

## Impact of Foliar-Applied Iron (Fe) and Zinc (Zn) Nanoparticles on Quinoa Growth and Biochemical Characteristics under Drought Stress

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### ABSTRACT

Amaranthaceae annual *Chenopodium quinoa* Willd seeds have more protein, fiber, B vitamins, and minerals than most seeds. In recent years, the cultivation area and consumption of quinoa have increased in the country due to its nutritional properties and ability to grow in harsh environmental conditions. Based on climate change scenarios, long periods of drought are expected, which emphasizes the need for planting and developing new plants that are adapted to these conditions. Quinoa's morphological, biochemical, and physiological responses to nanoparticle Fe and Zn foliar treatment during drought stress were examined. Quinoa development was also compared to zinc and iron. With nutrient supplementation, a 2019 drought experiment assessed quinoa growth and quality. Quinoa (Giza1 cultivar) was evaluated for morphological, biochemical, and physiological parameters throughout two reproductive stages (50 and 100% blooming stage) and two drought stress levels (85% and 85% soil water). Foliar micronutrient applications (control, Zn(ZnSO<sub>4</sub>), Fe(FeSO<sub>4</sub>), Zn+Fe, nano-Zn, nano-Fe, nano-Zn + nano-Fe) were studied. Drought stress greatly reduced plant height, main and lateral branch numbers, leaf number, inflorescence length, leaf, stem, and seed dry weight, wet and dry plant weights, and seed output. Foliar fertilizer increased plant height, main and lateral branch numbers, leaves, inflorescence length, stem, seed dry weights, and plant wet and dry weights. Iron and zinc nanoparticles were better for nutrition. Drought stress affects quinoa production less with fertilizer. Also, most metrics were negatively affected by drought stress; however, foliar nano-Fe and nano-Zn at 50% flowering minimized its negative effects. High protein, proline, soluble carbohydrates, water, photosynthetic pigments, antioxidant enzyme activity, and low malondialdehyde. Drought stress-application time-nutrient correlations were significant in most parameters. At 50% blooming, nano-Fe and nano-Zn treatments had the highest protein, proline, soluble carbohydrates, and antioxidant enzyme levels under drought stress.

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### 1. Introduction

One of the most pressing demands of contemporary society for human health is the replacement of chemical products with plant-based alternatives (Mirzaei *et al.*, 2020). Quinoa (*Chenopodium quinoa* Willd.), an ancient crop, has the potential to make a significant contribution to global food security. According to research by Pakbaz *et al.* (2021), the climate in Iran is among the most favorable for cultivating *Chenopodium* plants. Quinoa is a nutritious grain that grows well in marginal agricultural settings and is native to the

Andean, Chilean, and Peruvian regions. According to Adolf *et al.* (2013) and Alsaleem *et al.* (2024), it has been successfully cultivated as a new crop in numerous regions worldwide. Humans have utilized *chenopodium* species for their high protein content because of their balanced amino acid spectrum, which includes high levels of lysine (5.1-6.4) and methionine (0.4-1.0%). The FAO has likened quinoa seeds to a "superfood" due to their very high nutritional content (Adolf *et al.*, 2013). Reducing losses in food production as a result of several biotic and abiotic

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stressors is crucial for ensuring food security in the face of climate change (Anjum *et al.*, 2011).

The process by which plants adapt to drought stress involves various events that alter their growth and physio-biochemical processes. These changes include alterations in plant structure, growth rate, tissue osmotic potential, and antioxidant defense (Bascuñán-Godoy *et al.*, 2016). Therefore, it is essential to comprehend how crops respond to water deficits, their adaptation strategies, and effective methods for increasing crop yields under adverse environmental conditions (Chang *et al.*, 2002). According to several studies (Naghdi Badi *et al.*, 2017), plants have developed a number of antioxidant enzymes that help reduce oxidative stress by removing harmful oxygen species. According to Wojtyła *et al.* (2016), even drought-tolerant plants need to ramp up their defenses when faced with extreme drought stress. During drought stress, one important sign of drought tolerance is the ability to protect membranes and enzymes from damage by raising antioxidant levels (Aboueshaghi *et al.*, 2023). According to studies carried out by Omid *et al.* (2015), these chemicals serve several physiological and ecological purposes, including defense and free radical scavenging. There have been reports of increased production of these chemicals as a result of different physical and chemical stressors in the environment. For instance, research on Balangu, quinoa, and lemon balm has shown that plants produce more phenolic chemicals when subjected to various conditions (Ferreira *et al.*, 2015). Metabolic issues caused by insufficient or missing vital nutrients may lead to community-wide poor health, illness, and financial and social consequences. The use of nanotechnology in agriculture has the potential to improve global food production and quality via an ecologically friendly approach to soil and water remediation (Dhanapal *et al.*, 2024).

Afsahi *et al.* (2020) found that zinc and iron microelements have a big effect on many processes in plants, such as metabolism, transpiration rate, photosynthesis, enzyme dysfunction, nutrient absorption, plant wilting, water relations imbalance,

and crop quality and quantity. However, chemicals like zinc and iron increase plants' ability to withstand drought (Rafiee *et al.*, 2019). Finding the overall antioxidant capacity of vegetables allows one to test the hypothesis that the protection exhibited after consuming them includes protection against free radical damage. Prior and Cao (2000) propose that a family of antioxidant phytochemicals called flavonoids found in vegetables are responsible for their protective effects against illnesses including cancer and cardiovascular disorders.

Applying iron and zinc to quinoa plants has several purposes in this study, one of which is to learn how to boost the concentration of key active chemicals in drought-stressed plants. The study also looked into how nano-Fe and zinc oxide could lessen or control the effects of dehydration stress on the growth and production of quinoa seeds, as well as some biochemical and biophysical traits. This was done since iron and zinc are essential nutrients for medicinal plants. It was therefore hypothesized that quinoa. Plants would exhibit bioactivity, or effects on growth, stimulation, and secondary metabolite synthesis when treated foliarly with nano-Fe and zinc oxide. So, the goal of this study is to evaluate the spraying of Fe and Zn nanoparticle solutions on the growth process of quinoa plants under drought stress.

## 2. Materials and methods

### 2.1. Experimental field

An experiment with three repetitions was done in the greenhouse of the Faculty of Agriculture at Shahed University in the winter of 2020. A completely randomized design (CRD) was employed to investigate morphological, biochemical, and physiological factors. The greenhouse is situated at a latitude of 34° 35' N, a longitude of 51° 8' E, and an altitude of 1050 m above sea level.

### 2.2. Soil test

Prior to the experiment, a soil sample was collected from a depth of 0 to 30 centimeters and analyzed in the soil laboratory (Table 1).

**Table 1. Physical and chemical properties of the soil studied**

Soil texture	Clay	Silt	Sand	OC	N	P	K	Fe	Zn	pH	EC
			(%)					(mg kg <sup>-1</sup> )			(ds m <sup>-1</sup> )
Loam	20	33	44	0.56	0.05	8.1	247	6.01	4.1	7.8	3.58

pH: potential of hydrogen, EC: electrical conductivity, OC: Organic matter

### 2.3. Plant materials, experimental setup, and treatments

The plant seed (Code number: Giza1) used in this study was received from the Seed and Plant Research Institute, Karaj, Iran. Eighty-four plastic pots, each measuring 30 cm in height and 25 cm in diameter, were used for cultivation. All pots were filled with a homogeneous mixture of field soil, decomposed leaf soil, and sand in equal proportions. Three quinoa seeds, ranging from 3 to 5 cm in length, were planted at a depth of 3 to 5 cm in each pot. The seeds were then lightly covered with decomposed manure and watered. The plants were grown in a greenhouse that received natural sunlight. The relative humidity in the greenhouse fluctuated between 51% and 54%, while the average temperature remained constant at  $25 \pm 3^\circ\text{C}$  throughout the experimental period.

### 2.4. Drought stress application method

A moisture retention curve was generated using a pressure plate apparatus. The target moisture content for the drought stress treatments was calculated and applied based on this curve (Alizadeh, 2004).

### 2.5. Experimental design and treatments

The experiment was conducted as a completely randomized design (CRD) with three replications. The investigation utilized iron (II) sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 4 g  $\text{L}^{-1}$ ) and zinc sulfate heptahydrate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 3 g  $\text{L}^{-1}$ ) as sources of iron and zinc, respectively. These compounds were obtained from Germany (Merck & Co., Inc.). Iron oxide (FeO) and zinc oxide (ZnO) nanoparticles were acquired from Sigma-Aldrich in the USA at a concentration of 1 g  $\text{L}^{-1}$ . The experiment consisted of seven different treatments applied to quinoa plants (Giza1 cultivar). These treatments included foliar application of various substances such as Zn, Fe, Zn + Fe, nano-Zn, nano-Fe, and nano-Zn + nano-Fe. The application was done at two different stages of the plant's flowering process, specifically when it was at 50% and 100% flowering. Additionally, the plants were subjected to two levels of drought stress, with one group having 50% of the soil water drained (control) and the other group having 85% of the soil water drained (stress).

To apply irrigation treatments, soil samples were collected from the target field and their gravimetric water content was measured at various pressures using

a pressure plate apparatus. Based on the gravimetric water content, the irrigation amount for each treatment was determined.

Pots were weighed daily using a digital scale to monitor daily water loss. Some pots were irrigated to field capacity as a control. When the pot weight reached the desired threshold, plants were irrigated to 100% field capacity. During the stress period, pots in each treatment were randomly rearranged to reduce experimental error and ensure uniform growth conditions. Micro and nano-element doses were applied based on various sources (Shabanzadeh and Galavi, 2011; Saleem et al., 2022).

The micronutrients were applied to all the plants in the pots using a manual pump sprayer until they overflowed (Ghasemi et al., 2021). A surfactant, Tween 20, was used in all spray solutions at a concentration of 0.01% (v/v) to enhance the absorption of the spray by the leaves. The control plants were treated with distilled water (Omidi et al., 2015). The solution was sprayed in a quantity sufficient to make the droplets visible on the quinoa leaves. The spraying was conducted during the early morning hours, away from direct sunlight. To avoid the infiltration of external substances, the soil surface of the pots was covered with plastic. Following that, Prior and Cao (2000) described how to compute the amount of irrigation for each treatment. To minimize experimental error and provide consistent growth circumstances for all plants, the pots of each treatment were accidentally relocated during the stress phase. In order to enhance the absorption of foliar nutrients through the leaf surface, a surfactant was employed. This surfactant reduces the surface tension of water, expanding the contact area between the solution and the leaf surface, and resulting in increased nutrient uptake. Drought stress was imposed concurrently with the onset of flowering, and foliar spray was administered at 50% and 100% of flowering. Prior to this period, all pots were consistently irrigated. Following the application of treatments, leaf samples were randomly chosen from each plant. Various traits, such as plant antioxidant activity, total phenol content, flavonoid content, tannin content, and anthocyanin content, were measured. Additionally, the height of the plant from the soil surface to the end of the stem, as well as the height of the cluster, were measured using a ruler (Chang et al., 2002).

## 2.6. Measurement of traits

### 2.6.1. Physiological traits

Relative water content (RWC) was measured using the Barrs method (Barrs, 1968).

### 2.6.2. Biochemical and enzymatic traits

Chlorophyll a and b concentrations were calculated using the Arnon (1949) and Lichtenthaler and Wellburn (1985) methods, respectively. Free proline content was measured using the Bates et al. (1973) method. Protein concentration was determined by the Bradford method using a spectrophotometer (Bradford, 1986). We used the Chance and Maehly (1954) method to measure peroxidase activity, the Nakano and Asada (1981) method to measure ascorbate peroxidase activity, the Dhindsa et al. (1981) method to measure catalase activity, the Beauchamp and Fridovich (1971) method to measure superoxide dismutase activity, and the Davey et al. (2005) and Stewart and Bewley (1980) methods to measure malondialdehyde (MDA).

### 2.6.3. Morphological and growth traits

Plant height was measured from the soil surface to the tip of the stem using a ruler. To measure the number and wet weight of the plant, the yellow and dry leaves were removed from the plant after harvest, and the healthy leaves were counted. The wet weight of the plant was measured with a digital scale with an accuracy of one ten-thousandth. To calculate the dry weight, the samples were placed in an oven at 75 degrees Celsius for 24 hours, and then the dry weight

of the plant and leaves was measured with a digital scale with an accuracy of one ten-thousandth.

## 2.7. Statistical analysis

After checking the normality of the data distribution (Kolmogorov-Smirnov and Shapiro-Wilk tests), it was statistically analyzed by SAS version 9.4 software (SAS Institute, Cary, NC, USA). The analysis of the data was based on the GLM (General Linear Model) method, and Duncan tests were used for statistical analysis. Values of  $p \leq 0.05$  were considered significant indicators (Ghasemi et al., 2021).

## 3. Results and discussion

The results of the experiment showed that drought stress had a significant effect on all of the measured morphological and growth traits. The effects of drought stress were most pronounced on the traits that are involved in growth, such as plant height, number of leaves, and plant fresh weight.

### 3.1. Plant height

Results of the ANOVA showed that the effects of water stress, nutrient foliar application, and application time, as well as the interactive effects of water stress  $\times$  application time (DT), water stress  $\times$  nutrient foliar application (DN), application time  $\times$  nutrient foliar application (TN), and water stress  $\times$  application time  $\times$  nutrient foliar application (DTN), on plant height, were significantly different at the 1% probability level (Table 2).

**Table 2. Analysis of variance for the effect of foliar application of micronutrients at different times on growth characteristics of quinoa under drought stress conditions**

S.O.V	df	Mean Square (MS)								
		Plant height of main branches	Number of main branches	Number of secondary branches	Number of leaves	Inflorescence length	Dry weight of leaves	Dry weight of stem	Dry weight of seed	Total fresh weight
Drought stress (D)	1	44620.19**	36.54**	12.97**	6550.8**	346.16**	798.68**	20006.54**	12258.78**	1055264.58**
Spraying time (S)	1	11480.05**	10.86**	3.39**	2393.6**	45.73**	246.17**	4737.01**	1494.72**	25685.44**
Nutrient (N)	6	27337.38**	18.12**	8.69**	4724.99**	283.58**	516.12**	15569**	14038.23**	652286.61**
D $\times$ S	1	744.05*	0.65**	0.23**	511.23*	11.23**	27.89**	207.62**	416.39**	19657.44**
D $\times$ N	6	2006.86**	0.59**	0.41**	205.85**	5.57 <sup>ns</sup>	22.31**	738.37**	1263.3**	49968.75**
S $\times$ N	6	762.21**	0.13**	0.17**	171.56**	4.87 <sup>ns</sup>	14.1**	211.24**	1415.01**	18161.61**
D $\times$ S $\times$ N	6	882.21**	0.21**	0.15**	138.6**	3.79 <sup>ns</sup>	5.66**	23.63**	1045.16**	17778.27**
Error	56	119.05	0.034	0.03	40.6	2.62	1.17	4.83	19.25	1904.76
C.V. (%)		7.64	5.38	6.76	12.08	5.07	6.2	3.1	6.61	6.44

ns, \* and \*\*: non-significant, significant at 5% and 1%, respectively. df: degrees of freedom

When we looked at the average of the main effects, we saw that water stress significantly lowered plant height. The lowest height was linked to drought stress, with an average of 119.81 cm, which is 26.79% less

than the non-stress treatment (Table 3). The effect of foliar application at the time of 50% flowering increased the height of the quinoa plant by 15.13% compared to the time of 100% flowering (Table 3).

Also, foliar application of nutrients to the quinoa plant caused a significant increase in plant height, so that the highest height in the nutrient treatment was related to

the combination of nano-Fe and nano-Zn, with an average of 200.50 cm, which increased by 40.44% compared to the control treatment (Table 3).

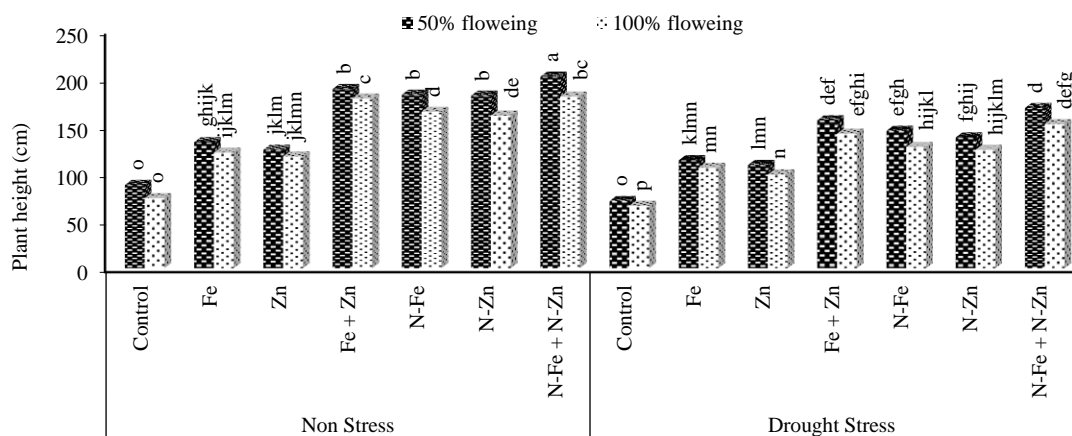
**Table 3. Mean comparison of the effects of foliar application of micronutrients at different times on growth characteristics of quinoa under drought stress conditions**

Treatment	Plant height (cm)	Number of main branches	Number of secondary branches	Number of leaves	Inflorescence length	Dry weight of leaves (g)	Dry weight of stem (g)	Dry weight of seed (g)	Total wet weight (g)	Total dry weight (g)
Drought stress										
Control	165.91 <sup>a</sup>	4.1 <sup>a</sup>	3.02 <sup>a</sup>	61.56 <sup>a</sup>	33.95 <sup>a</sup>	20.56 <sup>a</sup>	86.34 <sup>a</sup>	78.44 <sup>a</sup>	789.45 <sup>a</sup>	363.96 <sup>a</sup>
Drought stress	119.81 <sup>b</sup>	2.79 <sup>b</sup>	2.23 <sup>b</sup>	43.9 <sup>b</sup>	29.89 <sup>b</sup>	14.39 <sup>b</sup>	55.47 <sup>b</sup>	54.28 <sup>b</sup>	565.29 <sup>b</sup>	266.11 <sup>b</sup>
Foliar spraying time										
50% flowering	154.55 <sup>a</sup>	3.8 <sup>a</sup>	2.83 <sup>a</sup>	58.07 <sup>a</sup>	32.66 <sup>a</sup>	19.19 <sup>a</sup>	78.41 <sup>a</sup>	70.58 <sup>a</sup>	732.67 <sup>a</sup>	338.5 <sup>a</sup>
100% flowering	131.17 <sup>b</sup>	3.09 <sup>b</sup>	2.43 <sup>b</sup>	47.39 <sup>b</sup>	31.18 <sup>b</sup>	15.76 <sup>b</sup>	63.39 <sup>b</sup>	62.14 <sup>b</sup>	622.07 <sup>b</sup>	291.57 <sup>b</sup>
Micronutrients										
Control	119.42 <sup>e</sup>	2.35 <sup>e</sup>	1.85 <sup>f</sup>	35.55 <sup>f</sup>	24.51 <sup>g</sup>	12 <sup>g</sup>	44.2 <sup>g</sup>	38.18 <sup>f</sup>	548.5 <sup>e</sup>	256.85 <sup>f</sup>
Fe	148.42 <sup>d</sup>	2.83 <sup>d</sup>	2.23 <sup>d</sup>	45.1 <sup>d</sup>	30.28 <sup>e</sup>	17.48 <sup>e</sup>	64.13 <sup>e</sup>	62.91 <sup>d</sup>	683.5 <sup>d</sup>	328.87 <sup>d</sup>
Zn	141.42 <sup>d</sup>	2.68 <sup>d</sup>	1.9 <sup>e</sup>	39.65 <sup>e</sup>	27.66 <sup>f</sup>	15.15 <sup>f</sup>	58.86 <sup>f</sup>	57.24 <sup>e</sup>	659.75 <sup>d</sup>	303.74 <sup>e</sup>
Fe + Zn	181.17 <sup>b</sup>	4.4 <sup>b</sup>	3.3 <sup>b</sup>	67.3 <sup>b</sup>	35.95 <sup>b</sup>	22.63 <sup>b</sup>	95.64 <sup>b</sup>	89.74 <sup>b</sup>	868.5 <sup>b</sup>	400.13 <sup>b</sup>
Nano-Fe	164.92 <sup>c</sup>	4.03 <sup>c</sup>	3.05 <sup>c</sup>	62.15 <sup>bc</sup>	33.89 <sup>c</sup>	20.65 <sup>c</sup>	90.36 <sup>c</sup>	85.57 <sup>c</sup>	786 <sup>c</sup>	363.13 <sup>c</sup>
Nano-Zn	161.17 <sup>c</sup>	3.9 <sup>c</sup>	2.93 <sup>c</sup>	59.58 <sup>c</sup>	32.49 <sup>d</sup>	19.6 <sup>d</sup>	84.73 <sup>d</sup>	73.8 <sup>d</sup>	768.5 <sup>c</sup>	355.28 <sup>c</sup>
Nano-Fe + Nano-Zn	200.5 <sup>a</sup>	4.94 <sup>a</sup>	3.69 <sup>a</sup>	67.79 <sup>a</sup>	38.68 <sup>a</sup>	25.83 <sup>a</sup>	99.41 <sup>a</sup>	95.09 <sup>a</sup>	956.83 <sup>a</sup>	437.23 <sup>a</sup>

In each column, means having at least one same letter, are not significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

Comparing the mean of the DTN interaction effects showed that the highest plant height with an average of 201.67 cm was observed in the nutritional treatment of nano-Fe and nano-Zn at the stage of 50% flowering

under non-drought stress conditions, and other nutritional treatments had a significant decrease in the time of foliar application under 100 flowering and drought stress conditions (Fig. 1).



**Figure 1. Effect of foliar application of micronutrients on plant height of quinoa under drought stress conditions**

Drought stress decreases the allocation of photosynthates to the stem, leading to reduced plant height. Water deficit has been shown to disrupt various physiological processes, including carbohydrate and nitrogen metabolism, protein structure and enzyme activity. These disruptions can lead to the accumulation of proline and a reduction in growth promoters, ultimately resulting in morphological changes in different plant parts (Kramer, 1983). Drought stress affects plant height by reducing plant growth rate

(Farooq et al., 2009). The results of this study were consistent with the results of Hosseini et al. (2021) on quinoa. Results of this study indicated that water stress at different growth stages significantly reduced plant height, number of branches, dry and fresh weight of the shoot, root volume, and root dry and fresh weight compared to the control (Hosseini et al., 2021). The results revealed that foliar application of zinc and iron significantly increased plant height under drought-stress conditions. Iron and zinc are essential elements



for plants that are involved in many enzymatic reactions and are essential for their proper growth and development. They also play a role in regulating protein and carbohydrate metabolism, which increases plant photosynthesis and, thus, height (Swietlik, 1998).

### 3.2. Main and lateral branch numbers

The results of the analysis of variance showed that the effects of drought stress, nutrient foliar application, and application time, as well as the interaction effects of drought stress  $\times$  application time (DT), drought stress  $\times$  nutrient foliar application (DN), application time  $\times$  nutrient foliar application (TN), and drought stress  $\times$  application time  $\times$  nutrient foliar application (DTN), on the main and lateral branch numbers, were significantly different at the 1% probability level (Table 2). Drought stress significantly decreased main and lateral branch numbers. The lowest numbers were found with drought stress, averaging 2.79 and 2.23, respectively. This represents a decrease of 31.95% and 26.16% compared to the control (Table 3). The effect of foliar application at the time of 50% flowering increased the main and lateral branch numbers by 18.68% and 14.13%, respectively, compared to the time of 100% flowering (Table 3). Also, foliar application with nutrients caused a significant increase in main and lateral branch numbers, so that the highest main and lateral branch numbers in the nutrient treatment were related to the combination of nano-Fe and nano-Zn, with an average of 4.94 and 3.69, respectively, which increased by 52.43% and 49.86%, respectively, compared to the control treatment (Table 3). Comparison of the mean of the DTN interaction effects showed that the highest main (6.07) and lateral branch numbers (4.33) were observed in the nutritional treatment and nano-Zn at the stage of 50% flowering under non-water stress conditions, and also that the main and lateral branch numbers decreased significantly in other nutritional treatments in the time of foliar application of 100 flowering and drought stress conditions.

Coriander's yield per unit area of vegetative organs (leaves and stems) went down when it was stressed by drought. This was clearly because the plant didn't accumulate as much dry matter and grow as fast during the growth stages (Ghassemi-Golezani et al., 2009). The growth rate of leaves and stems is very sensitive to drought stress because it is dependent on cell growth.

In line with our findings in this study, Ziaei et al. (2020), in a study on the quinoa plant, stated that the highest number of branches in the plant was obtained in the treatment of 50 mm of evaporation and the lowest in the treatment of 110 mm of evaporation from the evaporation pan, and also that the highest number of branches was obtained in the nutrient foliar application treatments and the lowest in the non-foliar application treatment.

### 3.3. Leaf number in the plant

The ANOVA revealed significant effects of drought stress, nutrient foliar application, application time, and their interactions on leaf number at the 1% level (Table 2). When we looked at the average of the main effects, we saw that drought stress significantly reduced the number of quinoa leaves. The treatment with the fewest leaves was drought stress, with an average of 43.90, which was 28.19% less than the control treatment (Table 3). The effect of foliar application at the time of 50% flowering increased the number of leaves in the quinoa plant by 18.39% compared to the time of 100% flowering (Table 3).

Adding nutrients to the leaves of quinoa plants significantly increased the number of leaves. The treatment with the most leaves had an average of 67.79 leaves, which is 41.66% more than the control treatment (Table 3). When the average of the DTN interaction effects was compared, they showed that the nutritional treatment of nano-Fe and nano-Zn had the most leaves (91.37) at the 50% flowering stage when there was no water stress. Other nutritional treatments had a significant decrease in the time of foliar application at 100 flowering and when there was drought stress.

While severe drought stress leads to wilting and even death of the plant and crop weakness, mild to moderate stress usually does not cause visible symptoms but significantly reduces plant growth and crop yield (Blum and Jordan, 1985). The lack of sufficient water reduces the amount of drought in the leaf and slows down leaf growth. Leaf growth reduction occurs over a long period before wilting and initially occurs by reducing the amount of cell expansion due to a decrease in turgor pressure (Choluj et al., 2004). In general, the most important result of the sensitivity of cell growth to moisture deficiency is a significant decrease in leaf growth and, as a result, leaf area. With a decrease in

leaf area and a decrease in photosynthesis, the biological yield of the plant decreases. In line with the results of this study, Hosseini et al. (2021) reported that the use of micro and nano fertilizers increases the number of leaves and leaf areas under drought-stress conditions.

### 3.4. Inflorescence length

The results of the analysis of variance showed that the effects of drought stress, nutrient foliar application, and application time were significant at the 1% probability level, and the effect of the interaction of drought stress  $\times$  application time (DT) on inflorescence length was significant at the 5% probability level (Table 2). In comparing the mean of the main effects, drought stress caused a significant decrease in the length of quinoa inflorescence, with the shortest length of inflorescence being related to drought stress, with an average of 29.89 cm, which showed a decrease of 11.96% compared to the control treatment (Table 3).

The effect of foliar application at the time of 50% flowering increased the length of quinoa inflorescence by 4.53% compared to the time of 100% flowering (Table 3). Also, foliar application with nutrients on quinoa plants caused a significant increase in the length of inflorescence, so that the longest inflorescence length in the nutrient treatment was related to the combination of nano-Fe and nano-Zn, with an average of 38.68 cm, which increased by 36.63% compared to the control treatment (Table 3). A comparison of the mean of the interaction effects of drought stress  $\times$  application time (DT) showed that the longest inflorescence length, with an average of 35.05 cm, was observed at the time of 50% flowering under non-water stress conditions.

Application of water stress during the flowering period of quinoa, even with the addition of fertilizer, reduces yield and water productivity. The plant undergoes various mechanisms to cope with the drought stress that is applied to it in order to survive and function under stressful conditions (Keshtkar et al., 2021). In these conditions, the plant reduces its green area to reduce the amount of water that is lost through evaporation and transpiration and also closes its stomata to reduce transpiration (Pinto et al., 2021). Under stress conditions, the plant uses a larger share of the energy it produces from photosynthesis to absorb water and nutrients through the roots, and the reduction

in height and weight of aerial organs under drought stress is due to this (Alvar-Beltrán et al., 2021; Jamali et al., 2023). Since the plant uses more of the energy it produces to absorb water, it has less of the produced sap available, and as a result, reproductive growth is disrupted (Wang et al., 2020). Foliar application of fertilizer to plants under stress conditions reduces the negative effects of stress (Jamali et al., 2020). The results of this study are consistent with those of Jamali et al. (2020) and Keshtkar et al. (2021). The results of Beyrami et al. (2020) were also consistent with the results of this study.

### 3.5. Dry weight of leaves, stems, and seeds

The analysis of variance revealed significant effects (at the 1% probability level) of drought stress, foliar nutrient application, and application timing on the dry weight of leaves, stems, and seeds. Additionally, significant interaction effects were observed between drought stress and application timing (DT), drought stress and foliar nutrient application (DN), application timing and foliar nutrient application (TN), and all three factors combined (DTN) (Table 2). In comparing the mean of the main effects, drought stress caused a significant decrease in the dry weight of leaves, stems, and seeds of quinoa, with the lowest dry weight of leaves, stems, and seeds being related to drought stress with an average of (14.39 g), (55.47 g), and (54.28 g), respectively, which showed a decrease of (30%), (75.35%), and (80.30%) compared to the control treatment (Table 3). The effect of foliar application at the time of 50% flowering increased the dry weight of leaves, stems, and seeds of quinoa by 17.87%, 19.16%, and 11.96% compared to the time of 100% flowering (Table 2). Also, foliar application with nutrients on quinoa plants caused a significant increase in dry weight of leaves, stems, and seeds of quinoa, so that the highest dry weight of leaves, stems, and seeds in the nutrient treatment were related to the combination of nano-Fe and nano-Zn with an average of (25.83 g), (99.41 g), and (95.09 g), respectively, which increased by (53.54%), (54.55%), and (59.85%) compared to the control treatment (Table 3). Comparison of the mean of the interaction effects of drought stress  $\times$  application time  $\times$  elements (DTN) showed that the highest dry weight of leaves, stems, and seeds with an average of (32.03 g), (136.89 g), and (128.71 g), respectively, was observed in the nutritional treatment of nano-Fe and

nano-Zn at the stage of 50% flowering under non-water stress conditions, and other nutritional treatments had a significant decrease in the time of foliar application of 100 flowering and drought stress conditions.

A reduction in percent ground cover (PGC) due to drought stress can be attributed to the competition of plants for water and nutrients. Water stress has the greatest impact on biomass during the vegetative stages (Ghassemi-Golezani *et al.*, 2009). The reduction of PGC is probably due to reduced cell growth and increased leaf age, which are caused by reduced turgor pressure under water deficit conditions. In addition, it has been stated that the effect of reduced irrigation on the PGC of Ajowan may be related to the lower available moisture around the roots, which results in lower nutrient and water absorption and leads to lower biomass production (Singh *et al.*, 1997). When the plant is exposed to drought and salinity, the growth rate of developing leaves is reduced, and the appearance of new leaves is reduced. Also, if the stress continues, transpiration, photosynthesis, and stomatal conductance are also reduced in the leaves, and few tillers and branches are formed. Reduction of biological yield due to stress varies in different cultivars, and resistant cultivars have less weight loss than sensitive cultivars (Kafi *et al.*, 2012).

### 3.6. Plant fresh and dry weights

Drought stress, foliar nutrient application, and application timing significantly affected ( $p < 0.01$ ) both fresh and dry weight of quinoa plants. Interactions between these factors also played a crucial role (Table 2). The lowest weights were observed under drought stress conditions, averaging 29.565 g (fresh) and 11.266 g (dry), respectively. These values represent decreases of 39.28% and 88.26% compared to the

control treatment (Table 3). The effect of foliar application at the time of 50% flowering increased the fresh and dry weight of the quinoa plant by 15.10% and 13.86%, respectively, compared to the time of 100% flowering (Table 3). Also, foliar application with nutrients on quinoa plants caused a significant increase in the wet and dry weight of the plant, so that the highest wet and dry weight of the plant in the nutrient treatment was related to the combination of nano-Fe and nano-Zn with an average of 83.956g and 23.437g, respectively, which increased by 42.68% and 41.26% compared to the control treatment (Table 3; Fig. 2 and 3).

We compared the average effects of drought stress, application time, and elements (DTN). The plant with the highest wet and dry weight, on average 33.1204 g and 57.536 g, was given nano-Fe and nano-Zn nutrients when it was 50% flowering and not under drought stress. Other nutritional treatments had a significant decrease in the time of foliar application when the plant was 100% flowering and under drought stress. Drought stress in plants led to reduced vegetative growth, plant height, number of branches, and leaf area, especially at the upper leaf levels. Consequently, the plant's photosynthetic capacity and dry matter accumulation rate decreased. The reduction in plant dry matter, caused by soil water deficit, was due to reduced interception of incident radiation, decreased photosynthetic efficiency, or a combination of both (Li *et al.*, 2025). The results of Khalili Mahalleh and Roshdi (2008) showed that foliar application of zinc increased total dry matter, likely due to its influence on leaf chlorophyll content and indole-3-acetic acid (IAA) levels. The increased levels of chlorophyll a and b led to higher rates of photosynthesis, resulting in greater dry matter production and yield (Hemantaranjan and Garg, 1988).

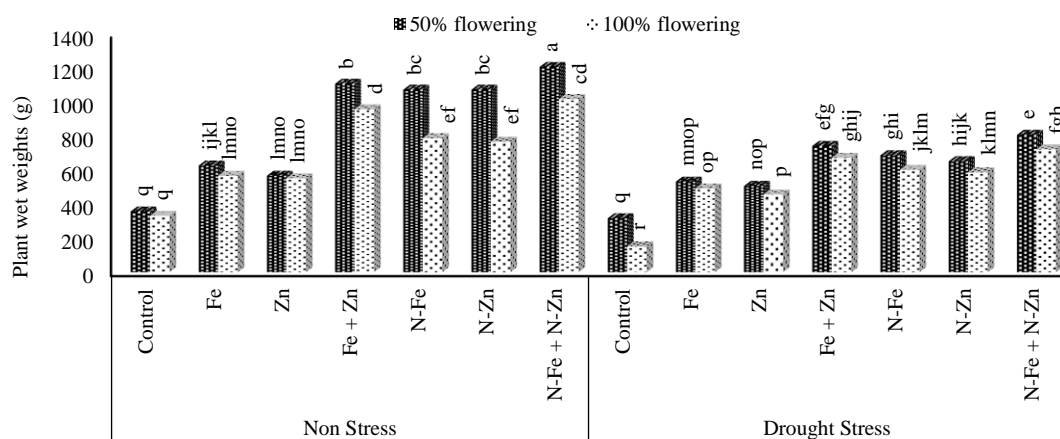


Figure 2. Effect of foliar application of micronutrients on plant wet weight of quinoa under drought stress conditions



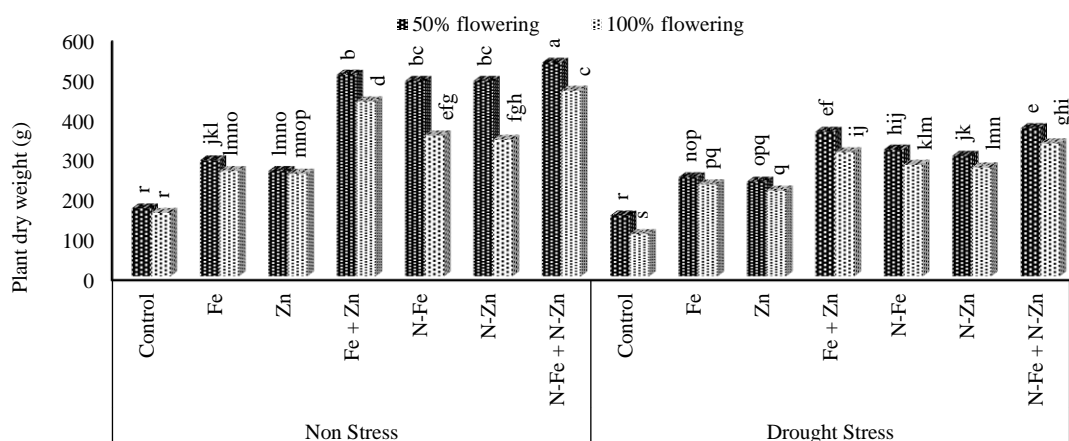


Figure 3. Effect of foliar application of micronutrients on plant dry weight of quinoa under drought stress conditions

### 3.7. Protein and proline

The analysis of variance revealed that drought stress, foliar fertilization, application timing, and their interactions significantly influenced ( $p \leq 0.01$ ) protein content. While drought stress and foliar fertilization, as well as drought stress, application timing and nutrients, showed strong interactions, the interaction between application timing and foliar fertilization was significant at a slightly lower level ( $p \leq 0.05$ ) (Table 4).

In the case of proline content, the results of the analysis of variance showed that the effects of drought stress, foliar fertilization, application time, and the interaction effects of drought stress  $\times$  application time (DT), drought stress  $\times$  foliar fertilization (DN), application time  $\times$  foliar fertilization (TN), and also the interaction effect of drought stress  $\times$  application time  $\times$  nutrients (DTN) were significant at the 1% probability level (Table 4).

Table 4. Analysis of variance for the effect of foliar application of micronutrient at different times on biochemical and physiological characteristics of quinoa under drought stress conditions

Mean Square (MS)														
S.O.V	df	Protein	Proline	Soluble carbohydrates	Malondialdehyde	Relative water content	Chla	Chlb	Total Chl	carotenoid	Catalase	Peroxidase	Superoxide dismutase	Ascorbate peroxidase
Drought stress (D)	1	39.61**	140.91**	9793.74**	28.13**	2048.32**	317.89**	1148.85**	2674.15**	5166926.26**	3.71**	68.97**	265.68**	2250.16**
Spraying time (S)	1	10.46**	52.33**	2778.34**	10.21**	566.17**	85.87**	569.24**	1096.36**	2071813.02**	0.89**	12.3**	63.32**	412.36**
Nutrient (N)	6	25.87**	81.1**	5383.03**	20.76**	1983.9**	262.28**	670.92**	1711.45**	2796674.21**	2.19**	58.24**	153.81**	1886.59**
D × S	1	0.3 <sup>ns</sup>	16.99**	490.35**	0.02 <sup>ns</sup>	1.03 <sup>ns</sup>	0.96 <sup>ns</sup>	214.05**	243.34**	665960.43**	0.29**	0.00 <sup>ns</sup>	75.2**	0.015 <sup>ns</sup>
D × N	6	0.59**	6.91**	405.85**	0.45**	51.18**	35.52**	137.56**	172.86**	427167.18**	0.09**	4.44**	133.1**	143.17**
S × N	6	0.36*	4.61**	131.41**	0.43**	6.31 <sup>ns</sup>	2.28 <sup>ns</sup>	138.15**	171.38**	449344.95**	0.2**	1.04**	45.9**	37.05**
D × S × N	6	0.5**	3.92**	165.31**	0.83**	13.43**	3.42 <sup>ns</sup>	127.63**	168.01**	253175.71**	0.1**	0.6**	20.2**	22.19**
Error	56	0.16	0.00	15.82	0.06	4.18	2.07	3.69	11.17	44935.34	0.02	0.07	1.43	3.86
C.V. (%)	-	3.91	2.27	6.82	5.93	3.11	4.51	9.26	6.34	8.69	11.84	4.63	12.85	6.11

ns, \* and \*\*: non-significant, significant at 5% and 1%, respectively. df: degrees of freedom

It has been reported that protein synthesis disorders, protein denaturation, and protein degradation are some of the major destructive effects of drought stress in plants (Horváth et al., 2007). The accumulation of proline in the tissues of stressed plants is due to increased synthesis of proline by pyrroline-5-carboxylate synthase and decreased degradation by proline oxidase (Chaves et al., 2002). Iron and zinc, as cofactors, play an important role in the activity of leaf enzymes, especially under stress conditions, so they have a strong

effect on the activity of the enzymes mentioned (Bağcı et al., 2007). Dehghanian et al. (2024) reported that drought stress on quinoa plants led to increased proline. Other researchers (Lum et al., 2014) have also reported increased proline levels with increasing stress. The mean comparison of the main effect of drought stress showed that drought stress caused an increase of 70.12% and 95.25% in protein and proline content, respectively, compared to the no-drought stress treatment (Table 5).

**Table 5. Mean comparison of the effects of foliar application of micronutrients at different times on biochemical and physiological characteristics of quinoa under drought stress conditions**

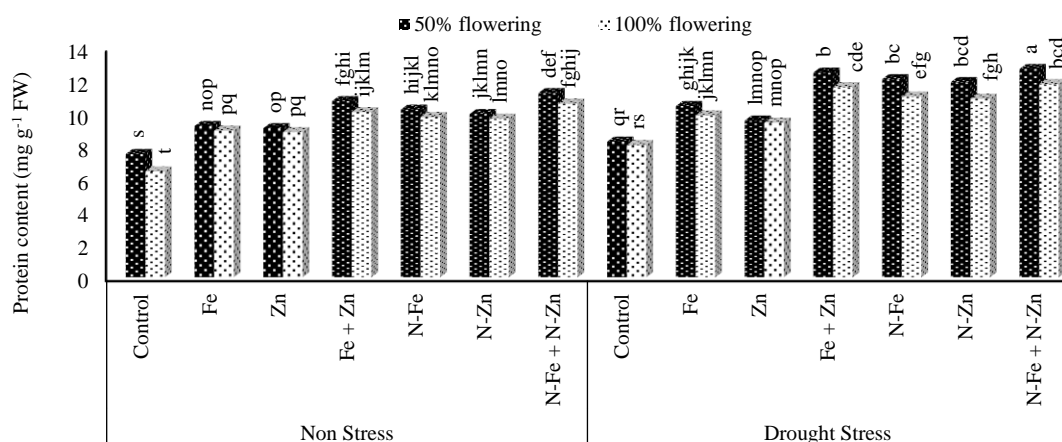
Treatment	Protein (mg g <sup>-1</sup> FW)	Proline (μmol g <sup>-1</sup> F)	Soluble carbohydrates (mg g <sup>-1</sup> Fw)	Malondialdehyde (μmol g <sup>-1</sup> FW)	Relative water content (%)	Chla (mg g <sup>-1</sup> FW)	Chlb (mg g <sup>-1</sup> FW)	Total Chl (mg g <sup>-1</sup> FW)	Carotenoid (mg g <sup>-1</sup> FW)	Catalase (U g <sup>-1</sup> FW)	Peroxidase (U g <sup>-1</sup> FW)	Superoxide dismutase (U g <sup>-1</sup> FW)	Ascorbate peroxidase (U g <sup>-1</sup> FW)
Drought stress													
Control	9.42 <sup>b</sup>	23.31 <sup>b</sup>	15.16 <sup>b</sup>	0.11 <sup>a</sup>	70.67 <sup>a</sup>	33.9 <sup>a</sup>	24.45 <sup>a</sup>	58.33 <sup>a</sup>	2686.29 <sup>a</sup>	1.04 <sup>b</sup>	4.76 <sup>b</sup>	7.52 <sup>b</sup>	2.7 <sup>b</sup>
Drought stress	10.79 <sup>a</sup>	30.13 <sup>a</sup>	21.36 <sup>a</sup>	0.15 <sup>a</sup>	60.8 <sup>b</sup>	30.01 <sup>b</sup>	17.05 <sup>b</sup>	47.05 <sup>b</sup>	2190.26 <sup>b</sup>	1.46 <sup>a</sup>	6.58 <sup>a</sup>	11.07 <sup>a</sup>	3.72 <sup>a</sup>
Foliar spraying time													
50% flowering	10.46 <sup>a</sup>	28.77 <sup>a</sup>	20.2 <sup>a</sup>	0.12 <sup>b</sup>	68.33 <sup>a</sup>	32.96 <sup>a</sup>	23.36 <sup>a</sup>	56.3 <sup>a</sup>	2595.32 <sup>a</sup>	1.35 <sup>a</sup>	6.05 <sup>a</sup>	10.16 <sup>a</sup>	3.42 <sup>a</sup>
100% flowering	9.75 <sup>b</sup>	23.27 <sup>b</sup>	16.62 <sup>b</sup>	0.14 <sup>a</sup>	63.14 <sup>b</sup>	30.94 <sup>b</sup>	18.15 <sup>b</sup>	49.08 <sup>b</sup>	2281.23 <sup>b</sup>	1.15 <sup>b</sup>	5.29 <sup>b</sup>	8.43 <sup>b</sup>	2.99 <sup>b</sup>
Micronutrients													
Control	7.45 <sup>f</sup>	18.79 <sup>g</sup>	11.26 <sup>f</sup>	0.18 <sup>a</sup>	44.04 <sup>g</sup>	23.65 <sup>f</sup>	14.08 <sup>f</sup>	37.72 <sup>f</sup>	1826.76 <sup>d</sup>	0.88 <sup>f</sup>	4.59 <sup>f</sup>	6.83 <sup>f</sup>	2.34 <sup>f</sup>
Fe	9.61 <sup>d</sup>	22.39 <sup>e</sup>	15.55 <sup>d</sup>	0.14 <sup>c</sup>	61.59 <sup>e</sup>	30.58 <sup>d</sup>	18.99 <sup>d</sup>	48.56 <sup>d</sup>	2204.72 <sup>c</sup>	1.25 <sup>d</sup>	5.96 <sup>d</sup>	9.58 <sup>d</sup>	2.95 <sup>d</sup>
Zn	9.23 <sup>e</sup>	20.15 <sup>f</sup>	14.11 <sup>e</sup>	0.15 <sup>b</sup>	54.62 <sup>f</sup>	28.62 <sup>e</sup>	16.65 <sup>e</sup>	44.26 <sup>e</sup>	2108.04 <sup>c</sup>	1.19 <sup>e</sup>	5.6 <sup>e</sup>	8.59 <sup>e</sup>	2.74 <sup>e</sup>
Fe + Zn	11.22 <sup>b</sup>	32.31 <sup>b</sup>	23.75 <sup>b</sup>	0.1 <sup>c</sup>	76.4 <sup>b</sup>	35.26 <sup>b</sup>	24.86 <sup>b</sup>	60.09 <sup>b</sup>	2685 <sup>b</sup>	1.58 <sup>b</sup>	7.42 <sup>b</sup>	11.98 <sup>b</sup>	4.2 <sup>b</sup>
Nano-Fe	10.77 <sup>c</sup>	29.88 <sup>c</sup>	21.37 <sup>c</sup>	0.11 <sup>d</sup>	73.09 <sup>c</sup>	34.37 <sup>bc</sup>	22.72 <sup>c</sup>	57.07 <sup>c</sup>	2578.8 <sup>b</sup>	1.46 <sup>bc</sup>	6.52 <sup>c</sup>	10.85 <sup>c</sup>	3.69 <sup>c</sup>
Nano-Zn	10.6 <sup>c</sup>	28.74 <sup>d</sup>	20.74 <sup>c</sup>	0.12 <sup>d</sup>	71.15 <sup>d</sup>	33.88 <sup>c</sup>	21.2 <sup>c</sup>	55.07 <sup>c</sup>	2526.17 <sup>b</sup>	1.4 <sup>c</sup>	6.37 <sup>c</sup>	10.24 <sup>c</sup>	3.61 <sup>c</sup>
Nano-Fe + Nano-Zn	11.83 <sup>a</sup>	37.62 <sup>a</sup>	26.73 <sup>a</sup>	0.09 <sup>f</sup>	79.28 <sup>a</sup>	37.31 <sup>a</sup>	27.77 <sup>a</sup>	64.07 <sup>a</sup>	2938.35 <sup>a</sup>	1.71 <sup>a</sup>	8.02 <sup>a</sup>	12.49 <sup>a</sup>	4.51 <sup>a</sup>

In each column, means having at least one same letter, are not significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

Previous studies on changes in the concentration of proline and betaine in the leaves of plants under drought stress show that sugar beet plants use these compounds to reduce drought stress. Both proline and betaine concentrations increased due to the increased drought. Proline concentration responded quickly and strongly to drought stress and increased significantly by 24% after the first day without irrigation, while plants did not show any visible signs of drought stress. After 6 days without irrigation, when plants were under severe drought stress, proline concentration increased more than 4 times compared to the control (Fugate et al., 2018).

The mean comparison of the interactive effects of drought stress  $\times$  foliar fertilization  $\times$  nutrients (DTN)

showed that the highest protein and proline contents were observed in the nano-Fe and nano-Zn foliar fertilization treatments at the 50% flowering stage under drought stress conditions, with average values of 70.12 mg g<sup>-1</sup> and 30.43 mg g<sup>-1</sup>, respectively. Other foliar fertilization treatments showed a significant decrease compared to the 100% flowering time treatment and the non-drought stress conditions (Fig. 4 and 5). The results of Abbasi et al. (2019) indicate increased proline synthesis with the application of zinc. The results of a study showed that the consumption of micronutrients (iron, zinc, and manganese) and potassium under drought stress conditions increased the amount of proline and protein percentage (Majlesy and Gholinezhad, 2014).

**Figure 4. Effect of foliar application of micronutrients on protein content of quinoa under drought stress conditions**

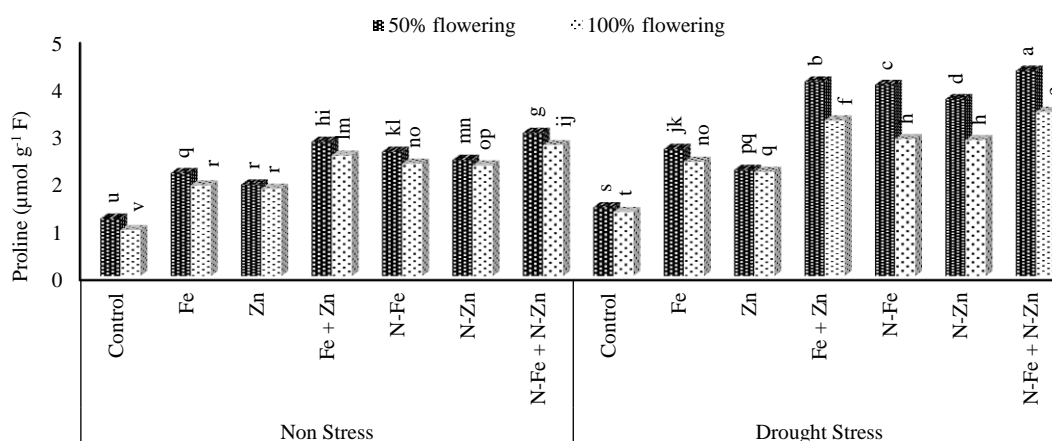


Figure 5. Effect of foliar application of micronutrients on proline content of quinoa under drought stress conditions

### 3.8. Soluble carbohydrates

The results of the analysis of variance showed that the effects of drought stress, foliar fertilization, and application time, as well as the interaction effects of drought stress  $\times$  application time (DT), drought  $\times$  foliar fertilization (DN), application time  $\times$  foliar fertilization (TN), and also the interaction effect of drought stress  $\times$  application time  $\times$  foliar fertilization (DTN) on soluble carbohydrates, were significant at the 1% probability level (Table 4).

The comparison of the mean effect of the main drought stress showed that drought stress caused an increase in soluble carbohydrates by 29.03% (Table 5). This result is completely consistent with the result of Lin and Chao (2021). It is worth mentioning that soluble carbohydrates increased with drought stress, which is consistent with the results of Dehghanian et al. (2024) on quinoa, Abedi Baba-Arabi et al. (2011) on safflower, and Rezayi Far et al. (2018) on wheat. The

effect of foliar application at the 50% flowering stage on soluble carbohydrates increased by 17.72% compared to the 100% flowering stage. The application of nutrients also improved the content of soluble carbohydrates, so that the highest content of soluble carbohydrates in the nutrient treatment was related to the combination of nano-Fe and nano-Zn treatment, with an average of 26.73 mg g<sup>-1</sup>, which increased by 57.87% compared to the control treatment (Table 4). The comparison of the mean effects of the interaction of drought  $\times$  time of foliar application  $\times$  nutrients (DTN) showed that the highest amount of soluble carbohydrates with an average of (32.61 mg g<sup>-1</sup>) was observed in the nano-Fe and nano-Zn nutritional treatments at the 50% flowering stage under drought stress conditions, and other nutritional treatments showed a significant decrease compared to the time of foliar application at 100 flowering and conditions without drought stress (Fig. 6).

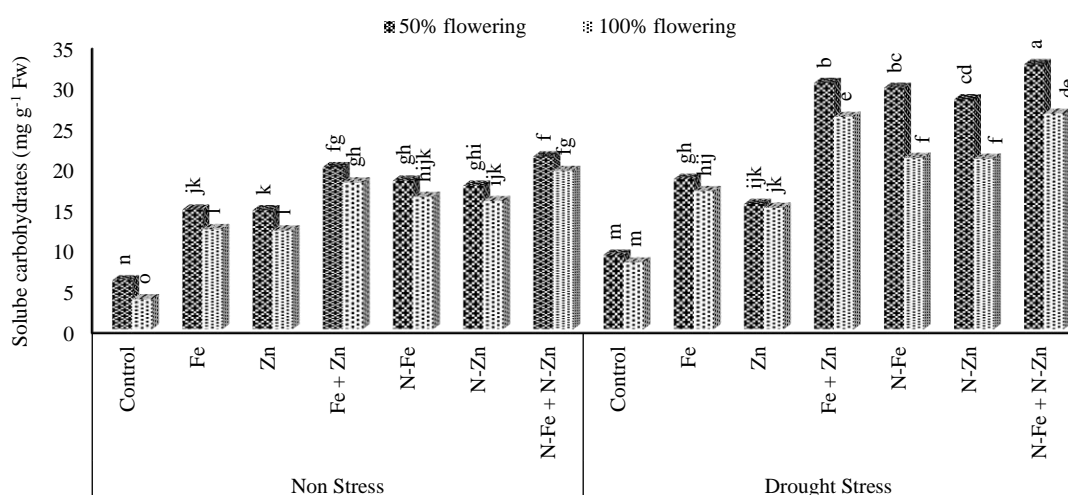


Figure 6. Effect of foliar application of micronutrients on soluble carbohydrates of quinoa under drought stress conditions

It reported that the foliar application treatment (iron, zinc, and manganese) on sunflower had the greatest effect on the synthesis and accumulation of carbohydrates. They also attributed the effect of zinc and iron to the role of these elements in the synthesis of carbohydrates and proteins (Crista et al., 2023). The presence of zinc affects the balance between starch production and protein and increases the amount of soluble sugars. Iron leads to increased photosynthetic activity. In fact, in chloroplasts with iron deficiency, the rate of photosynthetic carbon dioxide absorption decreases due to a decrease in photochemical capacity. Reduction of chlorophyll and damage to photosynthetic electron transport leads to a decrease in sugars and a decrease in growth (Marschner, 2011). The results of this study are consistent with the results of Amirinejad et al. (2015) on cumin.

### 3.9. Malondialdehyde (MDA)

The results of the analysis of variance showed that the effects of drought stress, foliar fertilization, application time, and the interaction effects of drought  $\times$  application time (DT), drought  $\times$  foliar fertilization (DN), application time  $\times$  foliar fertilization (TN), and the interaction effect of drought  $\times$  application time  $\times$  foliar fertilization (DTN) on the trait of malondialdehyde were significant at the 1% probability level (Table 4).

The effect of foliar application at the 50% flowering stage reduced the concentration of MDA by 14.29% compared to foliar application at the 100% flowering

stage. The application of nutrients also reduced the concentration of MDA and increased the leaf membrane stability index, so that the lowest concentration of MDA was obtained in the nutrient treatment related to the combination of nano-Fe and nano-Zn, with an average of  $0.09 \mu\text{mol g}^{-1}$ , which decreased by 49.99% compared to the control treatment. Nutritional treatments reduced MDA in both irrigated and drought-stressed plants (Table 5). When the average effects of drought, application time, and nutrients (DTN) were compared, it was found that the nutritional treatment of nano-Fe and nano-Zn had the lowest lipid peroxidation ( $0.065 \mu\text{mol g}^{-1}$ ) at the 50% flowering stage when there was no drought stress. Other nutritional treatments had a significant decrease at this stage when there was no drought stress (Fig. 7).

In this experiment, foliar application of zinc and iron in both stress conditions led to a significant decrease in malondialdehyde. So, it looks like applying zinc and iron to the leaves can help start the antioxidant system and get rid of free radicals or antioxidant enzymes. This lowers the damage done by reactive species, which in turn lowers membrane lipid peroxidation and the malondialdehyde that is made when membrane lipid peroxidation happens. Therefore, it seems that foliar application of zinc and iron by scavenging free radicals prevents the oxidation of fats, especially under drought stress conditions, and prevents the increase in malondialdehyde (Fathi et al., 2017). The results of this study are consistent with the results of Amirinejad et al. (2015) and Adib et al. (2020).

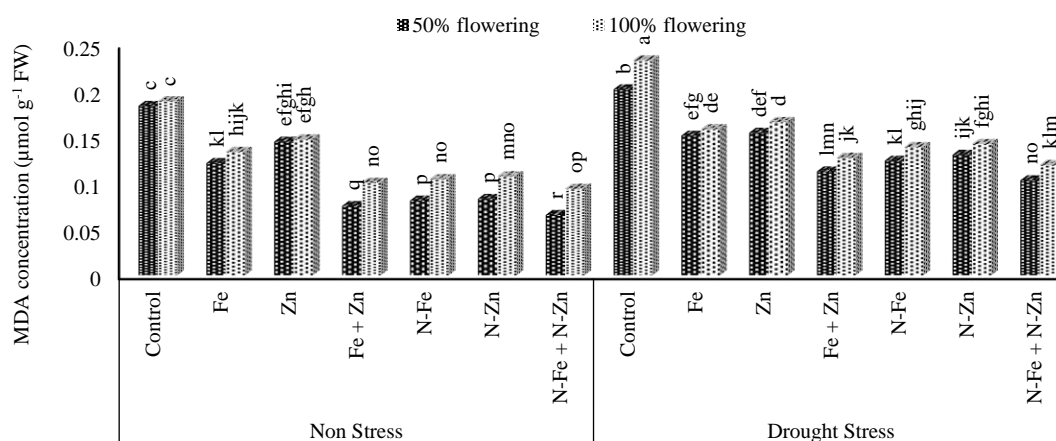


Figure 7. Effect of foliar application of micronutrients on MDA concentration of quinoa under drought stress conditions

### 3.10. Relative water content (RWC)

The results of the analysis of variance showed that the effects of drought stress, foliar fertilization,

application time, and the interaction effects of drought  $\times$  foliar fertilization (DN) and drought  $\times$  time  $\times$  foliar fertilization  $\times$  nutrients (DTN) on relative water



content were significant at the 1% probability level (Table 4). Relative water content was significantly affected by drought stress, and RWC decreased with increasing stress, so a decrease of 13.97% was observed in drought stress compared to the control treatment (Table 5). Relative water content is a complete indicator of plant water status that is used to evaluate osmotic stress tolerance (Ahmad and Satyawati, 2008).

The effect of foliar application at the 50% flowering stage increased relative water content by 7.60% compared to foliar application at the 100% flowering stage (Table 4). Also, the treatment with nutrients increased relative water content, so that the highest

relative water content was obtained in the nutrient treatments related to the nano-Fe and nano-Zn combination, with an average of 79.28 percent, which increased compared to the control treatment (44.45%). Nutritional treatments increased relative water content in both conditions of irrigated plants and under drought stress (Table 5). The average effects of drought, time, foliar fertilization, and nutrients (DTN) were compared. The nutritional treatment of nano-Fe and nano-Zn at the 50% flowering stage under non-drought stress conditions resulted in the highest relative water content, measuring 85.84%. Other nutritional treatments showed a significant increase compared to drought stress conditions (Fig. 8).

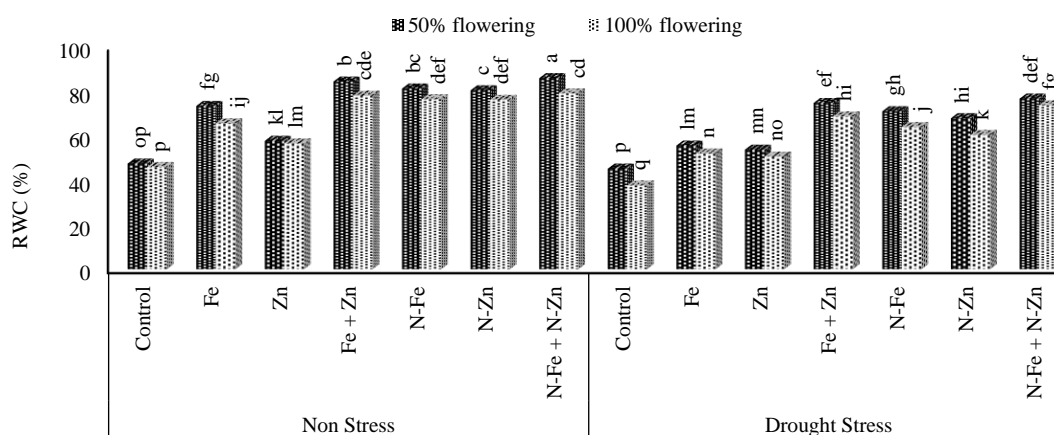


Figure 8. Effect of foliar application of micronutrients on relative water content (RWC) of quinoa under drought stress conditions

The decrease in leaf water content of plants under drought stress may be related to the decrease in plant force (Liu et al., 2004). Leaf relative water content decreases during the water deficit period, and this is due to the decrease in leaf water potential during drought stress. Most of the research conducted under drought stress conditions confirms that with the interruption of irrigation at different growth stages, the relative content of the plant decreases (Adamu and Baburai Nagesh, 2014). In line with the results of this study, the application of iron and zinc in drought-stressed fields had a significant effect on physiological characteristics such as relative humidity.

### 3.11. Photosynthetic pigments

It was found that drought stress, foliar fertilization, application time, and the interaction effects of drought  $\times$  application time (DT), drought  $\times$  foliar fertilization (DN), time  $\times$  foliar fertilization (TN), and drought  $\times$

time  $\times$  foliar fertilization  $\times$  nutrients (DTN) had a 1% chance of being significant on the amounts of chlorophyll a, b, total chlorophyll, and carotenoids (Table 4). For example, drought stress greatly decreased the amounts of chlorophyll a, b, total chlorophyll, and carotenoids, causing drops of 11.47 percent, 30.27 percent, 19.33 percent, and 18.47 percent, respectively, compared to the control treatment (Table 5).

Chlorophyll content is widely used as an index to show the level of abiotic stress tolerance in plants. Protection of chloroplasts and photosynthetic machinery, including chlorophyll content, is the first target of defense under stress conditions (Anjum et al., 2011). In this study, under drought stress, the levels of chlorophyll a, b, total chlorophyll, and carotenoids were significantly reduced. Chlorophyll reduction can be due to a decrease in chlorophyll synthesis as well as to its destruction. When reactive oxygen species and

the enzyme chlorophyllase work together, they separate the phytol chain from the porphyrin ring, which breaks down chlorophyll molecules (Ahmad and Satyawati, 2008).

The effect of foliar application at the 50% flowering stage increased the content of chlorophyll a, b, total chlorophyll, and carotenoids by (6.13%), (22.30%), (12.82%), and (12.10%), respectively, compared to foliar application at the 100% flowering stage (Table 5). Also, the treatment with nutrients increased the photosynthetic pigments, so that the highest content of chlorophyll a, b, total chlorophyll, and carotenoids in the nutrient treatments was obtained in the combination treatment of nano-Fe and nano-Zn, respectively, with an average of (37.31), (27.77), (64.07), and (2938.35) mg g<sup>-1</sup>, which increased by (36.61%), (49.30%), (41.70%), and (37.83%), respectively, compared to the control treatment. Nutritional treatments increased the content of photosynthetic pigments in both conditions of irrigated plants and under drought stress (Table 5).

A comparison of the mean effect of the interaction of drought × foliar fertilization (DN) on the content of chlorophyll a showed that the highest content of chlorophyll a, with an average of 39.85 mg g<sup>-1</sup>, was related to the nutritional treatment of nano-Fe and nano-Zn under non-drought stress conditions. A comparison of the average effects of drought, time, and foliar fertilization with nutrients (DTN) showed that the nutritional treatment of nano-Fe and nano-Zn at the 50% flowering stage under non-drought stress conditions had the highest levels of chlorophyll b, total chlorophyll, and carotenoids, with averages of (30.45), (74.25), and (3444.20) mg g<sup>-1</sup>.

Naderi Arefi (2020) studied the effect of drought stress on different cotton varieties and reported that drought reduced carotenoid levels in some varieties but increased them in others. In this experiment, iron deficiency led to a decrease in chlorophyll. In interpretation, it can be stated that iron is a metabolic component of the enzyme protochlorophyllide oxidoreductase. This enzyme is involved in the biosynthesis of alpha-aminolevulinic acid (ALA), which is a precursor of chlorophyll (Marschner, 2011). As a result, alpha-aminolevulinic acid and inhibition of protochlorophyllide reductase formation are likely mediators of chlorophyll biosynthesis inhibition, which is the cause of the decrease in chlorophyll storage in leaves (Wang et al., 2021). Zinc can also be effective

in plant tolerance to stress by inducing growth changes, affecting the synthesis of chlorophyll precursors, increasing metabolism, cell division, fertilization, sexual fertility, etc., and even more than most elements, it is involved in tolerance to drought stress (Ahmed et al., 2009). In a study, foliar application of iron and zinc increased the net assimilation rate and chlorophyll and carotenoid levels in the leaves (Grant et al., 1989).

### 3.12. Enzymes

A study of differences found that for all antioxidant enzymes, the effects of drought stress, foliar fertilization, and application time were important. The effects of drought on application time (DT), drought on foliar fertilization (DN), time on foliar fertilization (TN), and drought on application time on nutrients in foliar fertilization (DTN) were all significant at the 1% chance level for all antioxidant enzymes (Table 4). Drought stress significantly increased the activity of all antioxidant enzymes, so that the effect of drought stress on the activity of catalase, peroxidase, superoxide dismutase, and ascorbate peroxidase increased by (28.77%), (27.66%), (32.07%), and (27.42%), respectively, compared to the non-drought stress treatment (Table 5). The effect of foliar application at the 50% flowering stage increased the activity of catalase, peroxidase, superoxide dismutase, and ascorbate peroxidase by 14.81%, 12.56%, 17.03%, and 12.57%, respectively, compared to foliar application at the 100% flowering stage (Table 5). Foliar fertilization also increased the activity of all antioxidant enzymes, so that the highest activity of catalase, peroxidase, superoxide dismutase, and ascorbate peroxidase in the foliar fertilization treatments was related to the combination treatment of nano-Fe and nano-Zn, respectively, with an average of 1.71 units per gram of wet weight, 0.82 units per gram of wet weight, 1.249 units per gram of wet weight, and 4.51 units per gram of wet weight. These values were increased by 48.54%, 42.77%, 45.75%, and 48.12%, respectively, compared to the control treatment. Nutritional treatments increased the activity of all antioxidant enzymes in both conditions of irrigated plants and under drought stress (Table 5).

A study that looked at the average effects of drought, foliar application time, foliar fertilization, and nutrients (DTN) showed that the nano-Fe and nano-Zn nutritional treatment had the highest levels of catalase,

peroxidase, superoxide dismutase, and ascorbate peroxidase activity at the 50% flowering stage under drought stress. These levels were (2.16 units per gram of wet weight), (0.99 units per gram of wet weight), and

(1.607 units per gram of wet weight). Other nutritional treatments showed a significant increase in stress conditions compared to the non-drought stress conditions (Fig. 9-12).

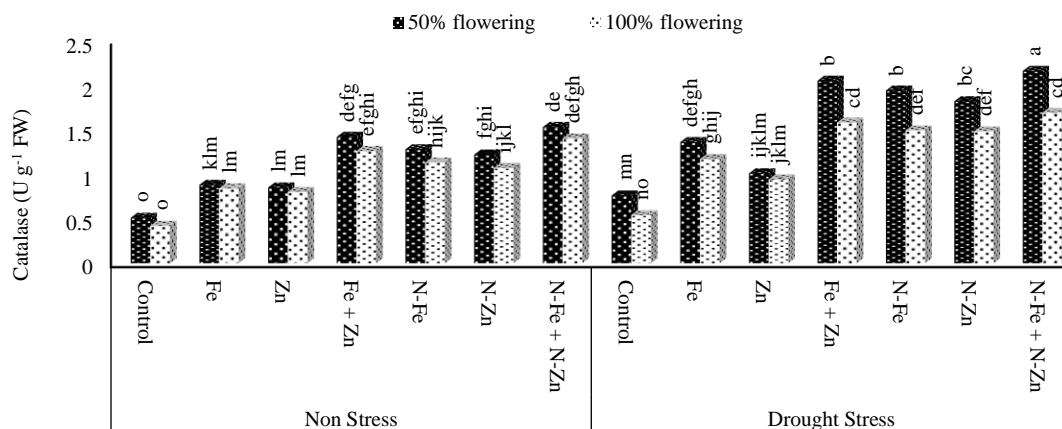


Figure 9. Effect of foliar application of micronutrients on catalase enzyme activity of quinoa under drought stress conditions

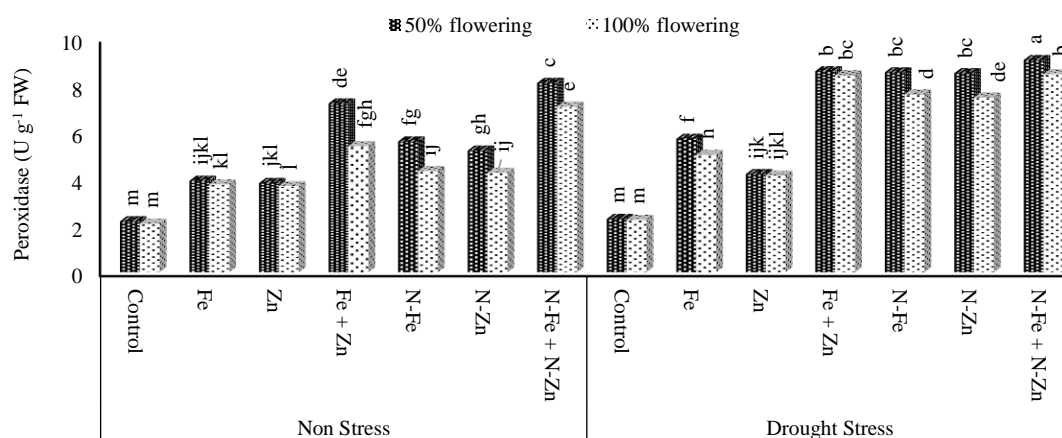


Figure 10. Effect of foliar application of micronutrients on peroxidase enzyme activity of quinoa under drought stress conditions

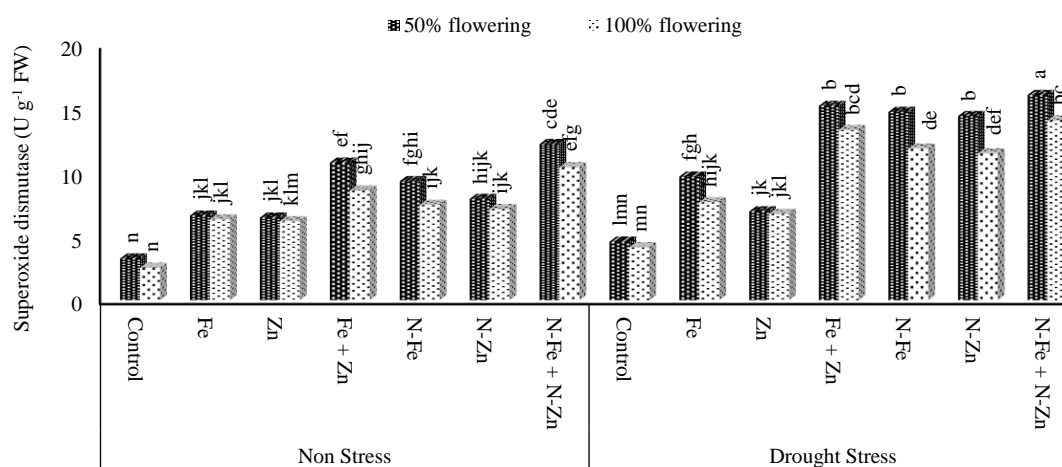


Figure 11. Effect of foliar application of micronutrients on superoxide dismutase enzyme activity of quinoa under drought stress conditions

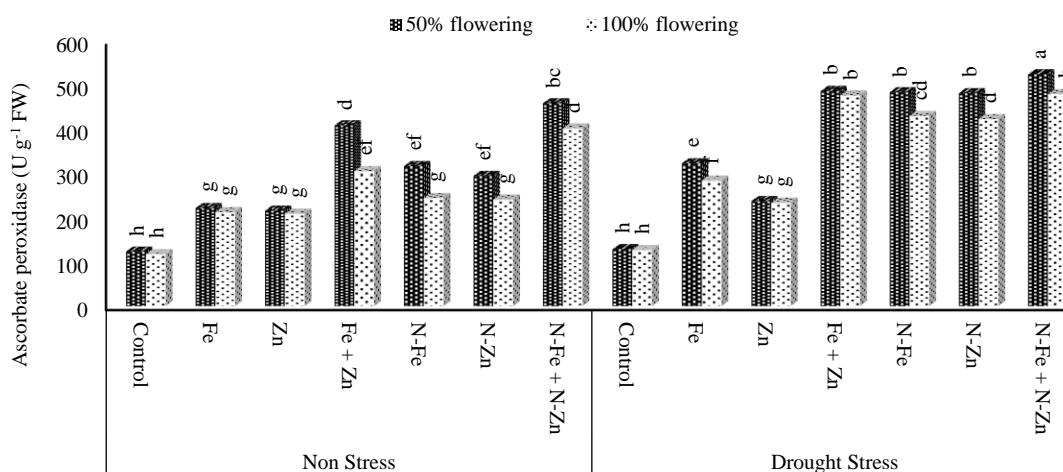


Figure 12. Effect of foliar application of micronutrients on ascorbate peroxidase enzyme activity of quinoa under drought stress conditions

Drought stress in the plant *Artemisia siberia* increased the activity of the enzymes ascorbate peroxidase, peroxidase, superoxide dismutase, and catalase (Abbasi *et al.*, 2019). Experimental results showed that the application of iron and zinc fertilizers in stressful conditions increased the activity of the enzyme catalase. This can be described as follows: since iron is an important factor in the structure of enzymes, the application of combined fertilizer provides the plant with the necessary iron and enables the plant to have the ability to produce the optimal amount of catalase enzyme in coping with free radicals under stress conditions. The results of a study showed that the highest activity of the enzyme catalase in the plant *Cuminum cyminum* was obtained from the treatment of severe drought stress with a combined foliar application of iron and zinc (Amirinejad *et al.*, 2015). The reason for this is that the element zinc is effective in the expression of genes that synthesize proteins and antioxidant enzymes and is also considered a cofactor for increasing the activity of this enzyme (Grewal and Williams, 2000). The role of the element zinc in the activity of many enzymes is well known. The increase in peroxidase enzyme activity with the use of zinc is consistent with the results of the research of Shoja *et al.* (2018).

#### 4. Conclusion

Based on the results of this study, the main effects of drought stress, foliar application, and foliar application time had a significant effect on most morphological and physiological traits of quinoa plants at the one percent level. The results of this experiment showed that

treatment with nutrients (nano-Fe and nano-Zn) under drought stress improved most of the traits under investigation. Among the nutritional treatments, the combination of nano-Fe and nano-Zn showed better results at drought stress levels. Among the levels of drought stress, there were also significant differences in most traits, with the non-drought stress treatment showing better results. Foliar application at the 50% flowering stage improved most of the traits under investigation, which could be due to increased dry matter remobilization.

In general, it can be stated that foliar application with nano-fertilizers improved the morphological and physiological properties of quinoa plants under drought stress in a greenhouse environment. It was found that quinoa uses mechanisms such as the accumulation of compounds, such as proline and soluble sugar and increased antioxidant enzyme activity to overcome stress. Therefore, it seems that in order to move towards sustainable agriculture and reduce the use of chemical fertilizers, improve physiological traits and nutrient uptake, reduce the effects of drought stress, and increase quinoa crop yield, especially under stress, foliar application of nano-fertilizers can be a logical and appropriate measure.

#### Conflict of interests

The authors declare that they have 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

The authors gave consent for the publication of the manuscript.

### Availability of data and material

All data supporting the findings of this study is available from the corresponding author on reasonable request.

### Authors' contributions

Heshmat Omid and Nasim Pakbaz designed experiment, performed it and analysed the data. Nasim Pakbaz and Mohammad Hosein Bijeh Keshavarzi wrote the manuscript with input from all authors. Hasanali Naghdibadi and Amir Bostani conceived the study and were in charge of overall direction and planning.

### Informed consent

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

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