



Evaluation of sulfur and foliar application of Zn and Fe on yield and biochemical factors of cumin (*Cuminum cyminum* L.) under irrigation regimes

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ABSTRACT

Introduction: Cumin, due to its food and medicinal properties, is one of the important plant species in the world. Moreover, water and nutrition deficiencies are serious abiotic stress factors. So, this experiment was conducted with the aim of investigating the effects of sulfur and foliar application of Fe and Zn on yield and biochemical characteristics of cumin under irrigation regimes.

Methods: The experiment was conducted as a split plot on the basis of a completely randomized block design during the 2016-2017 growing seasons with three replications. Experimental factors were arranged in irrigation regimes as main plots at three levels (I1: No stress (control), I2: irrigation based on 40% available water discharge, I3: 80% available water discharge) and foliar application of Zinc (Zn) and Iron (Fe) as subplots [F1: control (water-soluble), F2: Iron, F3: Zinc, F4: Zinc and Iron chelate] and sub-sub plots including sulfur fertilizer [S1: control (no use of sulfur), S2: sulfur fertilizer with *Thiobacillus*].

Results: Analyzed data showed that total phenol content and flavonoids were enhanced with the increase of drought intensity and the maximum amount was recorded under I3, while I3 caused a substantial reduction in grain yield. Flavonoid and grain yield significantly increased in F4. Total phenol content was the highest in F2 and F3 treatments. Application of sulfur fertilizer resulted in a significant increase in peroxidase, phenol and flavonoids. The highest amount of peroxidase was obtained in I3F4 and I3F3. The largest total soluble sugar (TSS) was resulted by I3S2 and the least by I1S1. Foliar application of Zn and Fe with sulfur fertilizer increased TSS.

Conclusion: The present study suggests that foliar application of Fe and Zn and sulfur fertilizer can improve the injurious effects of water deficiency on cumin plant through alteration in yield and biochemical characteristics.

Implication for health policy/practice/research/medical education:

Foliar application of Iron and Zinc and sulfur fertilizer can effectively reduce the damages of drought stress on cumin plant through alteration in biochemical characteristics.

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Introduction

One of the most important stresses is drought stress, adversely changing plant growth and yield production (1). Reports have indicated that plants may experience physiological and biochemical changes due to drought stress (2). Drought stress can damage the electron transport chain, which leads to the increase of reactive oxygen species as the subsidiary product of electron transport in chloroplasts, mitochondria, peroxisomes and

plasma membranes (3). When plant cells are under stress, free radicals are extremely detrimental for proteins, lipids and nucleic acids, ultimately leading to cell damage and death (4).

Under drought stress, nutrient availability and uptake by the root will get reduced. Therefore, foliar application of these elements has been considered in recent years. In this technique, nutrients are directly absorbed by leaves, so it is quicker than soil application. Besides, during the early

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stages of growth, when the system of roots has not been fully developed, foliar application of essential elements can lead to the increase of access to nutrients (5). For some plant types, studies have shown that foliar spraying leads to the improvement of their growth under drought stress (6). It also prevents the toxicity of these elements (7).

Zinc (Zn) is regarded as an important micronutrient, the shortage of which among many plants is a common issue (8). It is necessary for the activity of such enzymes as dehydrogenase, aldolase, isomerase, trans phosphorylase, RNA and DNA polymerase. It also plays a part in tryptophan synthesis, cell division, cell structure preservation and photosynthesis. As a cofactor, it gives rise to the synthesis of proteins, because of its role as a cofactor in many proteins (9). Fe is an essential element in plants due to its role as a cofactor in many proteins (10). Access to this element extremely affects the growth and efficiency of plant (11). It is largely required in chloroplast and mitochondria of plant cells. It contributes as a cofactor in several proteins of electron transport chain and with sulfur forms Fe-sulfur protein (12,13). However, Fe is one of the most abundant elements in the earth's crust. But its availability in alkaline and calcareous soils due to Fe^{+3} is very limited. It is therefore considered as a limiting element for the plant growth in such soils. Acidic soils account for the increase of Fe solution and conversion of Fe^{+3} into Fe^{+2} (14).

Applying sulfur is regarded as a method for reducing the soil pH (15). Elemental sulfur is oxidized by microorganisms and is converted to sulfate and hydrogen ion, and in this way, leads to the decrease of pH (15). In addition, as one of the essential nutrients for plant growth, sulfur is necessary for making protein and enzyme, by participating in the structure of the amino acids cysteine and methionine (16). Proper rate of oxidation of sulfur in soil is the main condition of its effectiveness, and the oxidation of sulfur is mainly considered as a biological process (17). In order to oxidize sulfur, a large population of sulfur-oxidizing micronutrients is essential, and *Thiobacillus* bacteria are one of the most effective types of such microorganisms (18). Sulfur containing products are important for plant fitness and their ability to cope with environmental stresses (19,20). The results indicated that application of biosulfur leads to a significant increase in grain yield and essential oil yield in cumin (21). Yield reduction of cumin due to drought stress is consistent with the results of previous researches (22,23). The results of Akbari et al (24) reported that foliar application of Zn+Fe for cumin causes a significant increase in both yield and yield components such as the number of umbrellas per plant, number of umbels per umbrella and 1000 seed

weight. Foliar application of Zn+Fe in the fields under drought stress had a significant effect on such physiological characteristics as relative water content, malondialdehyde, peroxide hydrogen, proline and protein content (24). The increase in grain yield with the application of Fe and Zn is congruent with the other results (25, 26). Results of another study indicated a significant increase of cumin yield with the application of Fe (27).

Cumin (*Cuminum cyminum* L.) is one of the important plant species (due to its food and medicinal properties) in the world. It is from the Apiaceae family and native to Egypt (28). It is cultivated in arid and semi-arid regions of Iran as a medicinal plant (29). Cumin has such various applications as a spice and also medicine for treatment of toothache, dyspepsia, diarrhoea, epilepsy and jaundice. It is also known as a natural antioxidant (30). The vegetative part of this plant contains phenolic compounds, particularly flavonoids and anthocyanins, which may be used as antioxidant (31). Besides, this plant has antibacterial activity (32). The aim of this study is investigating the effects of sulfur and foliar application of Fe and Zn on the yield and also on biochemical factors of cumin under irrigation regimes.

Materials and Methods

Experimental site

A field experiment (2016-2017) was conducted to investigate the effects of sulfur and foliar application of Zn and Fe on yield and physiological traits of cumin under different irrigation diets. The experiment was performed in a research farm, in the northern latitude of 35° and 34' and the eastern longitude of 51° and 8', 1050 m above the sea level in the southern part of Tehran province, Iran. This site is characterized by a semi-arid climate with the annual average temperature of 17.6°C. The physical and chemical properties of soil were measured using the standard methods before conducting the experiment (Table 1).

Plant material

Seeds of cumin (Sabzevar cultivar) were provided from Pakanbazar Company, Isfahan, Iran. Sowing was done in the month of February 2017. Seeds were planted by hand in 2×2 m plots having a density of 120 plants m⁻². Each plot consisted of 10 rows with 20 cm inter-row space. The field was fertilized according to soil testing using urea (170 kg/ha) and ammonium phosphate (180 kg/ha). The weeds were controlled during the growing season. The irrigation treatments were imposed after complete plant deployment in the 5-leaf stage (23,29). In this experiment, all macro elements (nitrogen, phosphorus, potassium, etc) and

Table 1. Some physicochemical properties of the soil of the experimental site

Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	P (mg/kg)	Electrical conductivity (dS/m)	pH	N	Soil texture
11.98	4.17	4.86	2.78	16	4.96	7.9	0.04	Sandy loam

micro (copper, manganese, etc) (except for experimental treatments) were applied on all test plots based on soil test. This was done in order to eliminate the negative effects of the deficiency of these elements on the quantitative and the qualitative yield of the plant (based on the Libibian law).

Treatments

The experiment was conducted as a split-split plot on the basis of a completely randomized block design with three replications. Experimental factors were arranged in irrigation regimes as main plots at three levels (I1: No stress, I2: irrigation based on 40% available water discharge, I3: Irrigation based on 80% available water discharge) and foliar application of Zn and Fe as subplots [F1: control (water-soluble), F2: Iron chelate, F3: Zinc chelate, F4: Zinc and Iron chelate] and sub-sub plots including sulfur fertilizer [S1: control (no use of sulfur), S2: petrochemical sulfur fertilizer 98% (300 kg ha⁻¹ based on soil test) with *Thiobacillus* (sulfur oxidizing bacteria)]. The exact water stress levels were applied after field analysis and determined the field capacity (FC) and permanent wilting point (PWP) of the soil sample. Soil moisture measurement was determined by gravimetric weighing method. It was done by sampling 30 centimeters of soil and determining the soil curve (33). The amount of Fe chelate was 400 g/ha in 300 L of water and the Zn chelate content was 17.1 kg ha⁻¹ in 187 L of water (34,35). The plants were treated with a foliar application of the freshly prepared aqueous solution of the Fe and Zn chelate. The first applications of Zn and Fe were at the early growing season (after the 7th to 10th leaf stage) and the second applications at the end of the vegetative stage (before flowering) using a hand-held sprayer (24,36). The control plants were sprayed with distilled water. *Thiobacillus* was purchased from Mehr Asia Co. Each 50 kg of sulfur fertilizer was added to the packaging of 1 kg of *Thiobacillus* fertilizer, according to the recommendation of the manufacturer. Soil inoculation with these bacteria increases the oxidation rate of sulfur (37).

Biochemical analysis

For the measurement of peroxidase, phenol, etc traits and the samples (fully expanded leaf) were immediately frozen in liquid nitrogen and kept in a freezer at -80°C before conducting the biochemical analyses.

Peroxidase

The activity of peroxidase enzyme was measured using the Chance and Maehly method (38). The measurement was based on the rate of oxidation of guayacol by this enzyme. In this method, 33 µmol of the extract was mixed with 1 ml of peroxidase solution containing 13mM guayacol, 5mM H₂O₂ and 50mM potassium phosphate buffer (pH = 7). Its absorption was read for one minute every 10 seconds by

spectrophotometer at the wavelength of 470 nm.

Total soluble sugars

TSSs were measured according to the method of Ndoumou et al (39). Briefly, 0.1 g of the sample was crushed in a mortar and 5 mL of 80% hot ethanol was added to it. Extraction was repeated 4 times with ethanol to evaporate the alcohol and the extract was placed at 70°C. Then, the extract was mixed with chloroform (1:5) to remove chlorophyll. After vortexing, it was left for 5 minutes. The upper transparent section was centrifuged for 10 minutes at 10000 rpm for determination of soluble sugars content according to the anthrone method by McCready et al method. TSS was determined by a LAMBDA 850 UV/Vis spectrophotometer at 620 nm. The concentration of TSS was calculated using the standard glucose curve and expressed in mg/g.dw⁻¹.

Phenols

Determination of phenol was conducted according to the following: 0.2 g of sample was mixed with 20 mL boiled water using a tube, which was then placed in a water bath for 1 hour. The samples were then passed through filter paper No. 1 and brought to the volume of 25 mL. Then 1 mL of extract was mixed with 5 mL indicator of Folin-Denis and 10 mL sodium carbonate (NaCO₃ 35%), and was brought up to volume using a 100-mL volumetric flask. The solution was left undisturbed under the room temperature for 45 minutes. Finally, the absorption of samples was measured using the standard curve of gallic acid (40).

Flavonoid

Flavonoids were determined using the method of Krizek et al (41). For this purpose, samples were powdered by liquid nitrogen to which ethanol acetic acid (99:1 v/v) was added. The mixture was then centrifuged at 8000 × g for 10 minutes. Ethanol acetic glacial acid (99:1 v/v) was used as the blank. The absorbance was read at three wavelengths of 300, 330 and 270 using a spectrophotometer.

Dried grain yield

Grain yield (GY) was determined by collecting plants in a 1-m area at physiological maturity. The grains were weighed accurately with a precision of 0.001 g.

Statistical analysis

Data were subjected to analysis of variance using SAS 9.4. The means were compared using Duncan's Multiple Range Test. *P* < 0.05 was considered significant.

Results

Irrigation regimes affected the level of phenol, TSS, activity of peroxidase enzyme, grain yield (*P* < 0.01) and flavonoid (*P* < 0.05). Phenol, flavonoid, TSS concentrations,

peroxidase and grain yield were significantly ($P < 0.01$) affected by foliar application of Zn and Fe (Table 2). Sulfur fertilizer was significant on phenol, flavonoid, peroxidase, TSS ($P < 0.01$) (Table 2). There was a significant interaction effect ($P < 0.01$) of irrigation regimes and foliar application of Zn and Fe throughout the experiment on the activity of peroxidase enzyme (Table 2). According to Table 2, the interaction between irrigation and sulfur fertilizer was significant ($P < 0.05$) on TSS. Also, interaction of spraying and sulfur fertilizer treatments significantly ($P < 0.01$) affected the TSS of cumin (Table 2).

Total phenol content

Water deficit stress increased total phenol content. The maximum amount of phenol content ($6.7 \mu\text{g gallic acid/g fw}^{-1}$) in cumin leaves was achieved under severe stress. Irrigation conditions based on discharges of 40 available

water and 80% available water resulted in 12 and 34% increase in total phenol compared to control treatment, respectively (Table 3). The treatment of plants with Fe and Zn increased by 43.8 and 49.2%, in total phenol content respectively, compared to untreated control plants (Table 3). Total phenol content increased with the application of sulfur fertilizer. Sulfur fertilizer had 9% higher phenol content as compared to control (Table 3).

Peroxidase

Stress and application of Fe and Zn separately increased the activity of peroxidase enzyme. The highest amount of peroxidase in cumin leaves was obtained under severe stress with the application of Fe+Zn (I3F4) ($3.72 \text{ mM H}_2\text{O}_2 \text{ mg}^{-1} \text{ min}^{-1}$) and Zn (I3F3) ($3.51 \text{ mM H}_2\text{O}_2 \text{ mg}^{-1} \text{ min}^{-1}$). Under mild and severe stress conditions, spraying with Zn and Zn+Fe increased peroxidase (Table 4). Application of

Table 2. Analysis of variance for the level of phenol, flavonoid, activity of peroxidase enzyme, TSS and the yield of cumin (*Cuminum cyminum* L.), subjected to irrigation regimes, sulfur and foliar application of Zn and Fe

Source of variation	df	Activity of peroxidase enzyme	Phenol	Flavonoid	TSS	Dried grain yield
Replication	2	0.05 ^{ns}	1.19*	0.23 ^{ns}	19.95 ^{ns}	31488.95 ^{ns}
Irrigation regimes (I)	2	53.93**	17.94**	1.70*	396.03**	133077.63**
Error a	4	0.08	0.15	0.17	21.90	9896.47
Spraying (F)	3	1.81**	17.73**	1.63**	590.84**	66956.58**
I×F	6	0.40**	0.63 ^{ns}	0.04 ^{ns}	32.49 ^{ns}	616.16 ^{ns}
Error b	18	0.10	0.62	0.17	22.47	4703.78
Sulfur (S)	1	0.47**	5.84**	2.45**	1062.22**	1845.28 ^{ns}
I×S	2	0.11 ^{ns}	0.17 ^{ns}	0.08 ^{ns}	77.11*	143.63 ^{ns}
F×S	3	0.05 ^{ns}	0.05 ^{ns}	0.02 ^{ns}	94.83**	187.65 ^{ns}
I×F×S	6	0.04 ^{ns}	0.17 ^{ns}	0.01 ^{ns}	18.95 ^{ns}	240.28 ^{ns}
Error c	24	0.04	0.65	0.16	21.27	4418.37
CV (%)		13.2	13.9	19.0	15.1	8.2

* and ** significant at $P \leq 0.05$ and $P \leq 0.01$ level, respectively; ns, not significant.

CV, coefficient of variation; Error a, Main plot error; Error b, Sub plot error; Error c, Sub-sub plot error; TSS, total soluble sugar.

Table 3. Mean phenol ($\mu\text{g gallic acid/g FW}^{-1}$), flavonoid (mmol/g FW^{-1}), activity of peroxidase enzyme ($\text{mM H}_2\text{O}_2 \text{ mg}^{-1} \text{ min}^{-1}$) and dried grain yield (kg/ha) in cumin plant under different irrigation regimes, spraying treatments and sulfur fertilizer

Irrigation regimes (I)	Dried grain yield	Phenol	Flavonoid	Activity of peroxidase enzyme
I1	878.5 ^a	4.99 ^c	1.77 ^b	-
I2	823.4 ^a	5.61 ^b	1.99 ^{ab}	-
I3	731.2 ^b	6.70 ^a	2.30 ^a	-
Spraying (F)				
F1	741.5 ^b	4.45 ^c	1.73 ^c	-
F2	779.3 ^b	6.41 ^a	2.09 ^b	-
F3	851.6 ^a	6.64 ^a	1.87 ^{bc}	-
F4	871.7 ^a	5.55 ^b	2.41 ^a	-
Sulfur fertilizer (S)				
S1	-	5.48 ^b	1.84 ^b	1.47 ^b
S2	-	6.05 ^a	2.21 ^a	1.64 ^a

The means with different letters in each column are significantly different for each sample ($P < 0.05$).

FW: fresh weight; I1: no stress, I2: irrigation based on 40% available water discharge, I3: irrigation based on 80% available water discharge, F1: control (water-soluble), F2: Iron chelate, F3: Zinc chelate, F4: Zinc and Iron chelate, S1: control (no use of sulfur), S2: petrochemical sulfur fertilizer 98% (300 kg ha^{-1} based on soil test) with *Thiobacillus*.

Table 4. The mean activity of peroxidase enzyme ($\text{mM H}_2\text{O}_2 \text{ mg}^{-1} \text{ min}^{-1}$) in cumin plant under different irrigation regimes and spraying treatments

Irrigation regimes (I)	Spraying (F)	Activity of peroxidase enzyme
I1	F1	0.20 ^f
	F2	0.22 ^f
	F3	0.29 ^f
	F4	0.32 ^f
I2	F1	0.88 ^e
	F2	0.91 ^e
	F3	1.42 ^d
	F4	1.64 ^d
I3	F1	2.51 ^c
	F2	3.06 ^b
	F3	3.51 ^a
	F4	3.72 ^a

The means with different letters in each column are significantly different for each sample ($P < 0.05$).

FW: fresh weight, I1: no stress, I2: irrigation based on 40% available water discharge, I3: irrigation based on 80% available water discharge. F1: control (water-soluble), F2: Iron chelate, F3: Zinc chelate, F4: Zinc and Iron chelate.

sulfur fertilizer significantly increased peroxidase ($1.64 \text{ mM H}_2\text{O}_2 \text{ mg}^{-1} \text{ min}^{-1}$), and the activity of peroxidase enzyme in this treatment increased 11.6% compared to the control treatment (Table 3).

Flavonoid

Irrigation based on 80% available water discharge (S3) caused a substantial increase in flavonoid content of cumin in comparison to the control treatment (S1). Flavonoid concentration gradually increased with the increase of drought stress, and the maximum amount of flavonoid ($2.3 \text{ mmol/g fw}^{-1}$) in cumin leaves was obtained under severe stress. Severe stress had 23% higher flavonoid as compared to control (Table 3). Foliar application of Zn and Fe resulted in an increase in total flavonoid content. The highest flavonoid content was achieved by the application of Zn and Fe (F4), which increased by 41.8% compared to the control treatment (Table 3). Application of sulfur fertilizer with *Thiobacillus* significantly increased the flavonoid content ($2.21 \text{ mmol/g fw}^{-1}$) and this trait increased by 20.1% compared to control treatment (Table 3).

Total soluble sugars (TSS)

TSSs significantly increased by application of sulfur fertilizer under all three irrigation conditions (no stress, mild stress and severe stress). The largest TSS ($40.78 \text{ mg/g fw}^{-1}$) was resulted from application of sulfur fertilizer under irrigation, based on 80% available water discharge (I3S2) and the least ($24.48 \text{ mg/g fw}^{-1}$) by no use of sulfur and normal irrigation conditions (Table 5). The mean comparison for the interaction between the spraying and

sulfur fertilizer showed that foliar application of Zn and Fe with sulfur fertilizer increased TSS. The largest value for TSS was $44.75 \text{ (mg/g fw}^{-1}\text{)}$, related to spraying with Zn+Fe and application of sulfur fertilizer (F4S2) and the least ($23.31 \text{ mg/g fw}^{-1}$) to control (F1S1) (Table 6).

Grain yield

Severe stress (Irrigation based on 80% available water discharge) significantly declined grain yield of cumin compared to control treatment. The lowest grain yield (731.2 kg/h) was achieved under severe stress. Severe stress had 16.8% lower grain yield as compared to control. There was no significant difference between mild and non-stressed conditions in grain yield (Table 3). Foliar application of Zn and Fe significantly improved grain yield. The largest grain yield was resulted by spraying with Fe+Zn (871.7 kg/h) and Zn (851.56 kg/h). The treatment of plants with Fe+Zn and Zn increased by 17.6 and 14.8%, in grain yield respectively, compared to untreated control plants (Table 3).

Table 5. Mean TSS (mg/g FW^{-1}) in cumin plant under different irrigation regimes and sulfur fertilizer

Irrigation regimes (I)	Sulfur fertilizer (S)	TSS
I1	S1	24.48 ^d
	S2	29.27 ^c
I2	S1	26.75 ^{cd}
	S2	33.32 ^b
I3	S1	29.09 ^c
	S2	40.78 ^a

The means with different letters in each column are significantly different for each sample ($P < 0.05$).

FW: fresh weight; I1: no stress, I2: irrigation based on 40% available water discharge, I3: irrigation based on 80% available water discharge. S1: control (no use of sulfur), S2: petrochemical sulfur fertilizer 98% (300 kg ha^{-1} based on soil test) with *Thiobacillus*.

Table 6. Mean TSS (mg/g FW^{-1}) in cumin plant under spraying treatments and sulfur fertilizer

Spraying (F)	Sulfur fertilizer (S)	TSS
F1	S1	23.31 ^e
	S2	26.84 ^{de}
F2	S1	25.79 ^{de}
	S2	36.23 ^b
F3	S1	25.95 ^{de}
	S2	30.00 ^{cd}
F4	S1	32.05 ^{bc}
	S2	44.75 ^a

The means with different letters in each column are significantly different for each sample ($P < 0.05$).

FW: fresh weight, F1: control (water-soluble), F2: Iron chelate, F3: Zinc chelate, F4: Zinc and Iron chelate, 1: control (no use of sulfur), S2: petrochemical sulfur fertilizer 98% (300 kg ha^{-1} based on soil test) with *Thiobacillus*, TSS: total soluble sugars.

Discussion

In the current study, total phenol content significantly increased under water stress. The phenolic contents of both seeds and aerial parts of cumin increased under water stress (29). Bettaieb et al (30), Davarpanah et al (42) and Azhar et al (43) reported that cumin seed phenolic contents were enhanced under severe or moderate drought. The enhancement of phenolic contents has also been reported under drought stress to accumulation of soluble carbohydrates in plants due to reduced transportation of soluble sugars (44). Furthermore, it was suggested that the enhanced production of phenolic compounds may result from the increased activity of phenylalanine ammonia lyase under water stress (45). Also, antioxidant activities of the phenolic components can counteract the stress induced ROS in plants (46). Furthermore, high light intensity, in combination with water shortage, may result in stomata closure. So, the CO₂ uptake is significantly reduced. As a result, the consumption of energy carriers such as "NADPH + H⁺" for CO₂ fixation through the Calvin cycle is considerably decreased, leading to overproduction of NADPH + H⁺. Accordingly, all metabolic processes lead to the synthesis of NADPH + H⁺ reducing compounds such as isoprenoids, phenols, or alkaloids (47). The treatment of plants with Fe and Zn, respectively increased the total phenol content by 43.8% and 49.2%. The positive effect of Zn has been previously reported on total phenolic contents (48,49). Authors have suggested that this increase is resulted from increased expression of responsible genes for biosynthesis of phenolic compounds (50). Total phenol content increased with the application of sulfur fertilizer. Sulfur-containing preparations in plants are of vital importance for the plant's competence and ability to cope with environmental stresses (19,20,51).

Our results suggested that drought stress conditions with application of Fe and Zn resulted in enhanced activity of peroxidase enzyme. Under mild and severe stress conditions, spraying with Zn and Zn+Fe increased peroxidase. The results of research by Sedghi et al (52) indicate that the activity of antioxidant enzymes in drought stress is increased. Studies have shown that water stress can increase the peroxidase activity in plants (53). The peroxidase enzyme has Fe in its structure (54). It has been reported that Fe deficient cells cannot thrive in the presence of a certain amount of hydrogen peroxide. However, cells with sufficient amounts of Fe were tolerant of hydrogen peroxide (55). Therefore, it seems that the abundance of Fe in the cells can control the amount of peroxidase in the plant. Zn is also effective in the expression of antioxidant enzyme synthesizing genes and is a cofactor of increased activity of these enzymes (56). The increase in peroxidase activity by the use of Zn is in line with the results of other research (57,58).

In our study, the flavonoid contents were enhanced

under drought stress. Severe stress had 23% higher flavonoid as compared to control. Flavonoids are secondary metabolites, which are produced in vacuoles and cytosols and of most plant cells (59). They can reduce lipid peroxidation and oxidative damage during water shortage (60). These flavonoids determine the quality of the herbs because of their antioxidant and anti-inflammatory activities (61). Our results, in this content, are agreement with the results of Yuan et al (62) in *Scutellaria baicalensis* and Alinian et al (29) in cumin who reported that flavonoid content increased under water stress. Consistent with our findings, Jaafar et al (63) explained that reduced irrigation increased leaf flavonoid content in *Labisia pumila*.

Application of sulfur fertilizer, significantly increased TSS of cumin in severe stress compared to other stress levels. Also, spraying with Zn+Fe and application of sulfur fertilizer increased TSS. According to the results obtained by other researchers, it can be stated that osmolytes accumulate in the cytosol to modulate osmotic pressure. This increase is also consistent with the amount of soluble sugars in line with proline, which is consistent with reports that these two are related to stress (64). During osmotic stress, soluble sugars are produced by hydrolysis of common carbohydrates (65). While well-watered plants can accumulate high amounts of 2-octulose (an eight carbon carbohydrate), in plants under osmotic stress, octulose is converted to sucrose (66). This type of carbohydrate transformation is a common phenomenon in many drought tolerant plants (67). In another experiment, the concentration of carbohydrates in flowers under stress was significantly increased compared to the control treatment (68). The results of several studies indicate carbohydrate accumulation during drought stress (69,70). The effect of Zn on total sugars can be due to its role in the starch and nucleic acid metabolism, and also to the activities of various enzymes involved in these biochemical reactions (71). In other hand, Zn is required for the formation and metabolism of carbohydrates (72). Most enzymes involved in carbohydrate metabolism are activated by Zn (73). Increase in total sugars was found after foliar applications of Zn and B (74). The increase in carbohydrate content with foliar application of Zn is consistent with other research results (42). Accumulation of soluble sugars can help maintain turgidity in stressed tissues by osmotic regulation, and micronutrients contribute to increased carbohydrate and nitrogen metabolism in plant soluble sugars in stress conditions (75). Fe results in increased photosynthetic activity. In fact, in Fe-deficient chloroplasts, the rate of photosynthetic carbon dioxide uptake is reduced due to a decrease in photochemical capacity. Reduction of chlorophyll and damage to the photosynthetic electron transfer reduces sugars and decreases growth (76).

Exposing cumin plants to serve stress led to a substantial

decrease in grain yield. Severe stress had 16.8% lower grain yield as compared to control. Decreased carbon metabolism rate, reduced stomatal conductance and decreased water uptake due to reduced root growth have been identified as contributing factors to yield loss under drought stress (77). The results of Vazin (23) showed that drought stress reduced yield and yield components of cumin. In severe drought, the yield of cumin decreased (30). This is in agreement with the results of Rahimi (78) and Tabatabai et al (22) studies on cumin. Foliar spraying with micro elements such as Zn, copper, manganese and Fe has a positive and very rapid effect on plants (11). The treatment of plants with Fe+Zn and Zn increased by 17.6% and 14.8%, in grain yield, respectively, compared to untreated control plants. Fe and Zn application led to the yield of cumin seed compared to control (25). According to the results of Yazdani Chamheidary et al (79), increasing drought stress decreased biological yield and grain yield of cumin. Also in this study, foliar application of Zn and Fe had a significant effect on grain yield of cumin. The increase in grain yield of cumin corresponds to the foliar application of Fe and Zn in accordance with the results of Akbari et al (24).

Conclusion

This research shows that irrigation based on 80% available water discharge with foliar application of Fe and Zn and sulfur fertilizer increased biochemical traits of cumin such as peroxidase enzyme, phenol, flavonoid and carbohydrate. Grain yield decreased under severe stress conditions, but the spraying with Zn +Fe and Zn significantly improved grain yield of cumin. Mild stress (Irrigation based on 40% available water discharge) had no significant effect on cumin grain yield. But, under mild stress conditions, the biochemical traits increased and this increase was more noticeable with the application of Fe, Zn and sulfur fertilizer.

Authors' contributions

SSA conceived and designed the experiments, MAD revised the manuscript, AR and AMN analyzed the data and SSA wrote the paper. All authors read and confirmed the publication of the article.

Conflict of interests

Authors declared that they have no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors. The protocol was confirmed by the authors.

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