

RESEARCH PAPER

Effect of Foliar Application of Iron Oxide Nanoparticles on Some Plant Enzymes of Chamomile (*Matricaria Chamomilla L.*) Under Different Irrigation Levels

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ABSTRACT

A plastic pot experiment was carried out in the courtyard of Al-Husseiniya Holy Shrine, which is situated in the Karbala Governorate, to investigate the effect of foliar application of iron oxide nanoparticles (Fe_2O_3) on certain plant enzymes in chamomile *Matricaria chamomilla* L. under different levels of irrigation levels. Two factors were included in the experiment. Iron oxide nanoparticles (represented by the letter F) applied topically at four different concentrations was the first factor (:) 0, 25, 50, and 100 mg L⁻¹. The second factor was salinity levels (denoted as S), applied at four levels:) 2, 4, 6, and 8 dS m⁻¹. Plants were grown in a loamy sand soil. Foliar spraying was carried out at four growth stages: the first at the stage of six leaves, the second at the stage of ten, the third at the stage of fifteen, and the fourth prior to flowering. A Completely Randomized Design (C.R.D.) with four repetitions was used to organize the experiment's 64 experimental units. The collected data underwent statistical analysis: The activities of antioxidant enzymes, such as catalase (CAT; katal g⁻¹ FW), superoxide dismutase (SOD; U g⁻¹ FW), peroxidase (POD; U g⁻¹ FW), and ascorbate peroxidase (APX; U g⁻¹ FW), were significantly impacted by foliar application of iron oxide nanoparticles under individual treatment. In comparison to the control treatment, the highest reported values were 51.2, 309.3, 0.298, and 0.3483 U g⁻¹ FW, respectively. Under salinity stress, a significant increase was also observed in the activities of CAT, SOD, POD, and APX enzymes under individual treatments. The enzyme activities reached their maximum values of)51.8, 353.7, 0.322, and 0.2596 U g⁻¹ FW(, respectively, compared to the control. Significant differences were detected under the interaction treatments for CAT, SOD, POD, and APX activities. The treatment F3S3 exhibited the highest enzyme activities, reaching)76.8, 598.0, 0.445, and 0.4695 U g⁻¹ FW(, respectively, compared with the control.

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INTRODUCTION

German chamomile *Matricaria chamomilla* L. has been known as a popular medicinal plant that has been widely used in traditional and

folk medicine today. Therapeutic, cosmetic and nutritional benefits backed by years of traditional practices as well as scientific inquiry [1]. A well-known medicinal and herbal remedy in the

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Asteraceae (Compositae) family being common, its various medicinal and aromatic properties are referred to as the “Star of Medicinal Plants [2].

However, its cultivation is severely hampered by environmental stresses. One of the most harmful abiotic stresses is salinity, which drastically lowers agricultural productivity and quality globally. Currently, salt stress affects more than 20% of all arable land on Earth, and this percentage is rising as a result of both natural and human-caused factors. Through a variety of mechanisms, such as nutritional imbalances, hormonal disruptions, ionic toxicity, osmotic stress, and oxidative damage, salinity negatively affects plant growth and development. As a result, these physiological disruptions make plants more vulnerable to certain illnesses [3]. As the world’s population grows, agricultural nanotechnology has become a useful tool for increasing crop yields and agricultural productivity. The use of nanoparticles (NPs) as bio-stimulants or nano-fertilizers has grown dramatically over the last ten years to enhance the morphological and biochemical characteristics of a variety of crops, especially in agricultural areas without sustainable management techniques. [4].

This study’s main goal is to assess how foliar application of iron oxide nanoparticles (Fe_2O_3 NPs) affects particular plant enzymes in chamomile *Matricaria chamomilla* L. at different salt stress levels.

MATERIALS AND METHODS

A pot experiment was conducted during the winter season of 2025–2026, following a four-replicate completely randomized design (C.R.D.). There were two primary components to the experiment:

1. Foliar Application of Fe_2O_3 NPs: Four concentrations were applied (0, 25, 50, and 100 mg/L^{-1}).

2. Salinity Stress: Four levels of saline irrigation were utilized (2, 4, 6 and 8 dS/m^{-1})

The treatments were applied over four stages, beginning at the 4–6 leaf stage. Seeds were sown on October 15, 2025, i15 kg plastic pots are available. Firstly, ten seeds were planted per pot, and seedlings were thinned to four plants per pot 20 days post-planting. Soil samples were collected prior to cultivation for baseline characterization (Table 1). Nano-iron oxide solutions were prepared

Table 1. Physicochemical properties of the experimental soil used in agriculture.

Property	Value	Unit
pH	7.5	----
Electrical conductivity Ece y	1.7	dS. m^{-1}
Organic Matter	6.7	
CaCO ₃	251.02	$\text{g. Kg}^{-1}\text{soil}$
Soluble Cations	Ca ⁺⁺	3.43
	Mg ⁺⁺	1.22
	Na ⁺	1.33
	K ⁺	1.26
Soluble Anions	HCO ₃ ⁻¹	2.35
	SO ₄ ⁻²	1.24
	Cl ⁻	3.85
Available Nitrogen	45.05	
Available Phosphorus	12.60	$\text{mg. kg}^{-1}\text{soil}$
Available Potassium	16.05	
Soil Separates	Sand	545
	Silt	282
	Clay	173
Texture	Loamy sand	----

*Analyzes in soil analysis laboratories at the Faculty of Agriculture – University of Baghdad

according to the required concentrations, The nano-iron oxide (Fe_2O_3 NPs) solutions were prepared according to the predetermined concentrations. The specific weight of the (Fe_2O_3) nanoparticles (powder form) was added to a measured volume of distilled water. The mixture was thoroughly agitated (Shaken) to ensure proper dispersion and reach the target concentration.

To enhance the efficacy of the foliar treatment, a commercial surfactant (detergent) was added to the solution as a spreading agent to ensure maximum wetting of the vegetative parts. Foliar spraying was conducted during the early morning hours using a 3-liter manual sprayer until the foliage was completely saturated. For the control group, Only distilled water was used to shower the plants. There were 64 experimental units in the experimental setup.

Estimation of catalase enzyme (CAT) Activity (katal / g f.wt)

The following equation was used to calculate CAT activity using the method outlined by [5]:

$$\text{Catalase Activity of Test kU} = (2.303/t) \times \log S^0/S$$

Estimation of Superoxide Dismutase (SOD) Activity (U/g.f.wt)

The following formulas were used to calculate SOD activity in accordance with the approach of [6]:

$$\Delta A_{\text{control}} - \Delta A_{\text{test}}$$

$$\% \text{ Inhibition of pyrogallol autoxidation} = X \ 100\% \Delta A_{\text{control}}$$

$$(\text{Cu-Zn}) \text{ SOD Activity (U/ml)} = \% \text{ inhibition of pyrogallol autoxidation}/50\%$$

Estimation of Peroxidase (POD) Activity (U/g.f.wt)

POD activity was calculated using the following equation:

$$\Delta A_{420} / 20\text{sec} \times V_t \times \text{dilution factor}$$

$$\text{Volume activity (U/ml)} = V_s \times 12$$

Where, V_t = Total reaction volume (3.0 ml), V_s = Total sample volume (0.10 ml), 12 = A constant representing the absorbance of 1 mg/ml solution of purpurogallin at 420 nm, Volume activity (U/ml) = $\Delta A_{420} / \text{sec} \times 2.5 \times \text{dilution factor}$.

$$\left(\frac{U}{\text{mg material}} \right) = \frac{U/\text{ml}}{\text{mg material}/\text{ml}} \quad (1)$$

Estimation of Ascorbate Peroxidase (APX) Activity (U/g.f.wt)}

APX activity was determined using the following formula:

$$\text{APX activity (U/g tissue)} = [(\Delta A/\epsilon \times 1) \div t] \times D.F$$

APX activity (U/g tissue) = Enzyme activity, ΔA : Change in absorbance over time, ϵ : Molar extinction coefficient, D.F: Dilution Factor, (ϵ = extinction coefficient = $2.8 \text{ mm}^{-1} \text{ cm}^{-1}$).

RESULTS AND DISCUSSION

Catalase (CAT) Enzyme Activity (katal / g. f.wt)

Table 2 findings show a considerable impact of foliar application with iron oxide nanoparticles (Fe_2O_3 NPs) on the leaf catalase (CAT) content of chamomile *Matricaria chamomilla* L. under the various levels of salinity stress investigated in this study:

The results indicated a significant impact of irrigation water salinity levels on the leaf catalase (CAT) content. Increased salinity led to a corresponding rise in CAT activity, reaching 0.2073, 0.2445, and 0.2596 katal/g f.wt at salinity levels of 4, 6, and 8 dS/m^{-1} , in contrast to 0.1597 katal/g f.wt at the control salinity level of 2 dS/m^{-1} .

The increase is due to the fact that high salt concentrations maintain the plant under environmental pressure, which causes an excess of H_2O_2 and O- reactive oxygen species (ROS). Antioxidant enzymes act to reduce these deleterious substances, and CAT, which cleaves H_2O_2 to O_2 and H_2O , protects the cell from oxidative injury [7]. These results are in line with those of [8,9], who found an increase rate of 18.25% at salinity levels of 3 and 6 dS/m^{-1} .

The results in the aforementioned table further indicate that foliar application of iron oxide nanoparticles (Fe_2O_3 NPs) had a significant effect on leaf CAT content. The activity increased to (0.1765, 0.1852, and 0.3403) (katal/g. f.wt) compared to the control treatment, which recorded 0.1611(katal/g. f.wt)

This enhancement is due to the role of nano-iron in stimulating the antioxidant defense system and reducing the accumulation of ROS [10]. These results align with the findings of [11] in their



study on *Arachis hypogaea*, where an increase in CAT activity was observed at a concentration of 50 mg/L. However, they contrast with [12] who reported a decrease in CAT concentration at 400 mg/L compared to the 200 mg/L treatment of iron oxide nanoparticles in *Carum copticum* L.

The leaf catalase enzyme concentration was significantly impacted by the binary interaction between the study conditions, irrigation water salinity and foliar spraying with nano-iron oxide. Treatment F3S3 had the greatest value, 0.4695 (g.f.wt), while treatment F3S3 had the lowest value F_0S_0 , which reached 0.1354 (g.f.wt). This is attributed to the fact that iron activated the defense system and increased catalase enzyme activity to reduce the damage resulting from salinity [13]. This is consistent with the findings of [14] upon applying nano-iron to *Zea mays* L. plants, as it mitigated salinity stress and increased catalase enzyme activity by a rate of 48.5%.

Superoxide dismutase enzyme (SOD) (u/g.f.wt)

The findings displayed in Table 3 demonstrated a noteworthy impact of irrigation water salinity levels on the superoxide dismutase (SOD) enzyme content. Increased salinity levels led to an increase in SOD enzyme activity, which rose to (0.273, 0.285 and 0.322) (u/g.f.wt) at salinity levels of (4, 6, and 8 dS m⁻¹) respectively, compared to the irrigation water level of 2 (dS m⁻¹), which reached 0.261 (u/g.f.wt). The explanation is that the plant's physiological functions, including respiration and photosynthesis, are disrupted when it is exposed

to salinity, leading to the formation of reactive oxygen species (ROS) such as the superoxide ion {O₂⁻} and hydrogen peroxide {H₂O₂}. These compounds are toxic to cells, and the plant, as a defense mechanism, produces the SOD enzyme, which in turn converts the {O₂⁻} ion into hydrogen peroxide {H₂O₂} and oxygen. After that, other enzymes like catalases and peroxidases act on the degradation of the hydrogen peroxide [15]. Overall, this is similar to the observations made by [16] by [17] 7.5 and 15 dS m⁻¹ salinity level in *Phaseolus vulgaris* plants.

The data obtained in the above table indicated that foliar spraying of plants with nano-iron oxide had a significant effect on the leaf superoxide dissipation (SOD) enzyme content in compared to the control treatment (0.274 (u/g.f.wt)) and increased it to (0.284, 0.286, and 0.298) (u/g.f.wt). This is because the augmenting effects of nano-iron regulate the defence system, increase the SOD enzyme activity and reduce the oxidative stress in plants [10]. These results are consistent with Singh et al. who reported in *Eucalyptus tereticornis* plants. found that the activity of this enzyme in response to nano-iron oxide at a concentration of 25 mg L⁻¹ increased to a 3.8-fold (3.8 times) of control. The concentration of the leaf superoxide dismutase (SOD) enzyme was greatly modified by the binary interaction of the two experimental factors of this study, irrigation water salinity and foliar spraying with nano-iron oxide. F2S3 treatment (0.445 (u/g.f.wt)) and F0S3 treatment (0.153 (u/g.f.wt)) showed the highest and lowest

Table 3. Superoxide dismutase enzyme activity (SOD) is affected by foliar spraying with nano-iron oxide and irrigation with varying water salinity levels. Superoxide dismutase (u/g.f.wt)

F Nano-iron oxide concentration mg L ⁻¹	S Irrigation water salinity levels (dS m ⁻¹)				Mean effect of nano-iron oxide
	2	4	6	8	
0	0.1354	0.1540	0.1677	0.1875	0.1611
25	0.1402	0.2018	0.1617	0.2024	0.1765
50	0.1795	0.1892	0.1932	0.1791	0.1852
100	0.1840	0.2844	0.4555	0.4695	0.3483
Mean effect of salini	0.1598	0.2073	0.2445	0.2596	
Experimental factors	Nano-iron oxide F		Salinity S	Binary interaction S × F	
LSD 0.05	0.02728		0.02728	0.05457	

values of (u/g.f.wt), respectively. This is due to the fact nano iron increased the growth of the plant, reduced oxidative stress, and played a role in the stimulation of SOD enzyme activity [17]. Such is the case with the results obtained from the SOD enzyme in *Rubus baileyanus* plants, in which case the highest value was in concentrations of 2.8(mg L⁻¹) of nano-iron at a salinity level of 3.8 (dS m⁻¹).

Peroxidase Enzyme (POD) (u/g.f.wt)

The peroxidase (POD) enzyme concentration (u/g.f.wt) was significantly impacted by irrigation water salinity levels, according to the results displayed in Table 4. The POD enzyme increased as salt levels rose, reaching (225.7, 270.0, 353.7) (u/g.f.wt) at salinity levels of (4, 6, 8) (dS.m⁻¹), respectively, compared to the irrigation water level of 2 dS.m⁻¹, which was 207.5 (u/g.f.wt) The reason is that increased salt concentration causes plant stress, leading to an increase in reactive oxygen species (ROS) such as H₂O₂ and O₂^{·-}. To resist these toxic compounds, the plant activates the antioxidant enzyme (POD) as a defensive mechanism; the enzyme decomposes H₂O₂, reduces oxidative damage within the cells, and protects cellular membranes from damage, thereby increasing the plant’s ability to tolerate salinity [18,19] and it agrees with the findings of [20] in his study on *Solanum lycopersicum* plants, However, in comparison to the control treatment, irrigation with high salinity levels increased

peroxidase (POD) enzyme activity to twice its value.

The results in the mentioned table indicated that foliar spraying with nano-iron oxide significantly affected the leaf content of the peroxidase enzyme, as its content increased to (244.9, 297.0, 309.3)(u/g.f.wt) compared to the control treatment, which reached 205.6 (u/g.f.wt). This is attributed to the fact that nano-iron oxide possesses peroxidase-like activity and can catalyze oxidation reactions associated with hydrogen peroxide H₂O₂, indicating its ability to enhance peroxidase enzyme activity [21,22]. This is consistent with the findings of [23] on *Artemisia scoparia*, where the POD enzyme activity value reached 4.5(U/mg protein) at a concentration of 15 mg.L⁻¹ of nano-iron oxide.

The leaf peroxidase enzyme concentration was significantly impacted by the combined interaction of the study parameters, irrigation water salinity and foliar spraying with nano-iron oxide. The F2S0 treatment had the lowest value, 100.4 (u/g.f.wt), whereas the F2S3 treatment had the greatest value, 598.0 (u/g.f.wt). The rationale is that nano-iron increased the activity of the superoxide dismutase (SOD) enzyme, enhanced plant development, and decreased oxidative stress [24]. This is consistent with [12] and is consistent with [25] in his study on *Triticum aestivum*, showing a significant effect at nan o-iron oxide concentrations of (200, 500 mg.L⁻¹) and salinity levels of (7.5, 15 dS.m⁻¹).

Table 4. Effect of foliar spraying with nano-iron oxide, irrigation water salinity levels, and their interaction on Peroxidase (POD) enzyme activity (u/g.f.wt).

F Nano-iron oxide concentration mg L ⁻¹	S Irrigation water salinity levels (dS m ⁻¹)				Mean effect of nano-iron oxide
	2	4	6	8	
0	0.321	0.247	0.225	0.305	0.274
25	0.259	0.330	0.301	0.248	0.284
50	0.314	0.323	0.172	0.335	0.286
100	0.153	0.194	0.445	0.402	0.298
Mean effect of salinity	0.261	0.273	0.285	0.322	
Experimental factors	Nano-iron oxide F		Salinity S	Binary interaction S × F	
LSD 0.05	0.0233		0.0233	0.1573	

Ascorbate Peroxidase (APX) enzyme activity (U/g.f.wt)

Ascorbate peroxidase (APX) enzyme content (U/g.f.wt) was significantly impacted by irrigation water salinity levels, according to Table 5. The APX enzyme increased as salinity levels rose, reaching (38.6, 44.5, 51.8) u/g.f.wt at salinity levels of (4, 6, 8) dS.m⁻¹, respectively, compared to the irrigation water level of 2 dS.m⁻¹, which was 36.5 u/g.f.wt. The plant increases the activity of antioxidant enzymes like APX as a result of oxidative stress brought on by higher salt concentrations. As part of the plant's defense mechanism, this enzyme is involved in the ascorbate-glutathione cycle, which deals with the buildup of hydrogen peroxide and free radicals [26]. This is consistent with the studies of [27]. Brassica napus L. study by inHe et al. in 2025 recorded a 35% increment in the APX enzyme activity at salinity level of 15 (dS. Nano-iron oxide foliar spraying increased leaf content

of ascorbate peroxidase (APX) to (36.6, 48.5, and 51.2) u/g.f.wt in comparison with treatment control 36.1 u/g.f.wt, according to the results in Table 5 [28]. By treating the plant with nano-iron oxide, which improves APX enzyme activity, you can completely restore the balance between O₂⁻ and H₂O₂ in the plant and its photo-oxidative stress, thus helping maintain a green color. This role of solanimycin in relation to salt resistance for the plant remains to be established [29]. This is in line with the findings by [30] showed that nano-iron oxide in a concentration of 1 (mg. L⁻¹).

The amount of the ascorbate peroxidase enzyme was affected by the two-way interaction of the irrigation water salinity and foliar spraying with nano-iron oxide in leaves. The highest value (63.2) u/g.f.wt for the F2S3 treatment while the lowest value (17.6) u/g.f.wt was found for the F1S1 treatment. The causal explanation is that the APX enzyme is elevated to a higher position

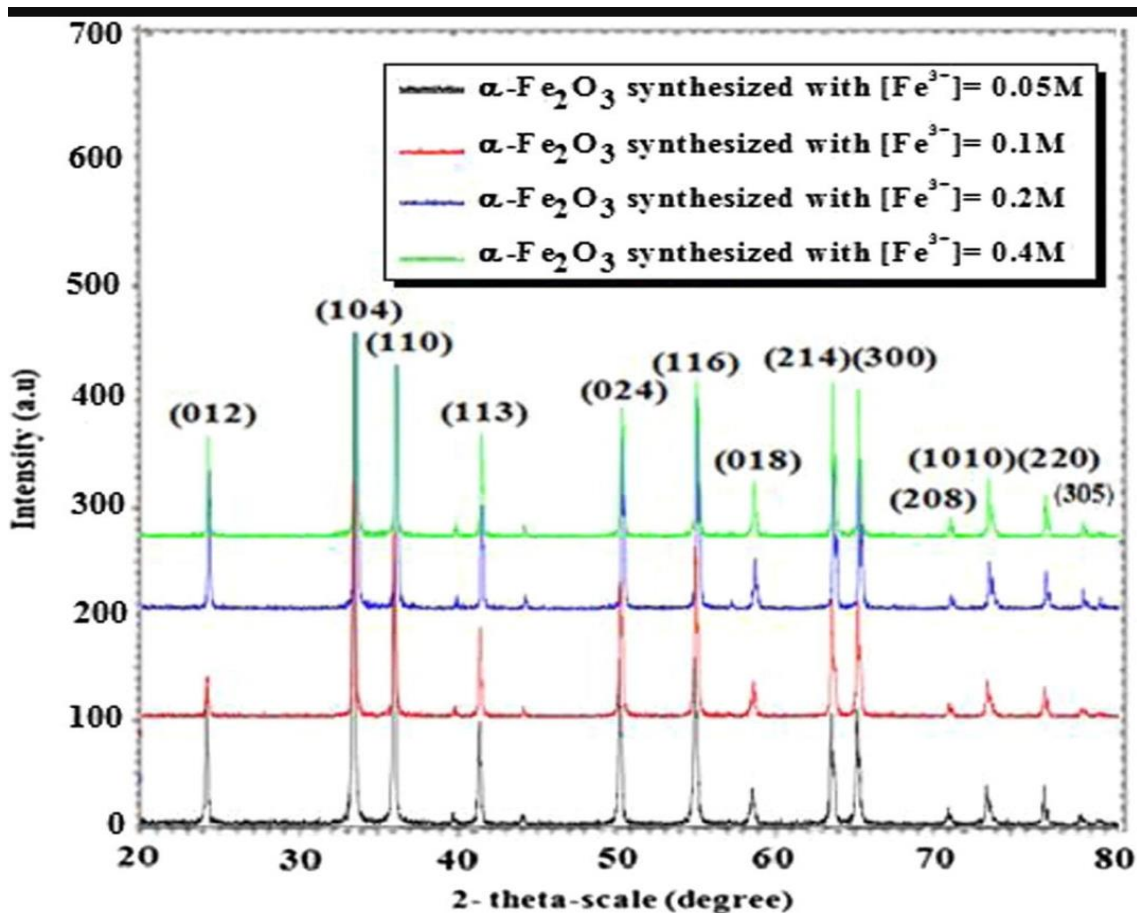


Fig. 1. X-ray diffraction (XRD) patterns of α -Fe₂O₃ nanoparticles synthesized at various Fe³⁺ precursor concentrations.

as a protective mechanism to eliminate reactive oxygen species (ROS), especially hydrogen peroxide. This activity increased further when

sprayed with nano-iron oxide [13]. These were consistent with the findings of [31] on the doses and have a significant effect with 1 mg in Phoenix

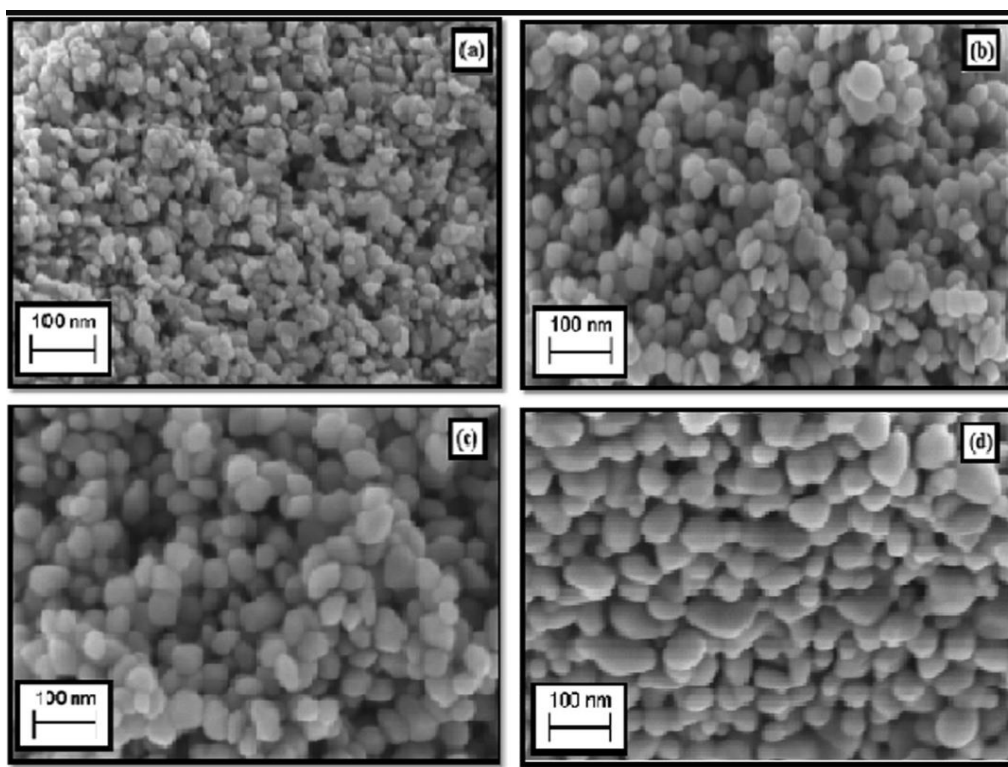


Fig. 2. SEM images of α -Fe₂O₃ nanoparticles synthesized using different Fe³⁺ concentrations.

Table 5. Effect of foliar spraying with nano-iron oxide, irrigation water salinity levels, and their interaction on Ascorbate Peroxidase (APX) enzyme activity

F Nano-iron oxide concentration mg L ⁻¹	S Irrigation water salinity levels (dS m ⁻¹)				effect of Mean nano-iron oxide
	2	4	6	8	
0	30.8	25.8	30.1	57.4	36.1
25	54.0	17.6	29.0	45.8	36.6
50	22.8	47.7	76.8	46.6	48.5
100	38.6	63.2	42.2	57.3	51.2
Mean effect of salinity	36.5	38.6	44.5	51.8	
Experimental factors	Nano-iron oxide F		Salinity S	Binary interaction S × F	
LSD 0.05	14.31		14.31	32.61	

dactylifera L. The highest increase in APX gene (OperonYR52587) was 3.28 U/g.f.wt at L-1 of nano-iron oxide.

CONCLUSION

The results obtained in this study emphasized the vital role of foliar spraying by iron oxide nanoparticles in the efficiency of the defense system of the chamomile plant in conditions of salt stress. NaCl-induced stress at different levels increases production of ROS and decreases the activity of the succus of the plant. On the contrary, iron oxide nanoparticles foliar spray increased antioxidant enzyme activities, especially APX, CAT and SOD activities, leading to oxidative damage reduction and restored cell membrane stability.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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