calculated using Equation 3.

(3) $$\text{EL} = (\text{EC1} / \text{EC2}) \times 100$$

Ultimately, the data analysis was conducted with SAS software (9.1) and mean comparison with Duncan’s test.

3. Results and discussion

3.1. Chl and essential oil

The results showed that the highest total Chl (2.88 mg. g^{-1} FW) was observed in 100% Evp irrigation level with 1 in 1000 Kadostim and Humiforte, which displayed no significant difference with total Chl in 1 in 1000 Humiforte without Kadostim (2.81 mg. g^{-1} FW) as well as 1 in 1000 Kadostim without Humiforte (2.73 mg. g^{-1} FW). On the other hand, as seen in Table 3, the highest essential oil was earned in 50% Evp irrigation level without treatment with Kadostim and Humiforte (23.77%), which was the maximum content compared with other treatments significantly.

Table 3. The interaction effect of irrigation level, Kadostim and Humiforte on the total Chl and essential oil in summer savory leaves

Irrigation level (%Evp)	Kadostim	Humiforte	Total Chl (mg. g^{-1} FW)	Essential oil (%)
100	0	0	2.65±0.05^{b}	4.25±0.43^{i}
		1 in 1000	2.81±0.01^{ab}	10.50±0.50^{g}
	1 in 1000	0	2.73±0.03^{ab}	13.48±0.03^{f}
		1 in 1000	2.88±0.04^{a}	9.24±0.25^{h}
75	0	0	2.13±0.15^{c}	19.24±1.10^{d}
		1 in 1000	2.10±0.10^{cd}	21.11±0.96^{c}
	1 in 1000	0	1.94±0.03^{d}	17.88±0.90^{e}
		1 in 1000	2.15±0.07^{c}	22.48±0.42^{b}
50	0	0	1.55±0.05^{ef}	23.77±0.75^{a}
		1 in 1000	1.61±0.02^{e}	9.25±0.43^{h}
	1 in 1000	0	1.42±0.03^{f}	11.22±0.19^{g}
		1 in 1000	1.50±0.03^{ef}	18.14±1.03^{de}

Different letters within each column indicate significant differences at $p \leq 0.05$ among the treatments, according to Duncan's test.

3.2. Total phenol, proline and total soluble sugar

Total phenol in 1 in 1000 Kadostim (9.52 nmol. g^{-1} FW) was higher than no treatment (8.59 nmol. g^{-1} FW) (Fig. 1).

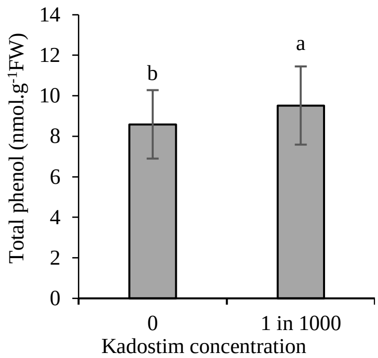

Figure 1. The effect of Kadostim concentration on total phenol in the leaves of summer savory

Furthermore, according to Fig. 2, concerning the interaction effect of irrigation level and Humiforte, the highest total phenol was observed in 50% Evp with 1 in 1000 Humiforte (12.22 nmol. g^{-1} FW), which was the highest total phenol against other treatments significantly.

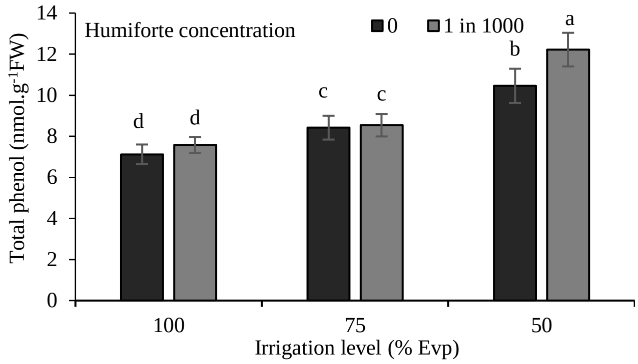

Figure 2. The interaction effect of Humiforte concentration and irrigation level on total phenol in the leaves of summer savory

As observable in Fig. 3, the highest proline was in 50% Evp with 1 in 1000 Humiforte (29.67 mg. g^{-1} FW), which was significantly the highest proline compared with other treatments. Also, Fig. 4, shows that the highest proline was in 50% Evp with Kadostim (29.25 mg. g^{-1} FW), indicating no significant difference with proline content in 50% Evp without Kadostim (27.5 mg. g^{-1} FW), while it was the highest proline against the other irrigation levels and treatments significantly.

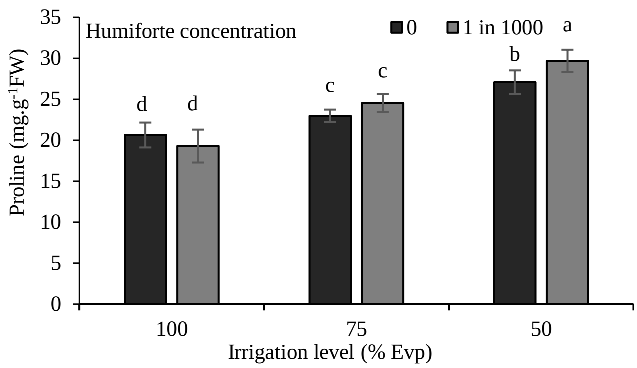

Figure 3. The interaction effect of Humiforte concentration and irrigation level on proline in the leaves of summer savory

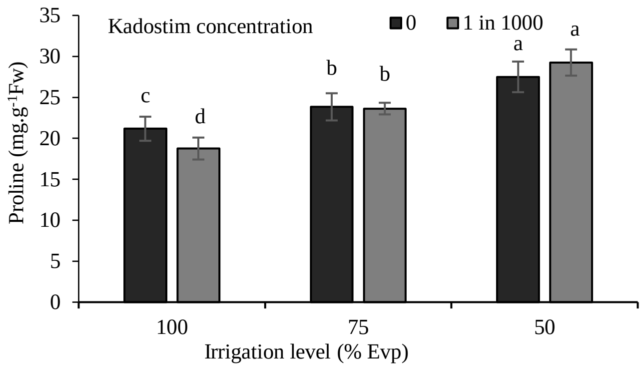

Figure 4. The interaction effect of Kadostim concentration and irrigation level on proline in the leaves of summer savory

Also, in the interaction effect of irrigation level and Humiforte (Fig. 5), the highest total soluble sugar resulted in 50% Evp with Humiforte (3.92 mg. g^{-1} FW) with no significant difference with total soluble sugar without Humiforte in 50% Evp (3.61 mg. g^{-1} FW), while these contents were the higher than other contents in other treatments significantly (Fig. 5). In Fig. 6, the highest total soluble sugar was in 50% Evp with Kadostim 1 in 1000 (3.87 mg. g^{-1} FW) that indicated no significant difference with total soluble sugar in 50% Evp without Kadostim (3.65 mg. g^{-1} FW), but it was significantly the higher content in comparison with other treatments.

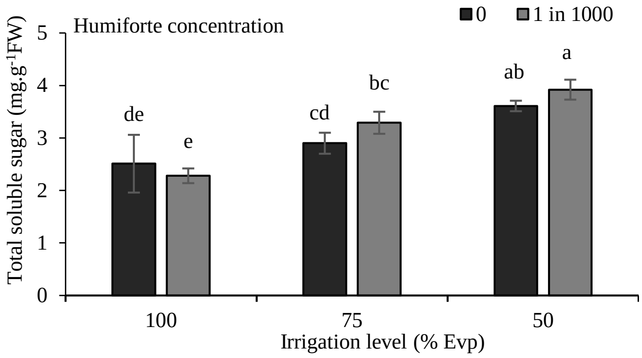

Figure 5. The interaction effect of Humiforte concentration and irrigation level on total soluble sugar in the leaves of summer savory

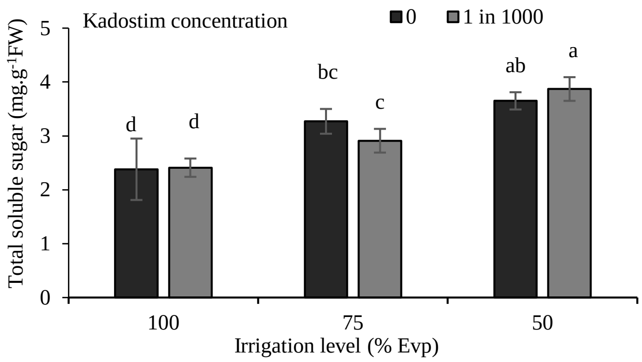

Figure 6. The interaction effect of Kadostim concentration and irrigation level on total soluble sugar in the leaves of summer savory

3.3. RWC

As shown in Fig. 7, the highest RWC was in 100% Evp with Kadostim (86.83%) without significant difference with RWC in the absence of Kadostim (86%), while it was the highest percent over other treatments significantly. As observed in Fig. 8, the use of the Humiforte could increase RWC and this parameter was higher in Humiforte application (73.39%), which was higher than RWC without Humiforte treatment (70.44%).

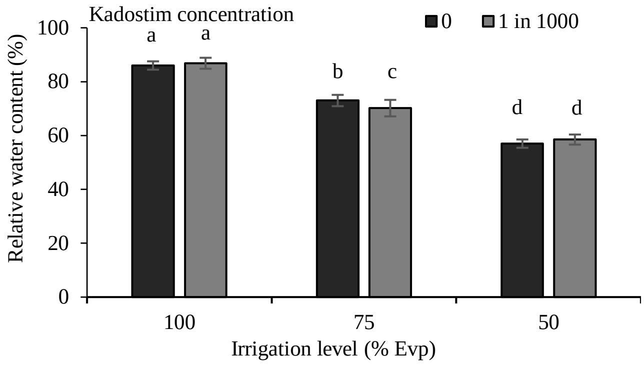

Figure 7. The interaction effect of Kadostim concentration and irrigation level on RWC in the leaves of summer savory

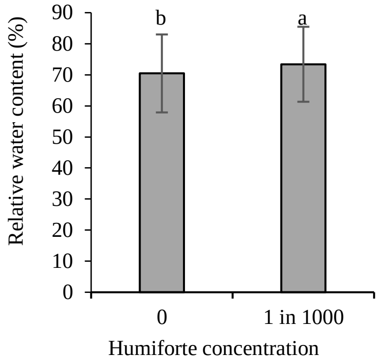

Figure 8. The effect of Humiforte concentration on RWC in the leaves of summer savory

3.4. MDA and EL

The highest MDA was in 50% Evp with 1 in 1000 Kadostim (2.9 nmol. g^{-1} FW), which was the highest MDA in comparison with other treatments (Fig. 9). In addition, in Fig. 10, EL was higher without Kadostim (45.55%) and the use of 1 in 1000 Kadostim could decrease this parameter to 42.22%. As a result of Fig. 11, the EL with no Humiforte was higher (45.33%) and the application of Humiforte could decline EL in Humiforte 1 in 1000 by 42.44%. Finally, the EL was affected by irrigation level (Fig. 12) and the highest EL was in 50% Evp (47.75%), which was the highest EL against other irrigation levels.

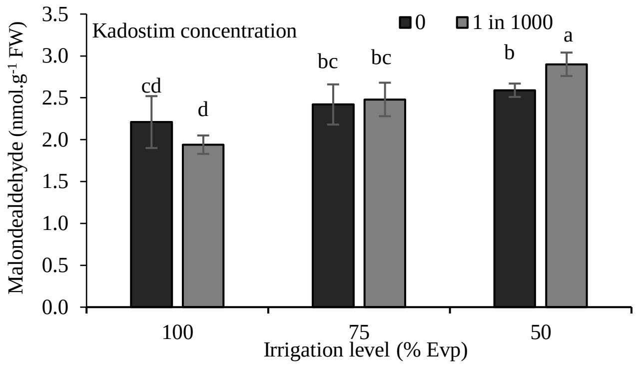

Figure 9. The interaction effect of Kadostim concentration and irrigation level on MDA in the leaves of summer savory

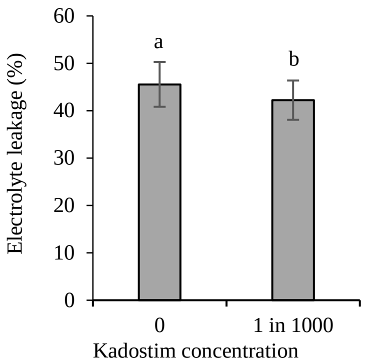

Figure 10. The effect of Kadostim concentration on total EL in the leaves of summer savory

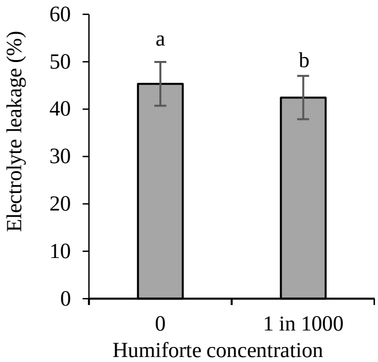

Figure 11. The effect of Humiforte concentration on EL in the leaves of summer savory

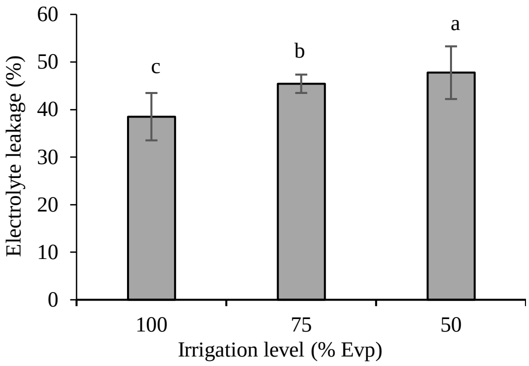

Figure 12. The effect of irrigation level on EL in the leaves of summer savory

The results of this experiment about the decline in RWC due to drought stress are in agreement with those reported in basil (Mulugeta and Radácsi, 2022) and rosemary (Shamsai *et al*., 2021). The decrease of RWC leads to turgidity loss and a decline in plant growth and biomass production (Beadle, 1985). The plant growth reduction under drought stress can lead to a reduction of leaf Chlorophyll amount, consequently decreasing light absorbance (Zarco-Tejada *et al*., 2000). In drought stress, the effective proteins in chloroplast synthesis face problems (Jin *et al*., 2015). Also, the activation of chlorophylase and Chlorophyll decomposition, as well as disturbance in enzymes involving Chlorophyll synthesis and ROS accumulation, cause Chlorophyll decrease (Cui *et al*., 2012). The enhancement of total phenol, proline and total soluble sugar in savory leaves in water deficiency was observed. Soluble sugar, as an osmoregulant, accumulates in stressful environments as a result of converting starch to simple and soluble sugars as well as less consumption of soluble sugars (Irigoyen *et al*., 2006). More proline in water deficiency is due to its osmolyte role as an amino acid and less oxidation of proline (Pedrol *et al*., 2000). Proline, as an antioxidant, plays a profound role in scavenging oxidative stress by improving catalase and peroxidase activity and the decline of hydroxyl. Also, proline has a protective role in cell structure (Osakabe *et al*., 2014).

Our findings are in accordance with those of Omidbaigi et al. (2003), who proved that by exacerbating drought stress severity, the yield of essential oil decreases. Similar to the obtained results, Farhoudi (2013) also found that drought stress causes elevated MDA concentration in rosemary. The membrane lipids are the first target for ROS and the peroxidation of membrane lipids leads to MDA production as a biomarker for sensitivity to stress (Turkan *et al*., 2005). Total phenol as a secondary metabolite has several roles –most notable- antioxidant and protective role (Andre *et al*., 2009), which its synthesis increases upon exposure of the plant to drought due to more of its gene expression (Schwambach *et al*., 2008) as observed in the present study. In this regard, in another study with more severity of drought stress, the total phenol content increased in linen (*Linum usitatissimum* L.) (Ghorbanli *et al*., 2012). The compositions, including amino acids such as Kadostim and Humiforte, were effective in improving protein, hormones, secondary metabolites and Chlorophyll synthesis and in general, resistance to environmental stresses (Seyedi *et al*., 2024). The production of amino acids consumes high energy and spraying them on plants decreases the requirement of the plant to their synthesis (Jacomassi *et al*., 2024). Humiforte and Kadostim improved the Chlorophyll content of mint leaves (*Menta spicat* L.) (Azarpira *et al*., 2020). The use of amino acids in drought stress, by elevated osmolytes, leads to a decrease of osmotic potential and contributes to water uptake in stressful conditions (Azarpira *et al*., 2020), similar to proline and soluble sugars in drought stress. The use of

Humiforte and Kadostim improves osmolytes such as proline and soluble sugar in mint. Proline contributes to the enhancement of the expression of proteins associated with improvement in resistance in plants (Khedr *et al*., 2003). The treatment with amino acids elevates Chlorophyll content due to an improved nitrogen supply (Bybordi, 2012). In spite of the obtained results about no effect of Kadostim and Humiforte on more accumulation of essential oil, these biostimulants lead to an increase in the accumulated essential oil in the medicinal plant basil (Rahimi Shokooh *et al*., 2013). The decrease of essential oil in drought stress is due to the fact that the plants have to decrease secondary metabolites, such as essential oil, in this condition to compensate for the loss of photosynthetic substances (Sangwan *et al*., 2001).

4. Conclusion

Biostimulants as eco-friendly substances, have no adverse effects on the environment, such as pollution from chemical fertilizers and also reduce production costs. According to the obtained results, the use of biostimulants such as Kadostim and Humiforte contributed to the improved biochemical characteristics in the medicinal plant summer savory exposed to drought stress. In conclusion, these biostimulants increased the resistance of the savory plants to water deficit through improved RWC, total Chlorophyll and osmolytes accumulation and these compounds could be promising treatments for savory in drought conditions (Fig. 13).

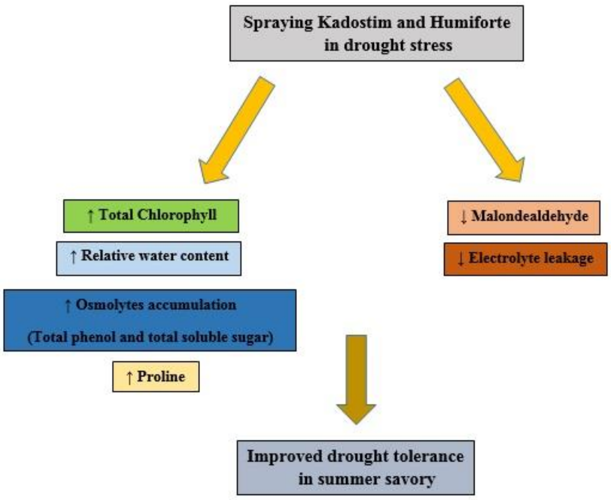

Figure 13. The effect of Kadostim and Humiforte application on summer savory exposed to drought stress

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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