The Effects of Zinc Oxide Nanoparticles on Drought Stress in *Moringa peregrina* Populations

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Abstract

Introduction: *Moringa peregrina* (Forssk.) Fiori, is a nutritionally and medicinally important desert tree, which is constantly exposed to drought stress. This study was accomplished to alleviate the adverse effects of drought stress on *M. peregrina* populations through the foliar application of zinc oxide nanoparticles (ZnO-NPs) by monitoring some physiological and biochemical alterations.

Methods: *Moringa peregrina* seeds were collected from the Southeast of Iran in 2014. Fourteen days after germination, the seedlings were subjected to drought stress by withholding watering until 50% field capacity (FC), followed by spraying 0.1% and 0.05% ZnO-NPs and no spraying (as control).

Results: The changes of sodium (Na), potassium (K), chlorophylls, total phenolic content (TPC) as well as antioxidant activity under drought stress condition varied based on *M. peregrina* populations. Drought stress significantly reduced chlorophylls content, while it enhanced TPC and antioxidant activity. ZnO-NPs treatment significantly inhibited chlorophylls degradation under drought stress conditions. It also enhanced chlorophyll content in well-watered plants. Moreover, it was revealed that TPC and antioxidant activity of *M. peregrina* populations significantly increased in response to foliar application of ZnO-NPs in both drought-stressed and unstressed plants.

Conclusion: Our results suggested that ZnO-NPs spray can augment drought tolerance of different populations of *M. peregrina* subjected to water deficit condition.

Keywords: Chlorophylls, Nutrients uptake, Phenol, Secondary metabolites, ZnO-NPs

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Introduction

Moringa is a small genus from the family of Moringaceae, consisting of 13 species from subtropical to tropical regions and ranging in size from small herbs to large trees. The *Moringa* trees are important food commodities as almost all plant parts such as fruits, flowers, leaves, and immature pods can be consumed as highly nutritive vegetables.¹ *Moringa peregrina* (Forssk.) Fiori, which is commonly known as horseradish tree, has economical and medicinal importance. The pharmacological actions were attributed to the presence of phenolic compounds in its leaf extract. It was revealed that the leaves of *M. peregrina* are rich sources

of vitamins, minerals, proteins, and phenolic compounds. It acts as a good source of natural antioxidants and also plays an important role in preventing and progression of many diseases.² *M. peregrina* can be grown on arid and semi-arid areas with high temperatures and low water availability where cultivation of other agricultural crops is difficult due to drought stress.³

Drought stress is one of the most serious abiotic stresses that limit plant growth. Numerous changes have been observed in plants as a result of water stress such as changes in mineral nutrients balance, bioactive compounds, and antioxidant activity. These changes depend on species,

genotypes, severity, duration, and time course of drought stress.⁴ Nowadays, the cultivation of medicinal plants is done in a drought stress condition to influence the secondary metabolites content such as phenols.⁵

Phenolic compounds as the largest groups of phytochemicals are present in foods. Flavonoids and other phenolic compounds are potent antioxidants and anticarcinogenic agents. Polyphenols exist in many plants and are especially abundant in the *Moringa* tree, whose dried leaves are used as antioxidant.⁶ Drought can also lead to pigment degradation, thus causing irreversible water-deficit damage to the photosynthetic apparatus. One of the indicators of the stress tolerance capacity of plants is chlorophyll stability index.⁷ In addition, it has been demonstrated that antioxidants affect the chlorophyll content of plants as well.⁸

The tolerance of plants to environmental stresses such as water deficit can be fortified by micronutrient fertilizers. Zinc (Zn) is an essential micronutrient required for metabolic activity, water balance, and stomatal regulation in plants. It adjusts various enzymatic functions and is required for biochemical reactions resulting in chlorophyll formation.⁹ Currently, the use of nano-materials such as zinc oxide nanoparticles (ZnO-NPs) has been expanded.¹⁰

It has been demonstrated that the use of micronutrient fertilizers in the form of NPs is an appropriate procedure to release required nutrients gradually and in a controlled way, which is essential to mitigate the problems of fertilizer pollutions. Due to high surface-to-volume ratio, highly active surface, unique size and shape, and physical, chemical, biological, and catalytic properties, NPs can modulate chemical and biological activities of cells.¹¹ It has been reported that ZnO-NPs can produce better results than the conventional ZnO.¹⁰ Additionally, ZnO-NPs possess excellent electrical properties.¹²

However, to the best of our knowledge, there is little or no reliable information about the physiological effects of foliar application of ZnO-NPs on mineral and biochemical content of medicinal plants under well-watered as well as drought stress conditions. Therefore, this study was carried out to evaluate the changes of mineral nutrients, chlorophylls as well as phenolic compounds, and

antioxidant activity of different *M. peregrina* populations under well-watered and drought stress conditions in response to foliar application of ZnO-NPs.

Materials and Methods

Plant Materials and Growth Conditions

The experiment was conducted in a greenhouse at the Faculty of Agriculture, University of Sistan and Baluchestan, Zahedan, Iran (latitude of 29°27'34" N, longitude of 60°51'10" E, 1385 m altitude, mean annual temperature of 18.3°C and rainfall of 72 mm), during the 2014 growing season. Ten populations of *M. peregrina* seeds were collected from different regions of Sistan and Baluchestan province, Southeast of Iran. The seeds were cleaned to remove damaged seeds, stones, leaves, wood, dust, and any other unknown materials. Cleaned seeds were stored in black plastic bags and labeled. The locations of all samples have been shown in Table 1. The regions where the populations were located are typically characterized by an arid and semi-arid climate.

Seeds were surface-sterilized with 0.5% (v/v) sodium hypochlorite and treated with benomyl solution for 30 minutes at 24°C and then washed three times with sterilized deionized water. Twenty seeds were placed in each 9-cm Petri dish on two sheets of filter paper moistened. The seeds were kept in a germination chamber under a photoperiod of 12 hours at 25°C. After germination, the seedlings were kept in a growth chamber for 14 days at 25°C, 70% relative humidity with a 12-hour photoperiod and watered daily. Later, the young plants were sown in plastic pots (30 cm height and 20 cm diameter) filled with steam-sterilized soil containing washed sand (3:1:1, v/v/v), clay horizon (red earth), and organic horizon soil (black soil). They were fully randomized, kept in a greenhouse and watered daily. Young plants, 40 days after germination, were exposed to drought stress and ZnO-NPs treatment.

Treatments

The field capacity (FC) of the medium in the pot was measured prior to each treatment. Three pots were saturated with water, covered with plastic to avoid

Table 1. The Geographical Location of *Moringa peregrina* Populations in Sistan and Baluchestan Province, Iran

Population Number	Positions	Location of Collected	Latitude	Longitude
1	Nikshahr	Konardan	29°16'69" N	21°79'56" E
2	Nikshahr	Keshik	29°13'84" N	23°27'94" E
3	Nikshahr	Shegim	29°29'04" N	21°87'17" E
4	Nikshahr	Desk	29°17'75" N	21°92'40" E
5	Nikshahr	Nesfuran	29°18'55" N	78°40'95" E
6	Fanuj	Girls Seven	29°19'72" N	75°26'06" E
7	Fanuj	Tange Fanuj Entrance	29°38'89" N	76°27'30" E
8	Fanuj	Tange Fanuj	29°35'68" N	76°29'73" E
9	Fanuj	Madohi village	29°29'45" N	76°26'47" E
10	Fanuj	Dahan village	29°15'45" N	75°39'26" E

evaporation and allowed to drain for 24 hours. The moisture content was determined after soil sampling from each pot. Average moisture content was calculated according to Kramer and Boyer.¹³ The performance of drought stress was evaluated at 40 days after germination. All the pots were maintained at 100% FC (well-watered) and 50% FC (drought stress) during the period of treatment.

At 7 days after drought stress, plants were sprayed with the different concentration of ZnO-NPs [0 (as control), 0.05, and 0.1%]. The second foliar application of ZnO-NPs was carried out one week later. Plants were sprayed until the leaves were completely wet and the solution ran off the leaves. The ZnO-NPs was purchased from Iranian Nanomaterials Pioneers Company, Iran. Plants were harvested at 30 days after ZnO-NPs spraying and were further processed for proximate analysis.

Sodium and Potassium Content

Before the measurement of Na⁺ and K⁺, leaf samples were exactly washed by tap water and then rinsed with deionized water to remove all surface remnants. Fruit flesh samples, after air-drying, were taken from the equatorial section of each fruit quarter, oven-dried at 70°C for 48 hours and milled to pass through a 40-mesh sieve. A portion of fine powder weighing 2 g was dry washed in a furnace at 550°C for 4 hours and then the ash was dissolved in 10 mL of 2 M hydrochloric acid (HCl). Through a Whatman No. 40 filter paper, the digested samples were filtered and used for the analysis of Na⁺ and K⁺. Na⁺ and K⁺ content was determined by flame photometric (Biotech Engineering Management Co. Ltd., UK) method as already described.¹⁴

Chlorophyll Assay

The content of chlorophyll a (Chl-a), chlorophyll b (Chl-b), and total chlorophyll (T-Chl) was determined by UV-visible spectrophotometry (PG Instrument Ltd., Leicester, UK) as described by Rajalakshmi and Banu.¹⁵ Briefly, 10 mL of acetone 80% was added to 0.1 g of homogenized freeze-dried herbage samples. The samples were centrifuged at 6000 g for 10 minutes. The supernatant was filtered through a Whatman No.1 filter paper and the absorbances were read at 645 and 663 nm using UV-visible spectrophotometer. The amounts of Chl-a, Chl-b and T-Chl were calculated according to the following formulas:

$$\text{Chl-a} = [(19.3 \times A_{663} - 0.86 \times A_{645}) \times V] / 100W$$

$$\text{Chl-b} = [(19.3 \times A_{645} - 3.6 \times A_{663}) \times V] / 100W$$

$$\text{T-Chl} = \text{Chl-a} + \text{Chl-b}$$

Where A_{645} and A_{663} are the absorbances at 645 and 663 nm, "V" is sample volume in absorbance, and "W" is the fresh weight (FW) of sample in grams.

Total Phenol Content

The total phenolic content (TPC) was determined using

Folin-Ciocalteu method with minor modifications.¹⁶ TPC extraction was carried out by 10 mL acidic methanol added to 1 g of the leaf powder with the mixture and then filtered through ordinary filter paper. Afterwards, 500 µL of this extract was diluted with 5 mL of Folin-Ciocalteu reagent (1:10 g mL⁻¹), and then 4 mL of Na₂CO₃ (1 M) was added to the mixture. This reaction solution was shaken in a shaker and kept in dark for 15 minutes. The absorbance of samples was taken at 765 nm using UV/Visible spectrophotometer (model PG Instrument +80, Leicester, UK). Gallic acid was used as standard to obtain calibration curve. Data were expressed as milligram gallic acid equivalent (mg GAE) per 1 g of fruit FW.

Antioxidant Activity

The antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method with some modifications.¹⁶ The absorbance of the samples was measured at 515 nm and the antioxidant activity was expressed as the percentage of the decline of the absorbance, relative to the control, corresponding to the percentage of DPPH that was scavenged. The percentage of DPPH, which was scavenged (%DPPHsc), was calculated using:

$$\%DPPHsc = [(A_{\text{cont}} - A_{\text{samp}}) / A_{\text{cont}}] \times 100$$

Where A_{cont} is the absorbance of the control, and A_{samp} is the absorbance of the sample.

Statistical Analysis

The experiments were carried out according to a 3-factor linear model based on a completely randomized design with three replications. Data were statistically analyzed using analysis of variance (ANOVA) by SAS software (version 9.1 2002-2003, SAS Institute, Cary, NC, USA). Before analysis of variance, data were tested for normality and homoscedasticity using the Shapiro-Wilk test. Least significant difference (LSD) test at $P \leq 0.01$ probability level was considered as the statistical significance level.

Results

Minerals Content

The results showed that drought stress reduced Na⁺ and K⁺ content in all *M. peregrina* populations (Table 2). The highest Na and K contents were found at 50% FC in population number 7 (0.89 and 13.69 mg g⁻¹ DW, respectively). Under well-watered conditions, untreated plants (controls) had higher Na content, whereas under drought conditions, ZnO-NPs treatment slightly enhanced Na content compared with control (Figure 1). However, it was revealed that the interaction effects of drought stress and ZnO-NPs treatment on the K content of *M. peregrina* populations were not significant ($P \leq 0.01$) according to the LSD test (Figure 2).

Chlorophylls Content

Table 2. Changes of Some Biochemical Traits of Ten *Moringa peregrina* Populations under Well-Watered (100%FC) and Drought Stress (50%FC) Conditions

Populations	Na (mg g ⁻¹ DW) 100% FC	Na (mg g ⁻¹ DW) 50% FC	K (mg g ⁻¹ DW) 100% FC	K (mg g ⁻¹ DW) 50% FC	Chl-a (mg g ⁻¹ FW) 100% FC	Chl-a (mg g ⁻¹ FW) 50% FC	Chl-b (mg g ⁻¹ FW) 100% FC	Chl-b (mg g ⁻¹ FW) 50% FC	T-Chl (mg g ⁻¹ FW) 100% FC	T-Chl (mg g ⁻¹ FW) 50% FC	TPC (mg g ⁻¹ FW) 100% FC	TPC (mg g ⁻¹ FW) 50% FC	Antioxidant activity (%DPPHsc) 100% FC	Antioxidant activity (%DPPHsc) 50% FC
1	0.75±0.02 ^{Ns}	0.76±0.05	8.27 ±0.2*	7.42±0.2	2.10±0.01 ^{Ns}	1.63±0.01	1.96±0.02 ^{Ns}	1.58±0.11	4.06±0.12*	3.21±0.01	34.5±1.02*	44.7±2.65	34.3±1.05*	52.9±2.01
2	0.63±0.05*	0.52±0.1	9.77 ±0.3 ^{Ns}	9.02±0.3	0.66±0.02 ^{Ns}	0.35±0.02	1.15±0.01*	0.52±0.01	1.81±0.12*	0.87±0.01	41.6±2.03*	54.9±1.13	50.0±2.11**	79.7±2.31
3	0.72±0.11*	0.57±0.13	9.84 ±1.1*	7.87±0.12	2.18±0.01*	1.55±0.01	1.43±0.03*	0.82±0.02	3.61±0.21*	2.37±0.25	24.4±1.05*	35.5±1.06	54.6±1.13*	68.6±1.62
4	0.70±0.06 ^{Ns}	0.72±0.14	8.79 ±1.2 ^{Ns}	8.85±0.03	2.05±0.01*	1.55±0.11	1.23±0.11*	0.53±0.03	3.28±0.11*	2.08±0.31	31.9±1.25*	42.5±1.04	57.2±1.23*	87.5±1.56
5	0.77±0.12*	0.53±0.01	8.97 ±0.2 ^{Ns}	8.85±0.12	2.09±0.02*	1.28±0.02	1.08±0.02*	0.45±0.05	3.17±0.08**	1.73±0.05	43.6±1.05*	54.0±2.03	45.4±0.06*	59.3±1.24
6	0.50±0.01*	0.70±0.01	8.25 ±0.3*	10.52±0.23	1.79±0.03**	0.32±0.002	1.10±0.03*	0.60±0.07	2.89±0.21**	0.92±0.01	40.8±2.12*	52.5±1.01	50.6±1.25*	71.2±2.06
7	0.58±0.03*	0.89±0.16	9.22 ±0.2*	13.69±1.3	1.67±0.11**	0.47±0.01	1.49±0.12*	0.90±0.11	3.16±0.12*	1.37±0.03	29.3±0.65 ^{Ns}	32.8±0.91	43.1±1.41*	59.0±0.95
8	0.70±0.12*	0.57±0.02	10.22±1.3*	8.59±0.14	0.52±0.01 ^{Ns}	0.30±0.01	0.91±0.01 ^{Ns}	0.49±0.06	1.43±0.11*	0.79±0.04	32.0±1.02**	51.8±1.23	56.3±2.11**	95.8±2.48
9	0.67±0.03*	0.60±0.03	10.35±1.2*	12.04±1.1	0.85±0.02*	0.41±0.01	0.96±0.02*	0.44±0.02	1.81±0.07*	0.85±0.03	35.1±1.23*	48.4±1.13	51.0±0.99*	67.7±1.76
10	0.53±0.10 ^{Ns}	0.53±0.05	9.95±0.5*	7.67±0.21	0.87±0.02 ^{Ns}	0.51±0.01	0.97±0.01*	0.40±0.01	1.84±0.03*	0.91±0.02	33.2±1.06	53.7±2.21	46.6±1.05*	59.1±0.85

For each column, means followed with the same letters are not significantly different at $P<0.01$ according to the LSD test. FC: Field capacity, DW: Dry weight, FW: Fresh weight, mg GAE: milligram gallic acid equivalent, DPPHsc: 2, 2-diphenyl-1-picrylhydrazyl which was scavenged.

* Significant difference at $P<0.05$, ** Significant difference at $P<0.01$, Ns: No significant difference.

Figure 1. The Effect of Zinc Oxide Nanoparticles (ZnO-NPs) Treatment on the Na Content of *M. peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) Conditions. The values are shown as the means ($n=3$) $\pm$ standard error. Different letters indicate significant difference ($P \leq 0.01$) according to the LSD test. FC: Field capacity, DW: Dry weight.

Figure 2. The Effect of Zinc Oxide Nanoparticles (ZnO-NPs) Treatment on the K Content of *M. peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) Conditions. The values are shown as the means ($n=3$) $\pm$ standard error. Different letters indicate significant difference ($P \leq 0.01$) according to the LSD test. FC: Field capacity, DW: Dry weight.

Figure 2. The Effect of Zinc Oxide Nanoparticles (ZnO-NPs) Treatment on the K Content of *M. peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) Conditions. The values are shown as the means ($n=3$) $\pm$ standard error. Different letters indicate significant difference ($P \leq 0.01$) according to the LSD test. FC: Field capacity, DW: Dry weight.

Chlorophylls content significantly differed in various *M. peregrina* populations, and population number 1 had the highest T-Chl content ($4.06 \text{ mg g}^{-1} \text{ FW}$) under well-watered condition. In addition, drought stress significantly reduced chlorophyll content, as compared with others. The population number 1 had the highest T-Chl content ($3.21 \text{ mg g}^{-1} \text{ FW}$) in drought stress condition (Table 2). Our results showed that foliar spray of ZnO-NPs not only prevented chlorophylls degradation but also significantly enhanced Chl-a, Chl-b as well as T-Chl content in both drought-stressed and unstressed plants. The 0.10% ZnO-NPs concentration was most effective in enhancing chlorophylls content (Figure 3).

Total Phenol Content

In well-watered plants, TPC varied from 24.4 to 43.6 $\text{mg GAE g}^{-1} \text{ FW}$ depending on *M. peregrina* populations. Furthermore, TPC significantly increased in response to drought stress (Table 2). Under both well-watered and drought conditions, foliar application of ZnO-NPs significantly increased TPC (Figure 4). The highest TPC was obtained in stressed plants that were treated with 0.05% ZnO-NPs ($81.37 \text{ mg GAE g}^{-1} \text{ FW}$).

Antioxidant Activity

In well-watered conditions, population number 4 and 8 had the highest antioxidant activity (57.2 and 56.8 % DPPHsc, respectively). The antioxidant activity of *M. peregrina* populations significantly increased under drought stress, and the highest antioxidant activity was observed in population number 8 and 4 (95.8 and 87.5 % DPPHsc, respectively). Similar pattern was found in both drought-stressed and unstressed plants in response

Figure 4. The Effect of Zinc Oxide Nanoparticles (ZnO-NPs) Treatment on the Total Phenol Content (TPC) of *M. peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) Conditions. The values are shown as the means ($n=3$) $\pm$ standard error. Different letters indicate significant difference ($P \leq 0.01$) according to the LSD test. FC: Field capacity, mg GAE: milligram gallic acid equivalent.

Figure 5. The Effect of Zinc Oxide Nanoparticles (ZnO-NPs) Treatment on the Antioxidant Activity of *M. peregrina* Under Well-Watered (100% FC) and Drought Stress (50% FC) Conditions. The values are shown as the means ($n=3$) $\pm$ standard error. Different letters indicate significant difference ($P \leq 0.01$) according to the LSD test. FC: Field capacity, DPPHsc: 2, 2-diphenyl-1-picrylhydrazyl which was scavenged.

to foliar application of ZnO-NPs (Figure 5). ZnO-NPs treatment significantly enhanced the antioxidant activity of *M. peregrina* populations as compared with untreated ones. The antioxidant activity significantly increased at 0.05% ZnO-NPs and then reduced at 0.10 % ZnO-NPs, while it was still higher compared to control plants. The highest antioxidant activity (82.1 % DPPHsc) of *M. peregrina* was obtained under drought stress conditions in plants treated with 0.05% ZnO-NPs.

Discussion

The present study has revealed that ZnO-NPs spray can increase the tolerance of *M. peregrina* plant to drought stress by enhancing some secondary metabolites and antioxidant potential. Drought stress affects the minerals uptake by plant roots through influencing root growth and nutrient mobility in soil. Under drought stress, water availability is reduced, followed generally by reduction in total nutrient uptake and the concentrations of minerals in crops.¹⁷ The most important effect of drought stress is observed on the transport of nutrients to the root which affects root growth and extension. The content of nutrient elements is balanced by nutrient uptake and unloading mechanisms as well as transpiration flow.¹⁸ It has been revealed that drought stress significantly reduced Na and enhanced K uptake and ion uptake efficiency in different chickpea genotypes.¹⁹

According to our results, drought stress reduced Na⁺ and K⁺ content in all *M. peregrina* populations. However, under drought conditions, ZnO-NPs treatment increased Na⁺ content. Change in nutrients balance in response to Zn-NPs spray is in agreement with the findings of Soliman et al²⁰ who reported that foliar application of Zn-NPs significantly affects mineral nutrients content of *M. peregrina*. Zn seems to affect the capacity for water and nutrients uptake as well as transport in plants under different abiotic stresses.²¹ Our results revealed that Zn-NPs spray enhanced Na content in drought stress conditions, which was in agreement with the findings

of Martinez et al,²² who reported that plant tolerances to water deficit is due to a common mechanism of Na uptake for osmotic adjustment.

Our results showed that foliar spray of ZnO-NPs not only prevented chlorophylls degradation but also significantly enhanced Chl-a, Chl-b as well as T-Chl content in both drought-stressed and unstressed plants. Photosynthetic pigments control the energy balance through chlorophylls and therefore, they involve in the adaptation and survival of plants in drought.²³ Inhibition of chlorophyll biosynthesis, activation of chlorophyllase and/or destruction of chloroplast structure lowered the pigment content under abiotic stress conditions.²⁰ In addition, Mejri et al²⁴ reported that drought stress prevented from chloroplast activity and led to the breakdown of chlorophyll as well as changes in chlorophyll a to b ratio. It has been reported that the decrease in chlorophyll under drought stress is generally the consequence of chloroplast damages resulting from reactive oxygen species (ROS).²⁵ Another reason for the decline in chlorophyll is the application of a glutamate precursor for the biosynthesis of proline.²⁶

Enhancing chlorophylls content by ZnO-NPs treatment are in agreement with the results obtained by Fathi et al²⁷ and Kheirizadeh Arough et al²⁸ who mentioned that ZnO-NPs treated plants showed higher amount of total chlorophyll content than the control. Moreover, Sharma et al²⁹ reported that Zn treatment increased the growth of cabbage and improved the chlorophyll content and photosynthetic activity in the leaves. Zarrouk et al³⁰ showed a positive correlation between Zn concentrations and leaf chlorophyll content in plants. It has been demonstrated that ZnO NPs induced the expression of chlorophyll synthesis genes.³¹

Based on the present study, TPC increased in response to drought stress. However, foliar application of ZnO-NPs increased TPC under both well-watered and drought conditions. The increase in TPC under drought stress condition is due to the soluble carbohydrates accumulation

in plant cells because transportation of soluble sugars is reduced under water deficit.³² Phenolic compounds are biosynthesized through various mechanisms such as malonic acid and shikimic acid pathways. Simple carbohydrate precursors are converted into aromatic amino acids through shikimic acid pathway.³³ It has been reported that the increase of phenolic compounds is highly correlated with the balance between carbohydrate sources and sinks.^{32,34} Moreover, the TPC enhancement under drought stress is greatly related to the production and distribution of various antioxidants in the plant and the intensity and duration of stress.³⁵ Similarly, we found that there was a significant positive correlation between TPC and antioxidant activity ($r = 0.93$). The increase of TPC by foliar application of ZnO-NPs is in agreement with findings of Oloumi et al³⁶ who mentioned that the phenolic compounds contents in seedlings of *Glycyrrhiza glabra* L. significantly increased in response to 10 μ M ZnO-NPs. They suggested that it might be due to the ability of ZnO-NPs to affect metabolic activity.

In the present study, the antioxidant activity of *M. peregrina* populations remarkably increased under drought stress. Drought stress, at the cellular level, induces the accumulation of ROS such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals. The excess ROS are detrimental because they damage membranes, proteins, chlorophylls and nucleic acids. To reduce this oxidative damage, plants enhance their antioxidant defense systems including enzymatic and non-enzymatic scavenging mechanisms.²³ The antioxidant activity has a crucial role in maintaining the balance between the production and scavenging of free radicals, therefore, the number of antioxidants should be high to compensate and tolerate stress condition.³²

Our results indicated that ZnO-NPs treatment enhanced the antioxidant activity of *M. peregrina* populations as compared with untreated ones. It was revealed that the application of Zn under abiotic stress enhanced removing reactive oxygen species by increasing antioxidant enzymes activities. Zn ions bind to ligands containing sulfur, nitrogen, and to a lesser extent oxygen, and preferentially bind to the membrane proteins.²⁸ Our results are in agreement with the findings of Tavallali et al³⁷ who reported that Zn is able to facilitate the biosynthesis of antioxidant enzymes and enhance antioxidant activity in the leaves of pistachio under abiotic stress conditions. The balance between the generation and removal of free radicals determines the survival of the system. Therefore, Zn may have a role in modulating free radicals and their related damaging effects by enhancing the antioxidant activity of plants.³⁸

The antioxidant property of ZnO-NPs could be resulted from the transfer of electron density located at oxygen to the odd electron located at nitrogen atom in DPPH.³⁹ This characteristic is dependent on the structural configuration of oxygen atom, and it determines the

thermal stability of nanoparticle.⁴⁰ In addition, the antioxidant efficacy of ZnO-NPs against DPPH is likely due to the electrostatic attraction between positively charged NPs ($\text{ZnO} = \text{Zn}^{2+} + \text{O}^{2-}$) of plant extracts and negatively charged bioactive compounds (COO^- , O^-).⁴¹ Binding of ZnO-NPs to phytochemicals synergistically increase their bioactivity.⁴²

Conclusion

ZnO-NPs treatment effectively protected *M. peregrina* populations from drought stress by inhibiting the chlorophylls degradation and enhancing TCP and antioxidant activity. Hence, foliar application of ZnO-NPs is recommended for the growth of different *M. peregrina* populations under drought stress conditions.

Competing Interests

The authors declare that they have no competing financial, professional, or personal interests that might have influenced the performance or presentation of the study described in this manuscript.

Ethical Approval

Not applicable.

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