

Arginine-capped magnetite nanoparticles improve wheat growth and grain yield under salinity stress

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Abstract

Salinity stress affects the growth and biochemical characteristics of cereal crops, ultimately reducing agricultural productivity. This study investigated the effects of foliar application of arginine nano-magnetite conjugate on wheat growth and grain yield under saline conditions. A pot experiment was conducted during the wheat growing season 2023-2024 by using a completely randomized design (CRD) with three replications. For this purpose, 20 day-old plants were exposed to salt stress at 150 mM NaCl and after 30 days, foliar spray of Fe nanoparticles at control, nano-magnetite (50 mg L⁻¹), and arginine-capped nano-magnetite (50 mg L⁻¹) was performed. The data for various morpho-biochemical traits was collected after 15 days of spray. The results indicated that the application of Fe-nanoparticles improved growth traits of wheat under salt stress. Salinity stress negatively impacted all the indicators including soluble sugars. The yield attributes were also negatively affected by salt stress. In contrast, the exogenous application of arginine nano-magnetite improved soluble proteins, phenolics and leaf photosynthetic pigments, which caused improvements in 100-grain weight. Overall, the detrimental effects of salt stress on wheat plants were mitigated by the foliar spray of Fe-NPs. This study highlights that arginine nano-magnetite application to salt-stressed wheat plants could induce salt tolerance and recover crop yield.

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Introduction

Increased salt levels in the soil limit water intake and cause oxidative damage to plants which lower agricultural output and crop quality (Saleem et al., 2023; Dixit et al., 2024). Prominent salinity-induced factors affecting plant development on saline soils include ion toxicity, osmotic stress and nutritional issues, which increase the impact of salt stress on plants (Ragaey et al., 2022). With the rising global population and increased food demand, it is imperative to develop sustainable agricultural techniques that might lessen the detrimental effects of salinity on crop productivity. Wheat is the second most widely grown food crop globally, and it is one of the most essential edible cereals for human use. While China leads the world in wheat production with over 24 million hectares of croplands (El-Kassas et al., 2020), Pakistan is one of the top 10 wheat producing nations (Shah et al., 2024).

Nutrients are necessary for plant growth and net primary productivity, whereas their absence might restrict the development of plants. Among various nutrients, micronutrients are required by plants to perform physiological functions vital for growth and survival (Feng et al., 2022). Not only plants, but micronutrient deficiencies also affect one-third of the world's population, and Zn and Fe

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are the most prevalent mineral deficiencies (Kiani et al., 2022). It is reported that around 20% of deaths in children under five are caused by micronutrient deficiencies (Akman, 2023). Iron is a micronutrient that is necessary for several metabolic and biosynthetic activities including plant growth regulation and chlorophyll biosynthesis (Iannone et al., 2016). Iron is particularly important for healthy and green foliage, and it also improves wheat quality and production. Although, the amount of Fe is excessive in soils, plants cannot take it easily (Nasibi, 2016). However, plants growing on alkaline soils frequently experience iron deficiency symptoms due to Fe-unavailability (Hassan et al., 2023). By contrast, the application of nano-iron in different forms may effectively recover plant performances under Fe deficiency through improvement in plant Fe status (Konate et al., 2017).

In contrast to bulk counterparts, nanoparticles are smaller in size and can improve plant growth under stressful conditions (Wang et al., 2021). The absorption and transport of NPs in plants relies on numerous variables including particle size, surface modification, exposure duration and plant species (Khan et al., 2019). NPs can enter the plant system through stomata, root hairs, leaf spraying, branch injection and seed treatment (Cele, 2020). Iron fertilizers based on NPs have a lot of potential in agriculture, which can serve as a physiologically accessible iron pool for soil-grown plants (Sharma et al., 2023).

During the current study, we investigated the effects of arginine-capped Fe-nanoparticles on wheat growth. It is pertinent to mention here that metabolism of arginine is crucial for the distribution and recycling of nitrogen (Slocum, 2005; Winter et al., 2015). The only necessary limiting factor is nitrogen (Hussein et al., 2022). Arginine makes up 40-50% of the total energy stored in seeds (Li et al., 2024). It is the main source of nitrogen storage in trees as well as in the underground storage organs and roots of other plants (Ragaey et al., 2022). Keeping in view these reports, we hypothesized that the foliar application of arginine-capped Fe-nanoparticles could improve Fe and N nutrition in salt stressed wheat leading to improvements in growth and grain yield. Thus, the primary objective of the current investigation was to assess that up to what extent arginine-capped Fe-nanoparticles would improve growth and yield as well as nutritional status of wheat plants grown in saline regimes.

Materials and Methods

Experimental design

A pot experiment was conducted from November 2023 to April 2024 at the Botanical Garden of the Government College University, Lahore with three replicates arranged in a completely randomized design. Wheat seeds (cv. Subhani-21) were acquired from the Federal Seed Certification and Registration Department. Surface sterilized seeds were sown in the plastic pots that were filled each with 9 kg soil. After seed germination, five seedlings per pot were retained. The seedlings were exposed to control (no NaCl added) and 150 mM NaCl stress (applied gradually to prevent osmotic shock) through half-strength Hoagland's nutrient solution.

Foliar spray of capped-iron Nanoparticles

The foliar application of Fe-nanoparticles was performed at 0 and 50 mg/L concentrations. For this, 0.05 g of nano-magnetite and arginine nano-magnetite each were suspended in 100 mL de-ionized water and then sonicated for 10 minutes at 40 Hz to avoid any particle aggregation. After sonication, 1-2 drops of tween-20 were added to the solution, and the foliar application was performed after 30 d of salinity stress.

Analyses of plant biomass

Data was collected at the vegetative and maturity stages, that for various growth and biochemical parameters were recorded at the vegetative stage. Growth analysis included root length, root fresh and dry weights, shoot length, shoot fresh and dry weights, number of leaves, leaf area, and number of tillers per plant.

Determination of biochemical parameters

Photosynthetic pigments

Fresh fully expanded leaves from the plants in each treatment were used to determine the amount of chlorophyll. The Arnon (1949) method was used to determine the chlorophyll and carotenoid contents. For this purpose, 0.1 g of fresh leaf sample was weighed and cut into small pieces and 5 mL of 80% acetone were added to each of the plastic bottles and placed them in a

freezer for 24 h. Absorbance of the filtrate was recorded at 663, 645 and 480 nm using a spectrophotometer.

Phenolics

The phenolics were determined using the method described by Bray and Thorpe (1954). For this test, FC reagent and Na₂CO₃ were used. An aliquot of 1 mL leaf alcoholic extract was mixed with 1 mL FC reagent (1:15) and the mixture was added to test tubes. After that, an aliquot of 2 mL of sodium carbonate was added to each of test tubes and the tubes were incubated at room temperature for 30 minutes in a water bath. Finally, the absorbance was recorded at 765 nm using a spectrophotometer.

Flavonoids

The method described by Pekal and Pyrzyńska (2014) was used to determine total flavonoid content. Plant alcoholic extracts, each 1 mL was taken in a test tube. After that, 0.3 mL aluminum chloride (10%) and 0.3 mL of sodium nitrate (5%) were added to each test tube. Afterwards, 2 mL of sodium hydroxide (4%) were added to the test tubes followed by vortexing. The absorbance of all reaction mixtures was recorded at 510 nm using a spectrophotometer.

Soluble sugars

The soluble sugar content was estimated using the anthrone method (Yemm and Willis, 1954). For this, an aliquot of 2 mL of anthrone reagent was mixed with 1 mL of leaf alcoholic extract and then the mixture was incubated in a water bath for 5 min at 95 °C. The absorbance of the extract was recorded at 620 nm using a spectrophotometer.

Amino acids

Plant-alcoholic extracts were used to determine the concentration of amino acids using ninhydrin and pyridine as described elsewhere (Hamilton et al., 1943). Briefly, 1 mL of extract was mixed with 1 mL of ninhydrin reagent (2%) followed by the addition of 1 mL of pyridine solution (10%) and mixed. The test tubes were placed in a boiling water bath for 30 minutes. The OD of the resulting purple solution was recorded at 570 nm.

Soluble proteins

The Bradford reagent (Bradford, 1976) was used for the determination of soluble proteins. Fresh leaves, 0.2 g each sample, were extracted with 10 mL of 100 mM potassium phosphate buffer (pH 7.8) and centrifuged at 7000 rpm. For protein estimation, 200 µL of the extracts were added to 5 mL of the Bradford reagent and the mixture was vortexed. After incubation for 15 minutes, the absorbance of the treated samples was recorded at 595 nm.

Yield attributes

At maturity stage, various growth parameters such as spike weight, spike length, spikelets per spike, and 100-grain weight were recorded.

Statistical analysis

The experiment was conducted in triplicates following the Complete Randomized Design. The data were statistically analyzed using the two-way ANOVA test at $P \leq 0.05$ to examine the differences between the variables.

Results

Plant biomass

Foliar application of magnetite and arginine-doped magnetite at 50 mg/L enhanced the shoot dry and fresh weights under salinity stress. In the control and salt-stressed plants, no significant differences were noted ($P \leq 0.05$; Table 1), however, the foliar spray of magnetite treatments caused the roots to grow shorter than that of the control plants ($P \leq 0.05$). Fresh and dry weights of roots decreased under salt stress conditions, but there had been non-significant difference between the treatments. The foliar application of Fe₃O₄ NPs caused an increase in leaf number under control conditions ($P \leq 0.05$). The control wheat plants had larger leaf area than the salt-stressed plants, although this difference was non-significant ($P \leq 0.05$; Table 1).

Table 1. Influence of arginine-doped magnetite nanoparticles on growth attributes of salt stressed wheat plants

Levels	Treatments	Shoot length (cm)	Shoot FW (g)	Shoot DW (g)	No. of leaves
Control	No Spray	81.8 ^c ± 2.61	29.3 ^b ± 0.66	8.66 ^{bc} ± 0.66	26.0 ^a ± 4.50
	Fe ₃ O ₄ NPs	84.6 ^b ± 2.18	28.6 ^b ± 5.17	8.33 ^{bc} ± 1.33	26.0 ^a ± 3.51
	Arg-Fe ₃ O ₄ NPs	84.2 ^{bc} ± 0.38	24.3 ^b ± 4.09	6.66 ^c ± 1.66	22.0 ^b ± 1.52
Salinity (150 mM NaCl)	No Spray	77.5 ^d ± 2.29	25.6 ^b ± 2.90	7.76 ^{bc} ± 0.90	27.0 ^a ± 3.21
	Fe ₃ O ₄ NPs	89.2 ^a ± 0.80	38.0 ^a ± 6.42	11.66 ^a ± 1.45	23.0 ^b ± 2.51
	Arg-Fe ₃ O ₄ NPs	87.7 ^a ± 2.66	36.0 ^a ± 3.21	10.0 ^{ab} ± 1.52	24.66 ^{ab} ± 1.45
Levels	Treatments	Leaf area (cm ²)	Root length (cm)	Root FW (g)	Root DW (g)
Control	No Spray	38.7 ^a ± 2.45	9.16 ^{bc} ± 0.92	9.0 ^a ± 1.00	5.66 ^a ± 1.20
	Fe ₃ O ₄ NPs	38.6 ^a ± 5.43	9.63 ^{bc} ± 0.87	8.66 ^a ± 2.18	4.66 ^a ± 1.66
	Arg-Fe ₃ O ₄ NPs	34.5 ^{ab} ± 2.83	12.7 ^a ± 1.44	7.66 ^{ab} ± 2.18	4.33 ^{ab} ± 1.33
Salinity (150 mM NaCl)	No Spray	34.5 ^{ab} ± 2.97	9.06 ^{bc} ± 0.57	6.33 ^b ± 0.66	4.26 ^{ab} ± 0.93
	Fe ₃ O ₄ NPs	33.5 ^{ab} ± 7.07	8.63 ^c ± 0.58	8.66 ^a ± 0.33	5.0 ^a ± 0.57
	Arg-Fe ₃ O ₄ NPs	27.7 ^b ± 5.62	10.2 ^b ± 0.26	4.0 ^c ± 0.57	2.50 ^b ± 0.26

Mean ± SE; Different letters within a column indicate statistically significant difference

Biochemical traits

Chlorophyll

Plants under salt stress showed higher levels of chlorophyll *a* than those in the control plants. However, significant improvements ($P \leq 0.05$) were recorded when arginine-magnetite was applied compared to the untreated controls. Although it was non-significant across all treatments, generally, chlorophyll *b* increased under salinity conditions. In case of total chlorophyll, the control plants exhibited higher levels than those in the salt-stressed plants, and the arginine-magnetite treatment resulted in notable improvements ($P \leq 0.05$; Table 2). The foliar application of Arg-Fe₃O₄ NPs increased the chlorophyll *a/b* content in the control wheat plants. Furthermore, the carotenoid content in the salt stressed wheat plants showed a slight increase with respect to that in the control plants. The Car/Chl ratio in response to foliar application of nanoparticles increased under salinity stress ($P \leq 0.05$; Table 2).

Table 2. Influence of arginine acid-doped magnetite nanoparticles on photosynthetic pigments of salt-stressed wheat plants

Levels	Treatments	Chl <i>a</i> (mg/g FW)	Chl <i>b</i> (mg/g FW)	Total Chl (mg/g FW)
Control	No Spray	0.74 ^b ± 0.15	0.15 ^c ± 0.02	0.89 ^c ± 0.13
	Fe ₃ O ₄ NPs	1.07 ^a ± 0.09	0.16 ^c ± 0.01	1.24 ^b ± 0.11
	Arg-Fe ₃ O ₄ NPs	0.79 ^b ± 0.29	0.19 ^{bc} ± 0.09	0.98 ^c ± 0.27
Salinity (150 mM NaCl)	No Spray	0.40 ^c ± 0.05	0.24 ^{ab} ± 0.015	0.65 ^d ± 0.04
	Fe ₃ O ₄ NPs	1.09 ^a ± 0.05	0.26 ^a ± 0.011	1.36 ^{ab} ± 0.06
	Arg-Fe ₃ O ₄ NPs	1.28 ^a ± 0.27	0.23 ^{ab} ± 0.017	1.52 ^a ± 0.29
Levels	Treatments	Chl <i>a/b</i> ratio	Carotenoids (mg/g FW)	Car/Chl ratio
Control	No Spray	5.36 ^{bc} ± 1.64	0.051 ^{bc} ± 0.01	0.05 ^b ± 0.007
	Fe ₃ O ₄ NPs	6.45 ^{ab} ± 0.39	0.06 ^{ab} ± 0.005	0.05 ^b ± 0.000
	Arg-Fe ₃ O ₄ NPs	7.05 ^a ± 2.83	0.054 ^{bc} ± 0.02	0.054 ^b ± 0.01
Salinity (150 mM NaCl)	No Spray	1.71 ^d ± 0.29	0.03 ^c ± 0.01	0.07 ^a ± 0.009
	Fe ₃ O ₄ NPs	4.09 ^c ± 0.12	0.07 ^{ab} ± 0.00	0.05 ^{bc} ± 0.001
	Arg-Fe ₃ O ₄ NPs	5.32 ^{bc} ± 0.82	0.08 ^a ± 0.01	0.08 ^a ± 0.002

Mean ± SE; Different letters within a column indicate statistically significant difference

Metabolites

Free amino acids slightly decreased in the control plants as compared to those in the salt-stressed plants. Furthermore, free amino acids progressively decreased in the treated plants. The foliar application of Fe₃O₄ NPs and Arg-Fe₃O₄ NPs caused non-significant changes in leaf soluble sugars of both control and salt-stressed wheat plants (Table 3). In the salt stressed plants, the total soluble proteins exhibited a slight increase than that in the control plants. In addition, both nano-magnetite and arginine-magnetite spray treatments caused a significant ($P \leq 0.05$) increase in the total soluble proteins. Moreover, the control plants had higher amount of flavonoids than that in the salt-stressed plants ($P \leq 0.05$; Table 3).

Table 3. Influence of arginine-doped magnetite nanoparticles on leaf biochemical traits under salt stress

Levels	Treatments	Phenolics (mg GAE/100 g DW)	Total soluble sugars (mg/g FW)	Free amino acids (mg/g FW)
Control	No Spray	12.2 ^c ±0.46	8.01 ^{ab} ±0.45	56.5 ^a ±7.18
	Fe ₃ O ₄ NPs	16.4 ^b ±4.49	7.95 ^{ab} ±0.18	43.9 ^{bc} ±7.91
	Arg-Fe ₃ O ₄ NPs	16.0 ^b ±2.44	7.29 ^b ±0.53	41.5 ^c ±1.83
Salinity (150 mM NaCl)	No Spray	14.3 ^{bc} ±1.81	8.06 ^a ±0.60	47.6 ^b ±3.95
	Fe ₃ O ₄ NPs	16.7 ^d ±3.17	7.50 ^{ab} ±0.89	33.4 ^d ±1.41
	Arg-Fe ₃ O ₄ NPs	19.5 ^a ±1.18	7.29 ^b ±0.21	35.3 ^d ±7.15

Levels	Treatments	Soluble proteins (mg/g FW)	Flavonoids (mg QE/100 g DW)
Control	No Spray	0.34 ^{abc} ±0.007	1.52 ^b ±0.11
	Fe ₃ O ₄ NPs	0.34 ^c ±0.004	1.97 ^a ±0.208
	Arg-Fe ₃ O ₄ NPs	0.34 ^{bc} ±0.006	1.34 ^{bc} ±0.202
Salinity (150 mM NaCl)	No Spray	0.36 ^a ±0.003	1.34 ^{bc} ±0.26
	Fe ₃ O ₄ NPs	0.33 ^c ±0.004	1.00 ^d ±0.21
	Arg-Fe ₃ O ₄ NPs	0.35 ^{ab} ±0.004	1.25 ^c ±0.06

Mean ± SE; Different letters within a column indicate statistically significant difference

Yield attributes

Salinity stress significantly ($P \leq 0.05$) reduced the number of spikelets per spike in the wheat plants (Figure 1). However, the spike length and 100-grain weight remained unaffected. Foliar application of arginine-nano magnetite at 50 mg/L improved the number of spikelets per spike and 100-grain weight (Figure 1).

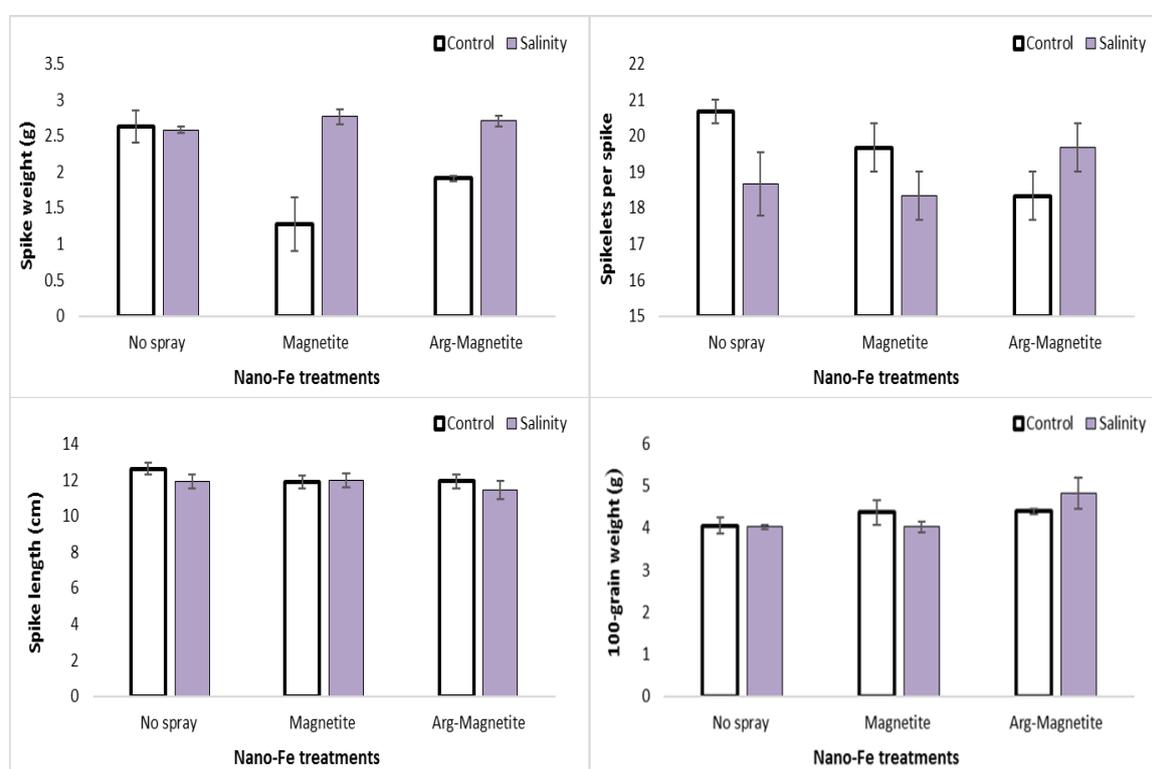


Figure 1. Influence of arginine-capped magnetite nanoparticles on yield attributes of salt stressed wheat plants

Discussion

In the present study, the wheat plants exposed to salinity stress exhibited a prominent reduction in the shoot length and root fresh biomass. Although, all the other growth parameters experienced negative effects of salinity stress, the changes were non-significant. Consistent with this, the salinity stress induced perturbations in growth of wheat plants have been reported earlier (Hasegawa, 2013; Shafiq et al., 2018, 2021). Basically, soil salinity impairs plant growth and development by affecting key metabolic functions through ion disequilibrium, Na⁺ toxicity and

excessive generation of ROS (Munns et al., 2020). Nano-magnetite treatments had a beneficial effect on wheat plant growth. Our data showed that in wheat plants, when the foliar spray of arginine-doped nano-magnetite was applied, the length of shoot increased; when comparing the treated and the untreated plants the shoot fresh and dry weights were better in the treated plants. Additionally, the foliar sprays of Fe₃O₄ and Arg-Fe₃O₄ NPs improved the development of the roots of wheat plants, but they did not affect number of leaves and leaf area. Also, in the non-stressed control plants, the number of tillers were also enhanced by the foliar treatment of NPs. In agreement with these findings, a previous study (El-Saber et al., 2021) demonstrated that salt stress had a negative impact on plant development, but treatments including Fe₃O₄ NPs improved the growth parameters. They showed how the application of nano-magnetite could mitigate negative effects of the salt stress on wheat plant development. Similar to this, *Majorana hortensis* seedlings treated with magnetite or in combination with silicon had significantly higher fresh and dry shoot weights and plant height than those of the untreated plants (Badawy et al., 2014). Iron is essentially required by plants for the photosynthetic electron transport chain and as a component of reaction centers (Balk et al., 2014; Boulard et al., 2019). However, plants cultivated on saline and alkaline soils often experience Fe-deficiency. The Fe-NPs mediated improvements in the wheat growth attributes under salinity stress could have been due to the improvements in Fe nutrition and better redox status of the chloroplast.

Salinity stress caused a considerable reduction in chlorophyll *a* and total chlorophyll contents in the wheat plants. However, an increase in chlorophyll *b* was recorded. In comparison with the control plants, the ratio of Chl *a/b* decreased in the foliar sprayed and salt stressed plants. Salinity induced reduction in the photosynthetic pigments has been well reported and linked to salt-induced physio-biochemical disturbances (Mohamed et al., 2017; Feghhenabi et al., 2022; Xin et al., 2024). Our data suggest that the arginine-doped magnetite supplementation significantly recovered the chlorophyll *a* and total chlorophyll contents in the wheat plants exposed to salinity stress. Furthermore, the control plants also had higher chlorophyll *a* due to nano-magnetite application. Moreover, carotenoids and total chlorophyll were also significantly affected by the foliar spray of Fe-nanoparticles. Consistent with our findings, the exogenous application of Fe₃O₄ nanoparticles improved photosynthetic pigments in salt-stressed wheat plants (Kreslavski et al., 2023). Similarly, Fe nanoparticles recovered chlorophyll in strawberry (Mozafari et al., 2018), spearmint (Hassanpouraghdam et al., 2023), maize (Fathi et al., 2017), and multiple other plant species. Enhancements in the photosynthetic pigments in the wheat plants under salinity stress was recorded in response to ZnO-NPs (Lalarukh et al., 2017). Therefore, exogenous application of nanoparticles can improve photosynthetic pigments in leaves.

There was a prominent reduction in leaf free amino acid concentration in the wheat plants under salinity stress. However, the concentration of total soluble sugars, soluble proteins, phenolics, and flavonoids remain unchanged under salinity. Moreover, wheat plants treated with nano-magnetite and arginine-magnetite under salt stress had higher phenolic contents. In this context, the application of Fe-nanoparticles has been shown to improve metabolic profile of *Moringa oleifera* under salinity stress (Tawfik et al., 2021).

Here in this study, we recorded a significant reduction in number of spikelets per spike of wheat plants exposed to salinity stress. Moreover, there was a slight reduction in the spike length as well, although it was statistically non-significant. It is well reported that the plants exposed to salinity stress experience a significant reduction in yield attributes and overall productivity (Iqbal and Ashraf, 2013; Munns and Gilliam, 2015; Munns et al., 2020; Shafiq et al., 2021). Most noticeably, the foliar spray of arginine-nano magnetite at 50 mg/L concentration improved the number of spikelets per spike, spike weight and 100-grain weight of wheat. Similar to this, the exogenous application of Fe-nanoparticles improved yield and salinity stress tolerance of ajowan (Ghassemi-Golezani and Abdoli, 2024). Interestingly, seed pre-treatment with glutamic-acid-functionalized iron nanoparticles conferred osmotic stress tolerance in *Vigna radiata* (Ul Haq et al., 2023). Likewise, Dola et al. (2022) reported that the nano-iron oxide improved growth and stress response of *Glycine max* under drought stress. Overall, these reports together with our findings suggest that the exogenous application of nano-iron oxide could be a beneficial approach to mitigate the harmful effects of salinity stress on different crops.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors

Planning and conduction of experiment: AF, FS. Conduction of research: AF. Data collection, visualization and interpretation: AF. Graphical presentation/visualization: AF, FS. Statistical analysis: AF. Preparation of initial draft: AF, FS. Review of initial draft: FS. Proof reading and approval of the final version: AF, FS. Revisions and corrections: AF, FS.

Ethical approval

This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material

No supplementary material is included with this manuscript.

Conflict of interest

The authors declare no conflict of interest.

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent

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It is declared that the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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