



## Research Article

# Effect of Disper Osmotic GS on Some Antioxidant Enzymes and Leaf Content of some Microelements of Date Palm *Phoenix dactylifera* L.c.v. Barhi Produced by Tissue Culture Under Salt Stress Conditions

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**Abstract** | This study was done in a private orchard in Nasiriyah district, the center of Thi Qar province, during 2024-2025. The aim was to study the effect of Disper Osmotic GS nutrient solution on some antioxidant enzymes and leaf content of zinc, copper, iron, and manganese elements in young date palm *Phoenix dactylifera* L. offshoots Barhi cultivar produced from tissue culture under salt stress conditions. Three concentrations of Disper Osmotic GS nutrient solution 0, 3, and 6 g L<sup>-1</sup> and three concentrations of sodium chloride 0, 50, and 100 mM, with three replicates for each treatment. The results showed that the treatment with Disper Osmotic GS nutrient solution at a concentration of 6 g L<sup>-1</sup> had a significant effect and achieved the highest averages of activity of the enzymes superoxide dismutase 62.00 unit min<sup>-1</sup> g<sup>-1</sup> f. w., peroxidase 181.89 unit min<sup>-1</sup> g<sup>-1</sup> f. w., and catalase 166.78 unit min<sup>-1</sup> g<sup>-1</sup> f. w., also recorded the highest averages of leaf content of zinc 71.02 ppm, copper 9.63 ppm, iron 210.55 ppm, and manganese 153.11 ppm, compared to the control treatment, which lowest were recorded. The treatment with sodium chloride at a concentration of 100 mM had a significant effect and achieved the highest averages of activity of the enzymes SOD 62.67 unit min<sup>-1</sup> g<sup>-1</sup> f. w., POD 182.56 unit min<sup>-1</sup> g<sup>-1</sup> f. w., and CAT 167.33 unit min<sup>-1</sup> g<sup>-1</sup> f. w., this treatment also led to a significant increase in the leaf content of zinc 70.25 ppm, copper 9.33 ppm, iron 213.62 ppm, and manganese 154.56 ppm, compared to the control treatment, which recorded the lowest averages. As for the interaction treatments between the study factors were concerned, some of them were significant in increasing the effectiveness of the studied antioxidant enzymes, as the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 6 g L<sup>-1</sup> and sodium chloride at a concentration of 100 mM was significantly superior and achieved the highest averages of the activities of the enzymes SOD 66.00 unit min<sup>-1</sup> g<sup>-1</sup> f. w., POD 185.67 unit min<sup>-1</sup> g<sup>-1</sup> f. w. and CAT 171.00 unit min<sup>-1</sup> g<sup>-1</sup> f. w., as well as achieving the highest averages of leaf content of zinc 74.69 ppm, copper 9.99 ppm, iron 222.45 ppm and manganese 157.67 ppm, compared to the control treatment, which recorded the lowest averages.

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**Keywords** | Superoxide dismutase, Peroxidase, Catalase, Zinc and Iron.



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## Introduction

The date palm *Phoenix dactylifera* L. is a monocotyledonous plant belonging to the palm family Arecaceae and order Palmae, which includes approximately 200 genera and more than 4,000 species, it is a perennial, evergreen tree, widely cultivated in tropical and subtropical regions between 10–39° north and south latitude (Al-Bakr, 1972; Ibrahim, 2014). Iraq is one of the oldest date palm cultivation sites in the world, with more than 627 cultivars grown commercially, locally, and as a rare species, the Barhi cultivar is characterized by its high sugar content and unique flavor and is consumed during the khalal, rutab, and tamr stages, it also has a moderate tolerance to salinity, drought, and frost, it is early in flowering and medium to late in maturing (Al-Mudhaffar, 2019).

Salinity is one of the most prominent agricultural problems affecting agricultural production globally, especially in arid and semi-arid regions (Munns and Tester, 2008; Alturki, 2018). Studies indicate that the use of saline water for irrigation affects nearly a third of irrigated lands around the world, whether in humid, arid, or semi-arid regions (Yaish and Kumar, 2015). Salinity affects plant growth through two main mechanisms: the first mechanism is direct effects represented by ionic imbalance and reducing water potential in the growth environment, and the second mechanism is indirect, including inhibition of cellular metabolism, disruption of protein synthesis, photosynthesis processes, hindering cell expansion, and increasing the production of reactive oxygen species ROS, as well as hormonal imbalance (Wani *et al.*, 2013; Acosta-Motos *et al.*, 2017).

Recent studies have shown that the use of chemical compounds, nutrients, and growth promoters contributes to mitigating the negative effects of salt stress by activating plant defense systems, plants rely on two main systems: the enzymatic system, which includes antioxidant enzymes such as superoxide dismutase SOD, peroxidase POD, catalase CAT, ascorbate peroxidase APX, and glutathione reductase GR, and the non-enzymatic system, which includes water-soluble compounds such as ascorbate, glutathione, phenols, and flavonoids, and fat-soluble compounds such as alpha-tocopherol, beta-carotene, and lycopene, these compounds are derived from primary metabolites such as lipids, and amino acids (Aziz *et al.*, 2023; Sghaier-Hammami *et al.*, 2023).

Despite the economic importance of the date palm, studies on strategies to enhance its tolerance to salt stress remain limited. Therefore, this study aims to test the effectiveness of the Disper Osmotic GS nutrient solution, produced by the Spanish company EDEN, as a qualitative addition to this field, this nutrient solution contains 65% humic extract 26% humic acid, 39% fulvic acid, 4.3% free amino acids, 4.2% water-soluble calcium CaO, 3% LS-chelated calcium, 1.2% EDTA-chelated calcium, 12.5% potassium K<sub>2</sub>O, and 9% sugars, this compound is specifically designed to enhance plant osmotic balance and mitigate the effects of salinity and water stress, thus improving plant resilience and increasing productivity w.w.w.disper.info.

## Materials and Methods

This study was done in a private orchard in Nasiriyah District, the center of Thi Qar province, during the 2024-2025 on 27 homogeneous offshoots of three-year-old tissue cultured date palm *P. dactylifera* L. c.v. Barhi. The experiment was implemented as a factorial experiment according to a Randomized Complete Block Design with a split-plot system RCBD-Split plot and with two factors: the first factor was the nutrient solution Disper Osmotic GS at three concentrations 0, 3, and 6 g L<sup>-1</sup>, and the second factor was sodium chloride at three concentrations 0, 50, and 100 mM. These were added to the soil alternately every 15 days for six times, with nine treatments and three replicates for each treatment and 27 experimental units.

### Enzyme activity assessment

#### Measuring superoxide dismutase activity

Superoxide dismutase activity was measured according to the method of Marklund and Marklund, 1974 based on the enzyme's ability to inhibit pyrogallol oxidation. Enzyme activity was calculated according to the following equation:

$$\text{Pyrogallol oxidation inhibition \%} = \frac{\text{Test A} - \text{Control A}}{\text{Control A}} \times 100$$

$$\text{SOD Activity unit min}^{-1}\text{g}^{-1} \text{ f.w.} = \frac{\text{Inhibition of pyrogallol oxidation \%}}{50 \%}$$

#### Measuring peroxidase activity

Peroxidase activity was estimated according to the method (Kim *et al.*, 1988). Absorbance was read

directly using a spectrophotometer at a wavelength of 470 nm, with three replicates for each treatment. Enzyme activity was estimated on the basis of units per minute per gram of fresh weight. Peroxidase activity was calculated using the following equation:

#### Device reading

Enzyme activity unit  $\text{min}^{-1}\text{g}^{-1}$  f.w. = Sample weight / extraction volume  $\times$  volume taken for reading

#### Measuring the activity of the catalase enzyme

The activity of the catalase enzyme was estimated according to the method (Hadwan and Kadhum, 2018), and on the basis of enzyme absorption unit per minute per gram of fresh weight. The activity of the catalase enzyme was calculated according to the following equation:

Catalase Activity of test unit  $\text{min}^{-1}\text{g}^{-1}$  f.w. =  $2.303 / t \times \log S^0 / S$

Note that:

$S^0$  is the absorbance of the standard tube.

$S$  is the absorbance of the sample test tube.

#### Determination of zinc, iron, manganese, and copper ppm

Zinc, iron, manganese, and copper in the leaves were determined by atomic absorption spectrometry according to the method Black, 1965.

#### Statistical analysis

The experiment was conducted in a RCBD for single-shot splinter plots, the data were statistically analyzed using the statistical analysis program Genstat 31 (2012), the significance of differences between means was tested using the least significant difference LSD test at a 5% probability level (Al-Rawi and Khalaf Allah, 1980).

## Results and Discussion

#### Superoxide dismutase activity unit $\text{min}^{-1}\text{g}^{-1}$ f. w.

The results showed that the nutrient solution Disper Osmotic GS at a concentration of  $6 \text{ g L}^{-1}$  significantly affected the activity of the superoxide dismutase enzyme, achieving the highest average of  $62.00 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w., followed by the control treatment, which recorded an average of  $58.44 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w., while the concentration treatment  $3 \text{ g L}^{-1}$  recorded the lowest average of  $55.89 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w.. Sodium chloride treatments significantly increased enzyme

activity with increasing concentration; the  $100 \text{ mM}$  treatment achieved the highest average  $62.67 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w., compared to the control treatment, which recorded the lowest average  $54.67 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w. The interaction effect between the study factors was significant in increasing superoxide dismutase activity, the interaction between the nutrient solution Disper Osmotic GS at a concentration of  $6 \text{ g L}^{-1}$  and sodium chloride at a concentration of  $100 \text{ mM}$  achieved the highest average  $66.00 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w., compared to the control treatment, which recorded the lowest average  $51.33 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w. (Table 1).

**Table 1:** The effect of disper osmotic gs, sodium chloride and the interaction between them on the activity of the superoxide dismutase enzyme (unit  $\text{min}^{-1} \text{ g}^{-1}$  f. w.).

| NaCl concentrations mM     | Disper osmotic GS concentrations $\text{g L}^{-1}$ |       |       | NaCl averages |
|----------------------------|----------------------------------------------------|-------|-------|---------------|
|                            | 0                                                  | 3     | 6     |               |
| 0                          | 51.33                                              | 54.67 | 58.00 | 54.67         |
| 50                         | 60.00                                              | 55.00 | 62.00 | 59.00         |
| 100                        | 64.00                                              | 58.00 | 66.00 | 62.67         |
| Disper osmotic GS averages | 58.44                                              | 55.89 | 62.00 |               |
| L.S.D <sub>0.05</sub>      | Disper osmotic GS NaCl                             |       |       | لخ ادبتلا     |
|                            | 1.055                                              | 1.023 | 1.649 |               |

#### Peroxidase enzyme activity unit $\text{min}^{-1} \text{ g}^{-1}$ f. w.

It is noted that the Disper Osmotic GS nutrient solution treatment at a concentration of  $6 \text{ g L}^{-1}$  significantly increased peroxidase enzyme activity, achieving the highest average of  $181.89 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w., this was followed by the control treatment, which recorded an average of  $178.33 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w., the concentration treatment  $3 \text{ g L}^{-1}$  recorded the lowest average of  $175.56 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w.. Sodium chloride treatments significantly increased peroxidase enzyme activity, the concentration treatment  $100 \text{ mM}$  achieved the highest average of  $182.56 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w., while the control treatment recorded the lowest average of  $174.56 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w. The effect of interaction treatments between the study factors was significant in the activity of the peroxidase enzyme, and the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of  $6 \text{ g L}^{-1}$  and sodium chloride at a concentration of  $100 \text{ mM}$  achieved the highest average of  $185.67 \text{ unit min}^{-1} \text{ g}^{-1}$  f. w., which did not differ significantly from the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of  $0 \text{ g L}^{-1}$

and sodium chloride at a concentration of 100 mM, which recorded an average of 184.00 unit min<sup>-1</sup> g<sup>-1</sup> f. w., while the control treatment recorded the lowest average of 171.00 unit min<sup>-1</sup> g<sup>-1</sup> f. w. (Table 2).

**Table 2:** The effect of disper osmotic gs, sodium chloride and the interaction between them on the activity of the peroxidase enzyme (unit min<sup>-1</sup> g<sup>-1</sup> f. w.).

| NaCl concentrations mM     | Disper osmotic GS concentrations g L <sup>-1</sup> |        |        | NaCl averages |
|----------------------------|----------------------------------------------------|--------|--------|---------------|
|                            | 0                                                  | 3      | 6      |               |
| 0                          | 171.00                                             | 174.67 | 178.00 | 174.56        |
| 50                         | 180.00                                             | 174.00 | 182.00 | 178.67        |
| 100                        | 184.00                                             | 178.00 | 185.67 | 182.56        |
| Disper osmotic GS averages | 178.33                                             | 175.56 | 181.89 |               |
| L.S.D <sub>0.05</sub>      | Disper osmotic GS                                  |        | NaCl   | لخ ادتلا      |
|                            | 1.556                                              |        | 1.708  | 2.508         |

**Table 3:** The effect of Disper Osmotic GS, sodium chloride and the interaction between them on the activity of the Catalase enzyme (unit min<sup>-1</sup> g<sup>-1</sup> f. w.).

| NaCl concentrations mM     | Disper osmotic GS concentrations g L <sup>-1</sup> |        |        | NaCl averages |
|----------------------------|----------------------------------------------------|--------|--------|---------------|
|                            | 0                                                  | 3      | 6      |               |
| 0                          | 156.33                                             | 159.33 | 161.33 | 159.00        |
| 50                         | 163.33                                             | 160.33 | 168.00 | 163.89        |
| 100                        | 167.00                                             | 164.00 | 171.00 | 167.33        |
| Disper osmotic GS averages | 162.22                                             | 161.22 | 166.78 |               |
| L.S.D <sub>0.05</sub>      | Disper osmotic GS                                  |        | NaCl   | لخ ادتلا      |
|                            | 1.405                                              |        | 1.868  | 2.413         |

#### Catalase enzyme activity unit min<sup>-1</sup> g<sup>-1</sup> f. w.

It is noted that the Disper Osmotic GS nutrient solution at a concentration of 6 g L<sup>-1</sup> significantly increased the activity of the catalase enzyme, achieving the highest average of 166.78 unit min<sup>-1</sup> g<sup>-1</sup> f. w., followed by the control treatment, which recorded an average of 162.22 unit min<sup>-1</sup> g<sup>-1</sup> f. w., which did not differ significantly from the concentration treatment 3 g L<sup>-1</sup>, which recorded the lowest average of 161.22 unit min<sup>-1</sup> g<sup>-1</sup> f. w. Sodium chloride treatments significantly increased the activity of the catalase enzyme with increasing sodium chloride concentration, the 100 mM concentration treatment achieved the highest average of 167.33 unit min<sup>-1</sup> g<sup>-1</sup> f. w., while the control treatment recorded the lowest average of 159.00 unit min<sup>-1</sup> g<sup>-1</sup> f. w.. The interaction

effect between the study factors was significant on the activity of the catalase enzyme, the interaction between the nutrient solution Disper Osmotic GS at a concentration of 6 g L<sup>-1</sup> and sodium chloride at a concentration of 100 mM achieved the highest average of 171.00 unit min<sup>-1</sup> g<sup>-1</sup> f. w., compared to the control treatment, which recorded the lowest average of 156.33 unit min<sup>-1</sup> g<sup>-1</sup> f. w. (Table 3).

#### Zinc concentration in leaves ppm

The results showed that the concentration of the Disper Osmotic GS nutrient solution 6 g L<sup>-1</sup> achieved the highest average, with a significant difference, in zinc content of leaves 71.02 ppm, while the concentration treatment 3 g L<sup>-1</sup> recorded the lowest average 62.02 ppm, compared to the control treatment, which recorded an average of 67.46 ppm. Sodium chloride treatments significantly affected zinc content of leaves, the concentration treatment 100 mM achieved the highest average 70.25 ppm, compared to the control treatment, which recorded the lowest average 62.77 ppm (Table 4).

**Table 4:** The effect of disper osmotic gs, sodium chloride and the interaction between them on the zinc concentration in leaves (ppm).

| NaCl concentrations mM     | Disper osmotic GS concentrations g L <sup>-1</sup> |       |       | NaCl averages |
|----------------------------|----------------------------------------------------|-------|-------|---------------|
|                            | 0                                                  | 3     | 6     |               |
| 0                          | 58.84                                              | 62.64 | 66.84 | 62.77         |
| 50                         | 69.89                                              | 61.03 | 71.53 | 67.48         |
| 100                        | 73.66                                              | 62.38 | 74.69 | 70.25         |
| Disper osmotic GS averages | 67.46                                              | 62.02 | 71.02 |               |
| L.S.D <sub>0.05</sub>      | Disper osmotic GS                                  |       | NaCl  | لخ ادتلا      |
|                            | 1.015                                              |       | 0.774 | 1.523         |

The effect of interaction treatments between the study factors was significant in the concentration of zinc in the leaves, and the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 6 g L<sup>-1</sup> and sodium chloride at a concentration of 100 mM achieved the highest average of 74.69 ppm, which did not differ significantly from the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 0 g L<sup>-1</sup> and sodium chloride at a concentration of 100 mM, which recorded an average of 73.66 ppm, compared to the control treatment, which recorded the lowest average of 58.84 ppm.

### Copper concentration in leaves ppm

The results showed that the Disper Osmotic GS nutrient solution significantly increased copper concentration with increasing added concentration, the 6 g L<sup>-1</sup> treatment achieved the highest average of 9.63 ppm, followed by the 3 g L<sup>-1</sup> treatment, which recorded an average of 7.77 ppm, this compares favorably with the control treatment, which recorded the lowest average of 7.02 ppm. Sodium chloride treatments significantly affected leaves copper content, the 100 mM treatment achieved the highest average of 9.33 ppm, while the control treatment recorded the lowest average of 6.95 ppm (Table 5).

**Table 5:** The effect of Disper Osmotic GS, sodium chloride and the interaction between them on the copper concentration in leaves (ppm).

| NaCl concentrations mm     | Disper osmotic GS concentrations g L <sup>-1</sup> |        |      | NaCl averages |
|----------------------------|----------------------------------------------------|--------|------|---------------|
|                            | 0                                                  | 3      | 6    |               |
| 0                          | 4.01                                               | 7.01   | 9.82 | 6.95          |
| 50                         | 7.10                                               | 8.24   | 9.08 | 8.14          |
| 100                        | 9.94                                               | 8.07   | 9.99 | 9.33          |
| Disper osmotic GS averages | 7.02                                               | 7.77   | 9.63 |               |
| L.S.D <sub>0.05</sub>      | Disper osmotic GS NaCl                             |        |      | لخ ادبتلا     |
|                            | 0.1648                                             | 0.2438 |      | 0.2963        |

As for the effect of the interaction treatments between the study factors, some of them were significant in the concentration of the copper element, and the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 6 g L<sup>-1</sup> and sodium chloride at a concentration of 100 mM achieved the highest average of 9.99 ppm, which did not differ significantly from the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 6 g L<sup>-1</sup> and sodium chloride at a concentration of 0 mM, which recorded an average of 9.82 ppm, compared to the control treatment, which recorded the lowest average of 4.01 ppm.

### Iron concentration in leaves ppm

The Disper Osmotic GS nutrient solution significantly increased iron concentration with increasing added concentration, the 6 g L<sup>-1</sup> concentration treatment achieved the highest average of 210.55 ppm, which did not significantly differ from the control treatment, which recorded an average of 208.73 ppm, and the 3g L<sup>-1</sup> concentration treatment recorded the lowest

average of 198.77 ppm (Table 6). Sodium chloride treatments significantly increased iron concentration with increasing sodium chloride concentration, the 100 mM concentration treatment achieved the highest average of 213.62 ppm, compared to the control treatment, which recorded the lowest average of 201.34 ppm, this did not significantly differ from the 50 mM concentration treatment, which recorded an average of 203.09 ppm.

**Table 6:** The effect of Disper Osmotic GS, sodium chloride and the interaction between them on the iron concentration in leaves (ppm).

| NaCl concentrations mm     | Disper osmotic GS concentrations g L <sup>-1</sup> |        |        | NaCl averages |
|----------------------------|----------------------------------------------------|--------|--------|---------------|
|                            | 0                                                  | 3      | 6      |               |
| 0                          | 192.82                                             | 202.59 | 208.61 | 201.34        |
| 50                         | 213.34                                             | 195.34 | 200.59 | 203.09        |
| 100                        | 220.02                                             | 198.37 | 222.45 | 213.62        |
| Disper osmotic GS averages | 208.73                                             | 198.77 | 210.55 |               |
| L.S.D <sub>0.05</sub>      | Disper osmotic GS NaCl                             |        |        | لخ ادبتلا     |
|                            | 2.742                                              |        | 3.414  | 4.596         |

The effect of the interaction treatments between the study factors was significant in the concentration of iron, and the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 6 g L<sup>-1</sup> and sodium chloride at a concentration of 100 mM achieved the highest average of 222.45 ppm, which did not differ significantly from the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 0 g L<sup>-1</sup> and sodium chloride at a concentration of 100 mM, which recorded an average of 220.02 ppm, compared to the control treatment, which recorded the lowest average of 192.82 ppm, which did not differ significantly from the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 3 g L<sup>-1</sup> and sodium chloride at a concentration of 50 mM, which recorded an average of 195.34 ppm.

### Manganese concentration in leaves ppm

The results showed that the Disper Osmotic GS nutrient solution significantly increased manganese concentration with increasing added concentration, the 6 g L<sup>-1</sup> concentration treatment achieved the highest average of 153.11 ppm, followed by the control treatment, which recorded an average of

148.00 ppm, this average did not differ from the 3g L<sup>-1</sup> concentration treatment, which caused a significant decrease and recorded the lowest average of 146.78 ppm (Table 7). The effect of sodium chloride treatments significantly increased iron concentration; the 100 mM sodium chloride treatment achieved the highest average of 154.11 ppm, while the control treatment recorded the lowest average of 143.56 ppm.

**Table 7:** *The effect of Disper Osmotic GS, sodium chloride and the interaction between them on the Manganese concentration in leaves (ppm).*

| NaCl concentrations mM     | Disper osmotic GS concentrations g L <sup>-1</sup> |        |        | NaCl averages |
|----------------------------|----------------------------------------------------|--------|--------|---------------|
|                            | 0                                                  | 3      | 6      |               |
| 0                          | 139.00                                             | 144.00 | 147.67 | 143.56        |
| 50                         | 150.00                                             | 145.33 | 154.00 | 149.78        |
| 100                        | 155.00                                             | 151.00 | 157.67 | 154.56        |
| Disper osmotic GS averages | 148.00                                             | 146.78 | 153.11 |               |
| L.S.D <sub>0.05</sub>      | Disper osmotic GS                                  | NaCl   | لخادتل |               |
|                            | 1.234                                              | 2.872  | 2.953  |               |

As for the effect of the interaction treatments between the study factors, some of them were significant in the concentration of iron element, and the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 6 g L<sup>-1</sup> and sodium chloride at a concentration of 100 mM achieved the highest average of 157.67 ppm, which did not differ significantly from the interaction treatment between the nutrient solution Disper Osmotic GS at a concentration of 0 g L<sup>-1</sup> and sodium chloride at a concentration of 100 mM, which recorded an average of 155.00 ppm, compared to the control treatment, which recorded the lowest average of 139.00 ppm.

It was noted from the results in Table 1, Table 2 and Table 3 that there was a significant increase in the activity of the enzymes SOD, POD and CAT and the leaves content of zinc, copper, iron and manganese, respectively, under the influence of the Disper Osmotic GS solution treatments. This may be attributed to the roles played by the components of this compound, represented by the humic extract at a percentage of 65% humic acid 26% and fulvic acid 39%, free amino acids at a percentage of 4.3%, total water-soluble calcium CaO at a percentage of 4.2%, calcium with LS at a percentage of 3%, calcium chelated with EDTA at a percentage of 1.2%, potassium K<sub>2</sub>O at

a percentage of 12.5% and sugars at a percentage of 9%, as these components provide nutrients well to the plant. w.w.w.disper.info. The significant increase in the activity of the enzymes SOD, POD and CAT under the influence of the nutrient solution Disper Osmotic GS may be attributed to the role of the components of the nutrient solution, especially humic extracts humic and fulvic, in improving the plant's tolerance to environmental stress conditions by acting as a protective substance that stimulates the plant's protective effects, the most important of which is increasing the activity of antioxidant enzymes, including SOD, POD and CAT enzymes (Fahim, 2019). Fulvic acid has a natural chelating ability, as it helps chelate and facilitate nutrients in the soil, which helps facilitate the microelements fixed in the soil, such as zinc, copper, iron and manganese, as a result of facilitating these microelements, as shown by the results of this study in tables 4, 5, 6 and 7, and since the enzyme SOD is a metal enzyme, it is present in plants in three types called isozymes, depending on the metal used as a cofactor, namely FeSOD, MnSOD and Cu/ZnSOD, there is a great similarity in the functions of these types, which is to remove toxins from the superoxide radical, but they are found in different places inside the cell (Morgan et al., 2008). Humic acid also has the ability to enhance the permeability of membranes and cells, which facilitates the absorption of water and nutrients by plants, this process, in turn, enables the transfer of cellular minerals that stimulate the activity of plant enzymes Dantas et al. (2007) stated that humic acid consists of a group of guanoates that act as hydrogen receptors and enhance enzyme activity.

The results showed a significant increase in the activity of the enzymes SOD, POD and CAT with increasing concentration of added sodium chloride. This may be due to the fact that the plant uses a defensive strategy in response to salt stress with the aim of mitigating reactive oxygen species ROS by increasing the production of enzymatic antioxidants, the most important of which are SOD, POD and CAT and non-enzymatic antioxidants, the most important of which are total soluble carbohydrates, proline, total soluble proteins and others, the increased activity of antioxidant enzymes is a response to changes in the structures of lipids, proteins, nucleic acids and cell membranes caused by reactive oxygen species ROS, which cause severe damage, the most important of which is damage to the plant's metabolic processes

due to the oxidative stress they cause to membrane lipids, proteins and nucleic acids, the increased activity of antioxidant enzymes is an adaptive feature of the plant in overcoming salinity damage and providing protection against oxidative stress (Sudhakar *et al.*, 2001; Chawla *et al.*, 2013).

They are able to remove or limit reactive oxygen species ROS, the balance between the enzymes SOD, POD, and CAT in cells plays a major role in removing ROS Bowler *et al.*, 1992, without these defenses, plants cannot efficiently convert light energy into mechanical energy, the enzyme SOD acts as a major defense, catalyzing the conversion of superoxide radicals into molecular oxygen and hydrogen peroxide, the enzymes POD and CAT then remove hydrogen peroxide and convert it to water and oxygen, it is worth noting that hydrogen peroxide increases in the cell as a result of the oxidative stress of salinity, in addition to its quantities resulting from the work of the enzyme SOD, in this way, cellular damage is avoided, mitochondria and chloroplasts contain mechanisms for removing reactive oxygen species ROS (Alscher *et al.*, 2003; Mittler *et al.*, 2004; Al Desuquy *et al.*, 2012).

The significant increase in the leaves content of zinc, copper, iron, and manganese due to the effect of both the Disper Osmotic GS nutrient solution and sodium chloride, as shown in Table 4, Table 5, Table 6, and Table 7, may be due to the fact that most of these trace elements are associated with antioxidant enzymes and are directly affected by these enzymes, as a result of the increased effectiveness of antioxidant enzymes SOD, POD, and CAT due to the effect of both the Disper Osmotic GS nutrient solution and sodium chloride, as shown in the results of this study in Table 1, Table 2, and Table 3, it is natural for the concentration of zinc, copper, iron, and manganese to increase in the leaves because they are associated with these enzymes, for example, the SOD enzyme is the first line of defense in the plant and plays an important role in protecting cells from oxidative damage caused by superoxide radicals, this enzyme is produced by all living organisms, from bacteria to humans, and is found in four types based on the metal ions present in the active site, which are Cu, Zn, Fe, and Mn Perry *et al.*, 2010. Plants contain Cu/Zn SOD which is found in the cytoplasm, chloroplasts and peroxisomes, while Fe SOD is found in chloroplasts (Morand *et al.*, 2013; Miller, 2012), while MnSOD is located in

the mitochondria and is the basis for the survival of aerobic life Carlioz and Touati, 1986; Miller, 2012. As a result of this association, it is natural for these antioxidant enzymes to play their roles in mitigating or reducing the effects of salt stress and increasing their effectiveness, as shown by the results of this study in Tables 1, Table 2 and Table 3, which were accompanied by an increase in the concentrations of copper, zinc, iron and manganese because they are linked to these enzymes and are directly affected by them.

The results of this study are consistent with what was reached (Al-Asadi and Al-Zubaidy, 2024; Al-Asadi and Al-Zubaidy, 2025) during their study of the effect of sodium chloride on the antioxidant enzymes of some date palm cultivars. The results of this study also agreed with what was found (Al-Badri, 2021; Gabash *et al.*, 2024) during their study on date palms.

The results of this study are consistent with (Alturki, 2021) stated that adding  $\text{CaNO}_3$  under salinity improved the absorption of Fe,  $\text{Zn}^{2+}$ , Mn and Cu. Hameed and Tama (2021) confirmed that humic increased the availability of microelements in saline soil. Shareef *et al.* (2020) also explained that spraying with iron and zinc increased their content in the leaves. Hakeem *et al.* (2023) and Alsudays *et al.* (2024) showed that humic and fulvic acid improved the absorption of microelements by improving the ionic balance under salt stress.

## Conclusions and Recommendations

This study concludes that adding concentrations of the nutrient solution Disper Osmotic GS improved the ability of Barhi date palm offshoots produced from tissue culture to withstand the harmful effects of sodium chloride, this was achieved through a significant increase in the activities of the antioxidant enzymes SOD, POD, and CAT, as well as a significant increase in the concentrations of zinc, copper, iron, and manganese, which bind to the active sites of these enzymes, thereby increasing their effectiveness.

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## Novelty Statement

The treatment with Disper Osmotic GS nutrient solution at a concentration of 6 g L<sup>-1</sup> had a significant effect and achieved the highest averages of activity of the enzymes superoxide dismutase 62.00 unit min<sup>-1</sup> g<sup>-1</sup> f. w., peroxidase 181.89 unit min<sup>-1</sup> g<sup>-1</sup> f. w., and catalase 166.78 unit min<sup>-1</sup> g<sup>-1</sup> f. w., also recorded the highest averages of leaf content of zinc 71.02 ppm, copper 9.63 ppm, iron 210.55 ppm, and manganese 153.11 ppm, compared to the control treatment, which lowest were recorded.

## Author's Contribution

**Sawsan Fathi Tayah Al-Husseinawi:** Wrote the first draft and collected the data

**Ahmed Dinar Khalaf Al-Asadi:** Analysed the data and edited the article.

### Generative AI or AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

### Conflict of interest

The authors have no conflict of interest.

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