Influence of Nano Iron Oxide Foliar Spray on Selected Phytohormones of Chamomile (Matricaria chamomilla L.) Under Salinity Stress

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Abstract

The experiment was carried out in a pot experiment method at nursery of The Holy Karbala Governorate (Al-Hafidh Street) during winter season 2025-2026 , to study the effect of foliar application of iron oxide nanoparticles (Fe_2O_3) on some phytohormones in chamomile plant (Matricaria chamomilla L.) grown in sandy loam soil. The experiment was designed as a two factor factorial trial in a Completely Randomized Design (CRD) with four replications. The first factor was salinity in four levels (S: 2, 4, 6, and 8 dS m^{-1}), and the second factor was four concentrations of iron oxide nanoparticles (F: 0, 25, 50, and 100 mg L^{-1}). The herbicide was foliar sprayed at 4 growth stages, i.e., 6-leaf stage, 10-leaf stage, 15-leaf stage and pre-flowering stage. A total of 64 experimental units were used in the experiment. Statistical analysis of the data indicated: The individual treatments were statistically significant in improving gibberellic acid (GA), abscisic acid (ABA), proline, and relative water content (RWC%), significantly in those treated with iron oxide nanoparticles (IONPs) via foliar application. The maximum concentrations were 73.02, 199.4, and 51.40 mg kg^{-1}, and 83.04% compared to the control treatment (63.41, 159.2, and 39.81 mg kg^{-1}, and 76.38%, respectively). Gibberellic acid, abscisic acid, proline content, and relative water content were significantly different in each of the treatments according salinity stress. The maximum values were 70.16, 205.1, 45.95 mg kg^{-1}, and 80.93%, while those of from the control were 63.78, 160.3, 43.14 mg kg^{-1}, and 77.92%, respectively. Interaction results of the foliar application of the iron oxide nanoparticles and irrigation with saline irrigation concentrations (levels)for all studied traits were significant (Table 2). For the treatments S_0F_3, S_2F_0, S_3F_0, and S_2F_3, the highest values recorded were 79.02, 259.5, and 60.14 mg kg^{-1}, as well as 87.81%, respectively, while the control values were 58.00, 151.31, and 33.61 mg kg^{-1}, as well as 73.89%, respectively.

Keywords : salt stress, iron oxide nanoparticles, chamomile, phytohormones.

Introduction

Chamomile *Matricaria chamomilla* L. is presently considered one of the most popularly used medicinal plants and used a lot in primary and traditional folk medicine. The various pharmacological, cosmetic and nutritional properties of has been well characterized over many years of traditional use and scientific validation Asteraceae is a well known medicinal species and because of its excellent medicinal and medicinal properties, it has been called the "star of medicinal plants Chamomile (*Matricaria chamomilla L.*) is one of the most important medicinal plants belonging to the Asteraceae family. It is widely used in traditional and modern medicine due to its rich content of bioactive compounds such as flavonoids, terpenoids, coumarins, and essential oils. Chamomile possesses several pharmacological properties including antioxidant, anti-inflammatory,

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antimicrobial, antispasmodic, and sedative activities [1].

Salinity is one of the most destructive environmental stresses that may severely decrease the crop yield and quality throughout the world. Salinity stress affects over 20% of the arable land, which is constantly expanding due to both natural and anthropogenic causes. Through a variety of mechanisms, salinity stress affects the growth and reproduction of plants, such as the imbalance of nutrients and hormones, ionic toxicity, oxidative and osmotic stress and enhanced susceptibility to diseases [2]. The swift growth of the world population has resulted in food shortages and increased interest in ways to increase agricultural productivity, and agricultural nanotechnology has recently proven viable in this context. In the last decade, the use of nanoparticles (NPs) as nanofertilizers, or biostimulants, has been widely reported on NPs enhancing morphological and biochemical traits in a number of crops, especially in the conventional agricultural systems lacking sustainable management [3] Iron oxide nanoparticles have been characterized in previous studies to be key elements in regulating the physiological and biochemical process of plants as they adapt to salinity stress conditions. Many studies have shown that foliar application of iron oxide nanoparticles not only ameliorates hormonal homeostasis but also stimulating the gibberellic acid activity, reducing excessive accumulation of abscisic acid and enhances antioxidant defense systems in a variety of crops. For instance, Zhang et al. Moreover, [21] demonstrated that iron oxide nanoparticles can improve metabolic osmotic regulation and photosynthesis of peanut seedlings against salt stress. Similarly, Reports suggest that Fe_3O_4 nanoparticles modulated hormone signal pathways in melon plants under saline-alkali stress [40]. In addition, [13] reported that application of iron oxide nanoparticles to sweet cherry plants enhances gibberellin activity. Even so, little is known about the effect of iron oxide nanoparticles on phytohormonal responses of the chamomile plants grown under salinity stress. Thus, this research was done to evaluate the effect of iron oxide nanoparticles (Fe_2O_3) foliar application on some phytohormones of chamomile *Matricaria chamomilla* L. in different salinity stress levels

Material and Methods

1. Materials and Methods

Experiment was carried out in pot during winter season 2025–2026 using Completely Randomized Design (CRD) with 4 replications. The interaction experiment involved two-factor, in which the first factor consisted of salinity stress at levels (2, 4, 6, and 8 dS m 1) and the second factor consisted of Foliar application Of iron oxide nanoparticles (Fe_2O_3) at concentrations of (0, 25, 50, and 100 mg L^{-1}) of all four growth stages.

Plastic pots, each with 15 kg of soil, containing ten seeds were prepared on October 15, 2025. Two weeks after seedling emergence, all but four plants in pots were thinned Selected physicochemical properties were determined on soil samples collected before planting (Table 1).

Iron oxide nanoparticle solutions were prepared by dissolving a known weight of Fe_2O_3 nanopowder into distilled water, while stirring and completed with distilled

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water to 1L. Tween 20 was then added to increase the absorption efficiency of the plant . Applications were performed in early morning through a 3-L hand sprayer with a vehicle-mounted backpack sprayer [21]. Only distilled water was sprayed on control plants. We had 64 total experimental units.

1.2 Assay of Gibberellic Acid (GA) and Abscisic Acid (ABA) in Leave (mg.kg-1)

The plant growth hormones of gibberellic acid (GA) and abscisic acid (ABA) was analyzed according to the method of [4]

2.2 Proline Content in Leaves (mg kg−1);

Estimation of proline content was done using the method of [5] with a spectrophotometer as described by [6] .

3.2 Relative Water Content (RWC%) of leaves:

Relative water content was calculated according to the method of [7].

$$\text{Relative Water Content} = \frac{FW-DW}{TW-DW} \times 100$$

Where:

- **RWC** = Relative Water Content
- **FW** = Fresh weight (g)
- **DW** = Dry weight (g)
- **TW** = Turgid weight (g)

Results and Discussion

1.3 Gibberellic Acid (GA) Content mg kg^{-1}

The effects of irrigation water salinity levels on gibberellic acid (GA) content are presented in Table (2). Increasing levels of salinity induced a decrease of GA concentration that reduced to 69.18; 65.13; and 63.78 mg kg^{-1} at salinity levels 4, 6 and 8 dS m^{-1} compared to 70.16 mg kg^{-1} at 2 dS m^{-1} which is the lowest salinity level. These reductions are mainly due to high Na^{+} and Cl^{-} ion levels and consequently lead to osmotic stress reducing the absorption of water and nutrients and impairing some metabolic processes required for gibberellin biosynthesis [8]. In addition, saliva produced by salinity may increase the activity of any genes or enzyme involved in turning active gibberellins into inactive versions, and thereby lowers the amount of immense degree biologically active GA in plants [9]. This is in line with the findings of [39] or 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55) and salinized by water, i.e water with a salinity level of 5 and 20 dS m^{-1} in rice in D as well as. [10] [11]

The outcomes additionally indicated that foliar use of iron oxide nanoparticles fundamentally expanded the content of GA in the leaves. The measured levels were 65.87, 65.95, and 73.02 mg kg^{-1}, against 63.41 mg kg^{-1} in the control treatment. The increase could be explained by the activity of iron oxide nanoparticles that improved photosynthesis efficiency, energy production, and nutrient uptake of nitrogen and iron, which are key components in metabolic pathways associated with gibberellin biosynthesis [12]. These results are in agreement with [13] that indicated a raise in GA levels of Prunus avium after applying iron oxide nanoparticles at 10 mg L^{-1}

Enormous connection between irrigation water salinity and foliar utility of iron oxide nanoparticles became additionally observed. The different GA contents ranged from 58.00-79.02 mg kg^{-1}, with the highest value (79.02 mg kg^{-1}) appearing in F_3 S_0 treatment and the lowest value being recorded (58.00 mg kg^{-1}) in F_0 S_3 treatment. The following interaction effect might be discussed based on the patriarchal assumption of salinity stress.

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2.3 Abscisic Acid (ABA) Content in Leaves (mg kg^{-1})

It is well known that iron application affects hormonal balance in plants by decreasing gibberellin levels and inhibiting growth, while that by foliar spraying may balance this suppression and keep the supply of a more functional source which is used for growth regulation under the condition of salinity stress so that plants will adapt more to the saline environment [14].

There were also a significant effect of irrigation water salinity levels on abscisic acid (ABA) content in leaves as illustrated in Table (3). With increasing salinity levels, ABA concentrations increased sharply, at the salinity of 4, 6 and 8 dS m^{-1} to the level of 163.0, 204.5 and 205.1 mg kg^{-1}, while in the control treatment (2 dS m^{-1}) the ABA level was 160.3 mg kg^{-1}. This increase could be explained as increase salinity lowers one of uptake of water by root and lowers also water content in cell. Interestingly, in response, plants increase ABA biosynthesis in both roots and leaves, which promotes stomatal closure, reduces transpiration and minimizes water loss to maintain the cellular water status [15] . Higher ABA levels also stimulate transduction of intracellular signals, mainly through kinase cascades and regulatory proteins, eventually leading to the transcription of stress-responsive genes related to osmotic adjustment and the cellular defense against salinity stress [16] [17]. The results are in keeping with those reported by [18]. on *Tricticum aestivum* L. grown on 20 dS m^{-1} high salinity for 6 days followed by increased ABA accumulation and [10] [19] [11]

Statistical analysis also showed that ABA content of leaves significantly affected with different levels of iron oxide nanoparticles foliar application, as ABA content decrease in the leaves 198.6, 176.0, and 159.2 mg kg^{-1} compared to 199.4 mg kg^{-1} in control treatment. This might be due to the effect of iron oxide nanoparticles on plant tolerance to salinity stress through enhancing photosynthetic efficiency, maintaining ionic balance in cells and reducing oxidative damage, all of which decrease ABA synthesis as the stress on the plant probably became less [20] . With the salinity levels and foliar application of iron oxide nanoparticles, a high interaction effect was observed, and the maximum value of ABA is recorded in treatment F_2 S_0 (259.5 mg kg^{-1}).

In relation to the lowest value recorded in the treatment F_3 S_1 , which reached 151.3 mg kg^{-1}, the quantity can be related to the action of iron oxide nanoparticles, which can act in enhancing the activity of antioxidant enzymes, including catalase, peroxidase and ascorbate peroxidase. These enzymes lower reactive oxygen species exhibited in the setup of oxidative stress which leads to decrease in the real signal of abscisic acid (ABA) accumulation. Under these conditions, iron oxide nanoparticles not only horseshoe the regulatory target of plant physiology but also are involved in modulating the ABA signalling pathway, acting by avoiding excess accumulation of ABA, which otherwise promotes growth inhibition, [21]. This observation was consistent with the results of the study conducted by [40]. Also, these results are in line with who marked that foliar application of 100 mg L^{-1} iron oxide nanoparticles improved *Cucumis melo* L. seedling growth and salinity stress tolerance.

3.3 Proline content (mg kg−1):

Results in Table (4) indicated a significant effect for salinity levels on proline content. The increase in salinity levels increased the leaf proline content reaching values (mg kg -1) of 43.86, 45.28 and 45.95 mg kg^{-1} at 4, 6 and 8 dS m^{-1}, respectively, compared to the lowest 43.14 mg kg^{-1} at the lowest

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salinity level (2 dS m⁻ ¹). The content of proline increased under salinity stress [22] [23] [24] is a small, water-soluble amino acid with a major role in stabilizing cellular osmotic pressure, in minimizing water loss, and also in protecting proteins, enzymes, and cellular membranes from damage These results are comparable to those of [25] [26] also reported significant correlation of proline accumulation with plant survival (up to 0.86) and stated that proline accumulation is one of the key factors for plant tolerance and survival in stress environment.

Statistical analysis results presented in the table below showed that the foliar spraying of nano iron oxide had a significant effect on the proline content of leaves (mg kg^{-1}), as its content on three levels (44.30, 42.59 and 39.81 mg kg^{-1}) is less than that 51.40 mg kg^{-1} recorded in the control treatment. This decrement, may be due to the utilisation of nano iron oxide in regulating ionic equilibrium, augmenting photosynthetic efficacy as well as stimulating antioxidant defence mechanisms, resulting in reduced oxidative stress and proline accumulation level [27]. This finding correlates with that previously reported by [28] who reported that proline concentration decreased by 43.9260–5650% in 100 mg L^{-1} iron oxide nanoparticles32 rice plants (treated plants) as compared to untreated salt-stressed plants.

The interaction of the two experimental factors: irrigation water salinity, foliar application of nano iron oxide on leaf proline content was very significant. The maximum value was produced from F_0S_3 which was 60.14 and the minimum from F_1S_0 that was 33.61 mg kg^{-1}. Foliar application of nano iron oxide on salt-stressed plants influences physiological and biochemical responses, and modifies osmolytes such as proline through better nutrient balance, antioxidant defense systems and by alleviating the negative effects of salinity stress [29] this probably explains the kinetics of the effect of foliar treatment on proline accumulation that was observed in this study. This is in line with the work of [30] used iron oxide and zinc oxide to activate both enzymatic and non-enzymatic antioxidants under salt stress so as to enhance plant defense mechanisms, especially proline.

4.3 Relative Water Content (RWC) (%)

As mentioned in table (5) data represented a highly significant influence of salinity of irrigation water treatments on relative water content (RWC%). The leaf relative water content showed a decline in its trend with a progressive increase in salinity levels which decreased from (80.93 - 80.18, 80.07 and 77.92%) for salinity levels (2-4, 6 and 8 dS m^{-1}) respectively. This reduction might be due to high concentrations of salt salts in soil reduce soil water potential and increase the adhesion of water molecules to ions, reducing the ability of roots to absorb water. Moreover, the resulted osmotic adjustment response by increased salinity outside the cell in order to move water molecules from inside to outside the cells causes the plants with less capacity to hold water which reduces relative water content in leaves [31] [32] . These findings are similar to that of [33] also reported reduced leaf relative water content in chamomile plants on salinity levels (1, 10 and 15 dS m^{-1}, with salinity).

The statistical analysis showed that foliar application of nano iron oxide significant difference on relative water content (%) as it elevated (78.25, 81.43, and 83.04%) comparing to the control treatment which recorded (76.38%) Table (5). The increase can be related to the use of iron in photosynthesis or other increase in cellular activities that lead to a higher osmotic potential in the cells (these change may increase the cells' ability to retain water [34]. For e.g., it enhances water relations of plants and relative water content in leaves [35] . This is in line with both [36]demonstrated that spraying iron nanoparticles at 50 mg L^{-1} on stressed chamomile plants raised relative water content by 21% compared to untreated plants.

This effect showed interaction with irrigation water salinity and foliar application of nano iron oxide. The value was the highest in treatment F_3S_2 which achieved 87.81%, and the lowest value was reached in treatment F_0S_0 which reached 73.89%. This could be due to presence of nano-iron oxide for potassium, iron uptake and sodium accumulation. This helps to increase water retention and restore osmotic balance in salt-stressed cells, as well as activate antioxidant systems that maintain membrane integrity and enhance the water-retaining ability of plant cells [37] . These findings are consistent with [38]have described the positive effect of iron nano-based foliar application (50 mg L^{-1}) on the relative water content and the overall growth of Arachis hypogaea L. under salinity (15 dS m^{-1}) stress, with an increase in the growth of the plants by 20.13–68.67 dS m^{-1} . [39] [40]

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

Property		Value	Unit
Ph		7.5	-----
Ece y Electrical conductivity		1.7	$dS.m^{-1}$
OrganicMatter		6.7	$g.Kg^{-1}soil$
$CaCO_3$		251.02	
Soluble Cations	Ca^{++}	3.43	$meg.L^{-1}$
	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	$mg.kg^{-1}soil$
Available Phosphorus		12.60	
Available Potassium		16.05	
Soil Separates	Sand	545	$g.Kg^{-1}soil$
	Silt	282	
	Clay	173	
Texture	Loamy sand		-----

*Analyzes in soil analysis laboratories at the Facuity of Agriculture University of Baghdad.

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Table (2): Effect of Foliar Application of Iron Oxide Nanoparticles and Irrigation Water Salinity Levels and Their Interaction on Gibberellic Acid (GA) Content (mg kg⁻ ¹) in Chamomile Plants

Iron Oxide Nanoparticle Concentration (mg L⁻ ¹)	Irrigation Water Salinity Levels (dS m⁻ ¹)				Mean of Iron Oxide Nanoparticles F
	2	**4**	**6**	**8**	
0	67.41	65.16	63.07	58.00	63.41
25	66.97	67.27	64.53	64.71	65.87
50	67.24	68.15	63.14	65.28	65.95
100	79.02	76.13	69.79	67.12	73.02
Mean of Salinity Levels	70.16	69.18	65.13	63.78	
Experimental Factors	Iron Oxide Nanoparticles		Salinity	**Interaction between Iron Oxide Nanoparticles and Salinity Levels**	
LSD 0.05	29.80		29.80	59.60	

Table (3): Influence of Foliar Spraying of Iron Oxide Nanoparticles and Irrigation Water Salinity Levels Their Interaction on Abscisic acid (ABA)(mg kg⁻ ¹) at Chamomile Plants

F Iron Oxide Nanoparticle Concentration (mg L⁻ ¹)	S Irrigation Water Salinity Levels (dS m⁻ ¹)				Mean of Iron Oxide Nanoparticles F
	2	4	6	8	
0	151.7	155.9	259.5	230.7	199.4
25	169.7	175.3	220.6	228.7	198.6
50	167.9	169.7	177.7	188.7	176.0
100	152.7	151.3	160.4	172.5	159.2
Mean of Salinity Levels S	160.3	163.0	204.5	205.1	
Experimental Factors	F: Iron Oxide Nanoparticles		Salinity	**Interaction between Iron Oxide Nanoparticles and Salinity Levels**	
LSD 0.05	29.80		29.80	59.60	

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Table 4: Effect of foliar application by nano iron oxide and irrigation salinity levels of water and their interaction in proline content mg kg^{-1} leaves of chamomile plant.

Iron Oxide Nanoparticle Concentration (mg L^{-1})	Irrigation Water Salinity Levels (dS m^{-1})				Mean of Iron Oxide Nanoparticles
	2	4	6	8	
0	51.26	45.95	48.52	60.14	51.40
25	33.61	42.32	58.19	43.41	44.30
50	46.95	45.48	37.80	40.07	42.59
100	40.76	41.69	36.62	40.20	39.81
Mean of Salinity Levels	43.14	43.86	45.28	45.95	
Experimental Factors	Iron Oxide Nanoparticles		Salinity	**Interaction between Iron Oxide Nanoparticles and Salinity Levels**	
LSD 0.05	29.80		29.80	59.60	

Table(5) Effect of foliar application with nano iron oxide, different levels of salinity irrigation in water and their interaction on relative water content (RWC)% in chamomile plant.

Iron Oxide Nanoparticle Concentration (mg L^{-1})	Irrigation Water Salinity Levels (dS m^{-1})				Mean of Iron Oxide Nanoparticles
	2	**4**	**6**	**8**	
0	73.89	76.72	75.83	79.11	76.38
25	79.60	78.71	79.12	75.57	78.25
50	85.07	82.51	77.55	80.60	81.43
100	85.16	82.78	87.81	76.42	83.04
Mean of Salinity Levels	80.93	80.18	80.07	77.92	
Experimental Factors	Iron Oxide Nanoparticles		Salinity	Interaction between Iron Oxide Nanoparticles and Salinity Levels	

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Conclusion

Based on the results obtained, it is clear that foliar spraying with iron oxide nanoparticles had a significant effect on modifying the physiological and biochemical responses of chamomile plants under salt stress conditions. Different concentrations of iron oxide nanoparticles contributed to improving plant hormone balance, as an increase in growth-promoting hormones such as gibberellin was observed, along with a relative decrease in the stress hormone (abscisic acid) as the treatment concentration increased up to a certain point. The results also showed that increased salt stress led to a disruption of hormonal balance, characterized by increased accumulation of abscisic acid and a decrease in growth-promoting hormones. However, treatment with iron oxide nanoparticles helped mitigate these negative effects, especially at moderate concentrations, indicating its role in enhancing the plant's ability to adapt to harsh environmental conditions.

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