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Research Article

Role of iron oxide nanoparticles in maize (*Zea mays* L.) to enhance salinity stress tolerance

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Abstract

Soils have been getting worse over time, which has led to lower crop yields and nutritional value. This is because of too many conventional fertilizers, anthropogenic activities, and climate change. Soil salinity is also a big problem and challenge for agricultural scientists. To address this issue, nanoparticles are gaining a reputation in agriculture that can enhance salinity tolerance in crops, especially at early growth stages. A pot experiment was conducted at Post Agricultural Research Station (PARS), Faisalabad to assess the impact of iron oxide nanoparticles (0, 15, and 30 ppm) and four levels of salinity (0, 50, 100 and 150 mM) on morphological, physiological and yield traits of maize (*Zea mays* L.) in salinity stress. Salinity stress significantly negatively affected the growth attributes, photosynthetic pigments, ion content (Na^+, K^+, and Ca^{2+}) of maize plants. Salinity has also increased the levels of MDA, H_2O_2, and Na^+ ions. The application of iron oxide nanoparticles through foliar spray had a notable impact in enhancing the growth and yield of the tested maize variety. It was achieved by promoting the activities of antioxidant enzymes, increasing photosynthetic pigments, and elevating K^+ and Ca^{2+} ion levels under both normal and salinity-stressed conditions. Additionally, iron oxide nanoparticles mitigated the adverse effects of salinity stress by effectively reducing Na^+ ion concentration, MDA levels, and H_2O_2 concentration. Among the different concentrations tested, 30 ppm of iron oxide nanoparticles proved best in alleviating the negative impacts of salinity stress in maize. Thus, field use of 30 ppm iron oxide nanoparticles as foliar spray could effectively mitigate salinity stress in maize.

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Keywords: antioxidants; FeO-NPs; ion homeostasis; maize; NaCl

Introduction

Maize (*Zea mays* L.) is among the most important cereals, cultivated after rice and wheat in the world (Seleiman *et al.,* 2017; Anwar *et al.*, 2022; Dimkpa *et al.*, 2023). This versatile crop can be grown over a diverse range of climatic conditions and is being used as animal feed , human diet and as a raw material for most of the industrial products (Seleiman *et al.,* 2012; Seleiman *et al*., 2013; Vijay *et al.*, 2022). It is cultivated in over 130 nations and is referred to as a "queen of cereals" because of its highest yield potential among cereals (Suganya *et al.*, 2020). Maize is gaining prominence due to its potential applications in the production of carbohydrates, resins, ethanol, sugar syrup and other products, in addition to its use as fodder and as a source of food (Rehman *et al.*, 2015; Ali *et al.,* 2023; Seleiman *et al.,* 2023b). In Pakistan, maize is cultivated on 1,720 thousand hectares having a production of 10,183 thousand tonnes with an improvement of 4.1% from than the previous year (GOP, 2023). Maize being the third most important cereal, contributes to 0.7% to gross domestic production (GDP) (GOP, 2023). Maize is a staple food for over 200 million people; however, the world population is continuously increasing and is expected to be over 8 billion by 2025. Moreover, it has the highest yield potential among cereals, it may be among the most important crops for overcoming global nutrition challenges (Rehman *et al.*, 2015).

Besides its importance in various aspects of life, its global production faces various challenges resulting from biotic and abiotic factors including extreme heavy metals, salinity, drought temperatures. These challenges lead researchers to emphasize prolonged ecological stability and improve crop versatility in such climatic conditions (He *et al.*, 2018). Yield reduction in maize due to abiotic stresses ranges from 50% to 70% (Dimkpa *et al.*, 2023). Among other environmental abiotic factors, salinity significantly threatens plant development and yield, particularly in arid climates (Taha *et al.,* 2021; Alhammad *et al.,* 2023b). Salt stress in plants is caused by three major factors like osmotic stress, nutritional imbalance and toxic ion exposure (Arif *et al.*, 2020; Seleiman *et al.,* 2023a; Alenazi *et al.,* 2024). Osmotic stress, toxic ion exposure, and nutritional imbalance are three major factors responsible for causing salt stress in plants (Seleiman *et al.,* 2020; Ibrahimova *et al.*, 2021). To address this problem, plants adopt different mechanisms as solute accumulation and toxic ions sequestration in certain tissues to preserve usual metabolic activity (Rahman *et al.*, 2022; Seleiman *et al.,* 2022). Osmotic imbalance and ion toxicity are caused by salinity stress due to increased Na^+ ion absorption and reduction in the Na^+/K^+ ratio in plant roots (Ahmad *et al.,* 2023; Asghar et al., 2023), caused by reduced osmotic potential (Rahman *et al.*, 2022). Moreover, it results in stomatal closure, which affects the chlorophyll content and ultimately reduces the photosystem I and II efficiency and the imbalance between ROS and antioxidant activities (Lima-Melo *et al.*, 2019; Zahra *et al.*, 2022). Salts concentrated in soil solution reduce water potential due to osmotic impact, which is considered an initial prerequisite for both cell division as well as turgidity (Yadav *et al.*, 2020; Alkharabsheh *et al.,* 2021).

There is a wide range of strategies for minimizing salinity effects in plants; among them, nanoparticles are gaining a reputation in agriculture that can enhance salinity tolerance in crops (Zulfiqar *et al.*, 2021; Elshayb *et al.,* 2022; Alhammad *et al*., 2023a). Nanoparticles are incredibly tiny particles with diameters that vary between 1 to 100 nm (Seleiman *et al.,* 2021). They have distinct physicochemical qualities that are unique to the same substance of larger sizes (Cele, 2020). Various findings have shown that nanoparticles have ability to provide salt resistance in various crop plants (Avestan *et al.*, 2019). In plants iron oxide NPs reduce salinity stress through a variety of mechanisms, such as ion homeostasis regulation, improved water absorption, and enhanced activity of antioxidants. In several studies, Iron oxide NPs have been demonstrated to boost growth and production under salt stress in various plants. According to Aazami *et al.* (2021), the administration of iron oxide NPs to tomato plants cultivated in saline conditions increased plant growth and photosynthesis. The potential of iron oxide NPs to aid in absorption of water, enhanced antioxidant enzyme activity, and preserve ion homeostasis in plant tissues was considered to be accountable for the beneficial effects observed in tomato plants administered iron oxide NPs in salt stress. Junedi *et al.* (2023) proved that applying iron oxide NPs to wheat plants facing salt stress responsible for increasing growth and yield. Therefore, the aim of this study is to provide a thorough investigation of how different doses of iron oxide NPs may encourage the growth and physio-chemical mechanism of maize plants under various saline environments.

Materials and Methods

Crop management

To investigate the response of maize to foliar application of iron oxide nanoparticles under saline conditions, an experiment was carried out in the botanical garden at Post Agricultural Research Station (PARS), Faisalabad. Seeds of maize variety 'H-636' were collected from the Ayoub Agricultural Research Institute, Faisalabad. The seeds were sown in plastic pots with each having 38 cm diameter containing 7 kg sand each, following the completely randomized design with three replicates. Sowing was done on 15 September. Ten seeds of maize were sown in each pot and three plants were maintained by thinning after fifteen days of sowing. Plants were grown under varying levels of salinity (0 mM, 50 mM, 100 mM and 150 mM) which was applied after 7 days of germination with increment of 50mM in equal interval of one day to attain 150mM salinity and sodium chloride (NaCl) was used for salinity imposition, and after one week of salinity application iron oxide (Fe_2O_3) nanoparticles were foliarly applied in three different concentrations (0, 15 and 30 ppm). During growth plants were supplied with nutrients, after 21 days of sowing in two different doses with an interval of 1 week, using Hoagland's nutrient solution till harvest. After completion of 21 days application of iron oxide nanoparticles data for different morpho-physiological attributes was recorded. Two plants from each replication were carefully removed and cleaned root and shoot length (cm) and fresh biomass (g) of both root and shoot were measured. Plants were kept in sunlight for 7 days and then at 65 °C oven dried. When fully dried dry biomass (g) of both shoots and roots were recorded from each replication. Additionally, the following attributes were analyzed using different protocols.

Iron oxide (Fe_2O_3) nanoparticles

For this study, iron oxide (Fe_2O_3) nanoparticles were prepared by adopting the methodology previously described in literature with few modifications (Debnath *et al.*, 2016). In a typical experimental procedure, 0.75 moles of NaOH were dissolved in 1 L of deionized water at room temperature to make a clear solution. Similarly, 0.15 moles of $FeCl_3.6H_2O$ were dissolved in 250 ml of deionized water by stirring at room temperature. Thereafter, ferric chloride solution was added dropwise into sodium hydroxide solution with gentle magnetic stirring and pH of the reaction mixture was set to 8.5. The reaction mixture turned brown which was an indication of formation of $Fe_2(OH)_3$ precipitates. After continuous stirring for 15 mins, the precipitate was separated by centrifugation (at 1000 rpm) and was washed several times with deionized water to remove the unreacted chemicals and to neutralize the pH to 7. The precipitates were then dried in hot air oven at 85 °C for 24 hours and then annealed at 400 °C for 8h and cooled down to room temperature slowly. The final brown product of iron oxide (Fe_2O_3) was grinded with mortar and pestle and was converted into a fine homogenous powder form before it was used in rest of the experiments after redispersing in water. The nanoparticles were characterized by transmission electron microscopy for their size and morphology.

Photosynthetic pigments

Carotenoids and chlorophyll *a* and *b* contents were determined by following the methods ascribed by (Arnon, 1949) . Fresh leaves weighing 0.1 g were crushed and put in sterilized plastic bottles having 10 ml of 80% acetone solution. After keeping overnight in dark at room temperature reading was noted at 480, 645 and 663 nm.

Hydrogen peroxide (H_2O_2) and antioxidants

Following Alexieva *et al.* (2001), standardization of fresh leaf sample (0.5 g), in chilled conditions was carried out with TCA, to measure hydrogen peroxide contents, and then was homogenized with K-phosphate buffer (100 mM), TCA (5 mL) and KI (2 mL) was observed at 390 nm.

To measure SOD activities procedure adopted by Giannopolitis *et al.* (1977) was followed. The sample mixture was in cuvettes in addition to specific amounts of riboflavin, L-methionine, Enzyme extract, Triton - X, NBT, H_2O and phosphate buffer, then cuvettes were heated for 15 minutes below a fluorescent lamp and reading was noted at 560 nm.

Following the Chance *et al.* (1955) methodology 50 mM of K_2HPO_4 buffer was used to standardize the 0.5 g of leaf sample. The peroxidase activity was measured by preparing the mixture of potassium phosphate buffer (Ph 7), H_2O_2 (40 mM), 0.1 ml of enzyme extract and guaiacol (20 mM), at 470 nm for 20 s. Likewise, the CAT activity was measured using the mixture containing 5.9 mM H_2O_2 0.1 ml of enzyme extract and 50 mM of potassium phosphate buffer and absorbance was measured at 240 nm after every 20 s.

Nutrient analysis

The acid digestion method for determination of nutrient contents was used as proposed by Wolf *et al.* (1982). Dried 0.1 g sample was digested by keeping overnight at room temperature in digestion flask containing 2 ml of H_2SO_4 and 1 ml of H_2O_2 and heating at 250 °C resulted in fumes and solution become colorless. After filtration the volume of each sample was made 50 mM with distilled water. Using a flame photometer, the mineral ions were measured.

Secondary metabolites

To measure total phenolics 80% of acetone was used to standardize the 0.5 g of fresh leaf sample, centrifuged and homogenate obtained was observed at 280 nm, as described by Ainsworth *et al.* (2007).

Riboflavin was extracted using the Okwu *et al.* (2006) method. A 0.1 g sample was homogenized in 2 mL of 50% ethanol and filtered. After a one-hour water bath, 1 mL of filtrate was mixed with 5% $KMnO_4$ and 30% H_2SO_4. After adding 0.2 mL of Na_2SO_4, the reaction mixture was diluted with distilled water up to 5 mL. After 5 minutes, the upper colorless layer was aspirated and its absorbance at 510 nm was measured with a spectrophotometer. As a control, 50% ethanol was used.

Singh *et al.* (2006) described a method for calculating alkaloid content. A plant sample of 0.1 g was extracted in 1 mL of methanol prior to being diluted with distilled water. 0.01 M sodium metaperiodate (SP1) and 0.5 mL acetic acid were added to 1 mL of extract. After boiling, 0.01 M 3-metyle-2-benzothiazol (MBIT) solution was added to the mixture. Extracted samples were placed in a microfuge tube after being placed in for 20 minutes in a water bath. The level of absorbance at 470 nm was measured after the sample was cooled to room temperature.

Flavo was calculated employing an approach proposed by Zhishen *et al.* (1999). A fresh sample of 0.1 grams was thoroughly standardized in 80% acetone. The test tubes were then filled with 1 mL solution and 4 mL distilled water. After waiting 5 minutes, 0.6 mL of 5% $NaNO_2$ and 0.5 mL of 10% $AlCl_3$ were put into each of the tubes. After a minute, 2 mL of 1 M NaOH was added. A 2.4 mL distilled water was used to dilute the reaction mixture. At 510 nm, absorbance was measured. We used 80% acetone for blank. The concentration of ascorbic acid within each sample was determined by plotting a standard curve with known ascorbic acid concentrations.

Statistical analysis

A complete block design (CRD) under a factorial arrangement having three replications was used. The analyzation and evaluation of data was done by using a statistical package (Statistix 8.1). HSD test was used to compare the treatment means.

Results

Growth parameters

Graphical data representing shoot dry weight displayed significant ($P \leq 0.05$) differences under SS and foliar treatments, though, interaction among them noted non-significant ($P > 0.05$) differences. Under SS, percent decrease for shoot dry weight (SDW) was up to 13.9 to 49.46% as compared to normal conditions, however, the Fe_2O_3 treatments by foliar application enhanced the (SDW) under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in shoot dry weight were as 12.66 and 14.23 % under 50 mM SS, 65.33 and 67.59% under 100 mM SS and 20.74 and 42.23% under 150 mM SS as compared to their respective controls (Table 1).

Significant ($P \leq 0.05$) differences in SS, and nanoparticle treatments were depicted for shoot fresh weight but, interaction between them was noted to be non-significant ($P > 0.05$). Under SS, percent decrease for shoot fresh weight up to 14.2 to 56.8% as compared to normal conditions, however, the Fe_2O_3 application increased SFW under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in shoot potassium were as 8.7 and 13.8% under 50 mM SS, 25.84 and 34.84% under 100 mM SS and 20.89 and 26.79% under 150 mM SS as compared to their respective controls (Table 1).

There was significant ($P \leq 0.05$) difference in salt stress, and nano particle treatments for root fresh weight but, interaction among these was non-significant ($P > 0.05$). Under SS, decrease trend for root fresh weight control < 50 mm SS < 100 mM SS, and 150 mM SS, however, the foliar application of iron oxide treatments increased the root fresh weight under all normal and stress condition. The trends of the percent increase in root fresh weight were as 10.97% (15 ppm Fe_2O_3) and 17.70% (30 ppm Fe_2O_3) under 50 mM SS, 8.4% (15 ppm Fe_2O_3) and 18.30% (30 ppm Fe_2O_3) under 100 mM SS and 18.31% (15 ppm Fe_2O_3) and 29.18% (30 ppm Fe_2O_3) under 150 mM SS as compared to their respective controls (Table 1).

A significant ($P \leq 0.05$) difference in SS, and nanoparticles for root dry weight was depicted by graphical data but, interaction between these was found to be non-significant ($P > 0.05$). Under SS, percent decrease for root dry weight up to 8.07 to 58.81% as compared to normal conditions, however, Fe_2O_3 treatments enhanced the RDW under all normal and stress condition. However, under SS the order for the root dry weight was obtained higher in 15 ppm Fe_2O_3 > 30 ppm Fe_2O_3 > 50 mM SS, 15 ppm Fe_2O_3 > 30 ppm Fe_2O_3 > 50 mM SS, and 15 ppm Fe_2O_3> 30 ppm Fe_2O_3> 50 mM SS (Table 1).

Root length depicted significant ($P \leq 0.05$) differences in salinity stress, and nanoparticle application, though, interaction among them noted non-significant ($P > 0.05$). Under salinity stress, percent decrease for root length up to 19.30 to 28.30% as compared to normal conditions, however, the foliar application of iron oxide treatments increased the root length under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in root length were as 6.49 and 9 % under 50 mM SS, 8.65 and 11.27% under 100 mM SS and 7.18 and 12.51% under 150 mM SS as compared to their respective controls (Table 1).

Graphical data depicted the significant ($P \leq 0.05$) increase in shoot length, when nanoparticles were applied under salt stress but, interaction between these was noted non-significant ($P > 0.05$) differences. Shoot length reduced from 21.82 to 52.50% when compared with control under salinity stress, however, the foliarly applied Fe_2O_3 nanoparticles increased the shoot length under both saline and control conditions. The percent increase in shoot length was 14.71 and 16.60% when 15 and 30 ppm Fe_2O_3 was applied under 50 mM SS, 19.02 and 26.82% under 100 mM SS and 32.29 and 45.97% under 150 mM SS when compared to their respective controls (Table 1).

Table 1. The influence of iron oxide nano particles on growth of root and shoot parameters of maize crop

Root length (cm)					**Shoot length (cm)**				
Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl
Control	21.3±0.26 b	17.2±0.52 d-f	16.65±0.98 e-g	15.27±0.35 g	Control	56.5±3.5 bc	44.1±1.33 e	34.16±1.15 g	26.8±7.52 h
15 ppm Fe_2O_3	23.2±1.13 a	18.3±0.67 cd	18.08±1.03 c-e	16.36±0.85 d-f	15 ppm Fe_2O_3	60.6±1.75 ab	50.6±0.76 d	40.66±1.25 ef	35.5±3.5 fg
30 ppm Fe_2O_3	23.1±0.72 a	18.7±1.65 c	18.52±0.34 cd	17.18±0.77 fg	30 ppm Fe_2O_3	62.8±2.08 a	51.5±3.6cd	43.33±0.57 e	39.2±2.46 e-g
Significance					Significance				
Nano particle (N)	0.000 **				Nano particle (N)	0.000 **			
Saline (S)	0.000 **				Saline (S)	0.000 **			
N×S	0.976ns				N×S	0.711 ns			
Root Fresh weight (g)					**Shoot Fresh weight (g)**				
Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl
Control	3.76±0.17 bc	3.27±0.40 c-f	2.91±0.11 ef	2.33±0.05 g	Control	18.65±4.32 ab	16.01±1.55 bc	11.75±1.2 de	8.05±0.76 f
15 ppm Fe_2O_3	3.96±0.27 ab	3.63±0.20 b-d	3.16±0.23 d-f	2.75±0.51 fg	Fe_2O_3 15 ppm	19.59±1.31 a	17.72±2.04 a-c	14.79±1.8 cd	9.74±3.17 ef
30 ppm Fe_2O_3	4.35±0.49 a	3.85±0.12 ab	3.45±0.19 b-e	3.01±0.65 ef	Fe_2O_3 30 ppm	20.48±0.52 a	18.05±0.85 ab	15.85±3.1 bc	10.22±0.74 ef
Significance					Significance				
Nano particle (N)	0.001**				Nano particle (N)	0.019 *			
Saline (S)	0.000 **				Saline (S)	0.000 **			
N×S	0.997 ns				N×S	0.968 ns			
Root Dry weight (g)					**Shoot Dry weight (g)**				
Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl
Control	1.91±0.14 a-c	1.75±0.32 bc	1.02±0.47 de	0.78±0.37 e	Control	7.29±0.44 ab	6.18±0.48 bc	3.09±0.65 d	2.23±0.26 d
Fe_2O_3 15 ppm	2.12±0.24 ab	1.91±0.29 a-c	1.25±0.14 d	1.21±0.16 de	Fe_2O_3 15 ppm	7.84±1.75 ab	6.96±1.87 ab	5.12±0.09 c	2.69±0.41 d
Fe_2O_3 30 ppm	2.28±0.23 a	2.10±0.14 ab	1.47±0.28 cd	1.29±0.19 d	Fe_2O_3 30 ppm	8.46±0.36 a	7.06±0.43 ab	5.19±1.19 c	3.17±1.53 d
Significance					Significance				
Nano particle (N)	0.003 **				Nano particle (N)	0.012*			
Saline (S)	0.000 **				Saline (S)	0.000 **			
S×N	0.987ns				S×N	0.826 ns			

Photosynthetic pigments and carotenoids

Graphical data for chlorophyll *a* depicted significant ($P \leq 0.05$) differences in saline conditions, and nanoparticles application, though, interaction among them noted non-significant ($P > 0.05$). Under salt stress, percent decrease for chlorophyll *a* up to 10.31 to 18.31% as compared to normal conditions, however, the foliar application of iron oxide treatments increased the chlorophyll *a* under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in chlorophyll *a* were as

6.19 and 23% under 50 mM SS, 7.07 and 21.21% under 100 mM SS and 7.77 and 26.66% under 150 mM SS as compared to their respective controls (Figure 1).

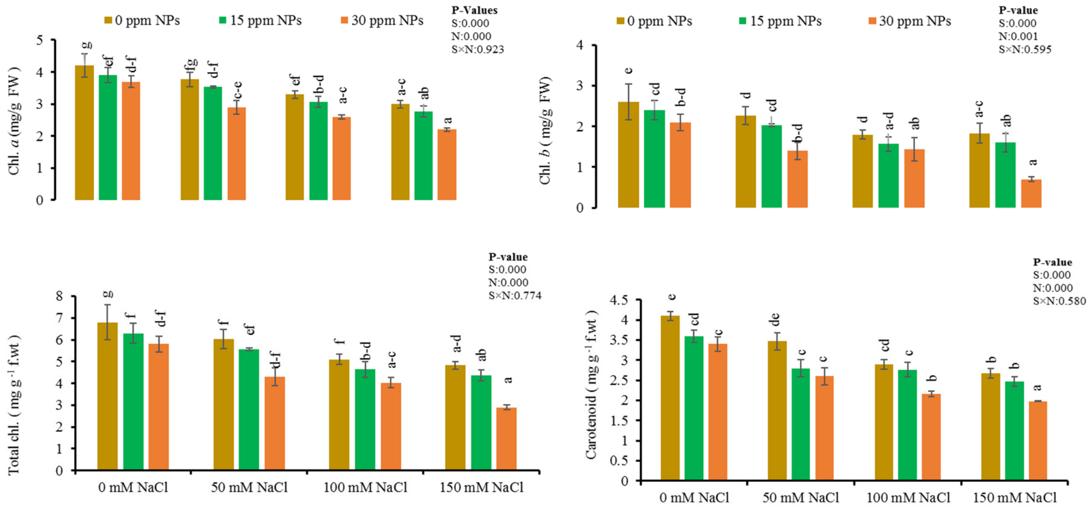

Figure 1. The influence of iron oxide nanoparticles on chlorophyll contents in maize crop
(Error bars = Standard errors, and bars with different letters (a, b, c, etc) are significantly differed from each other as indicated by Tukey test ($p \leq 0.05$)

There was significant ($P \leq 0.05$) difference in salt stress, and nanoparticles application for chlorophyll *b* but, interaction between these was found to be non-significant ($P > 0.05$). Under SS, decrease trend for chlorophyll *b* control < 50 mm SS < 100 mM SS, and 150 mM SS, however, the foliar application of iron oxide treatments increased the chlorophyll *b* under all normal and stress condition. The trends of the percent increase in chlorophyll *b* were as 10.29% (15 ppm Fe_2O_3) and 38.23% (30 ppm Fe_2O_3) under 50 mM SS, 12.96% (15 ppm Fe_2O_3) and 20.37% (30 ppm Fe_2O_3) under 100 mM SS and 12.72% (15 ppm Fe_2O_3) and 61.81% (30 ppm Fe_2O_3) under 150 mM SS as compared to their respective controls (Figure 1).

A significant ($P \leq 0.05$) difference in salinity, and nanoparticles application for total chlorophyll was depicted by graphical data but, interaction between these was found to be non-significant ($P > 0.05$) differences. Under SS, percent decrease for total chlorophyll up to 11.27 to 28.92% as compared to normal conditions, however, the Fe_2O_3 application increased the total chlorophyll under all normal and stress condition. The percent increase in total chlorophyll was 7.73 and 28.72% when 15 and 30 ppm Fe_2O_3 was applied under 50 mM SS, 9.15 and 20.91% under 100 mM SS and 9.65 and 40% under 150 mM SS when compared to their respective controls (Figure 1).

Significant ($P \leq 0.05$) differences in salinity, and nanoparticle applications were depicted by graphical data for carotenoids but, interaction between these was noted non-significant ($P > 0.05$) differences. Under SS, percent decrease for carotenoids up to 15.44 to 34.95% as compared to normal conditions, however, the Fe_2O_3 treatments increased the carotenoids under all control and stressed conditions. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in carotenoids were as 19.23 and 25 % under 50 mM SS, 4.59 and 25.28% under 100 mM SS and 7.5 and 25.75% under 150 mM SS as compared to their respective controls (Figure 1).

Nutrients contents

Graphical data for sodium in roots depicted the significant ($P \leq 0.05$) difference, when nanoparticles were applied under salt stress but, interaction between these noted non-significant ($P > 0.05$) differences. Root sodium increased from 18.46 to 52.30% when compared with control under salinity stress, however, the foliarly applied Fe_2O_3 nanoparticles increased the root sodium under both saline and control conditions. The percent decrease in root sodium was 15.58 and 22.07 when 15 and 30 ppm Fe_2O_3 was applied under 50 mM SS, 11.23 and 17.97 % % under 100 mM SS and 9.09 and 14.14% under 150 mM SS when compared to their respective controls (Table 2).

Table 2. The influence of iron oxide nano particles on root and shoot ions in maize crop

Root Ca^{2+} ($\mu g\ g^{-1}$ DW)					**Shoot Ca^{2+} ($\mu g\ g^{-1}$ DW)**				
Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl
Control	17±2 a-c	13.66±2.08 d-f	11.33±2.51 ef	8±1.2 g	Control	16.66±1.52 a-c	13.66±1.154 c-e	12±1 c-f	9.66±3.05 f
15 ppm Fe_2O_3	19±2.64 ab	16±1 b-d	14±1.73 c-f	11±1.73 fg	15 ppm Fe_2O_3	19±0.98 ab	15.66±0.57 b-d	13±2.64 c-f	11±2.1 ef
30 ppm Fe_2O_3	20±0.96 a	17.66±1.52 ab	14.33±0.57 c-e	12±2.64 ef	30 ppm Fe_2O_3	20.33±2.51 a	18.66±2.08 ab	15.33±3.51 b-d	12±3.21 d-f
Significance					Significance				
Nano particle (N)	0.000 ***				Nano particle (N)	0.000 ***			
Saline (S)	0.000***				Saline (S)	0.000 ***			
S×N	0.788 ns				S×N	0.789 ns			
Root Na^+ ($\mu g\ g^{-1}$ DW)					**Shoot Na^+ ($\mu g\ g^{-1}$ DW)**				
Treatments	Control	Treatment	Control	Treatment	Treatments	Control	Control	Treatment	Control
Control	21.67±1.53 ef	Control	21.67±1.53 ef	Control	Control	21.67±1.53 ef	21.67±1.53 ef	Control	21.67±1.53 ef
15 ppm Fe_2O_3	18.33±1.5 fg	15 ppm Fe_2O_3	18.33±1.5 fg	15 ppm Fe_2O_3	15 ppm Fe_2O_3	18.33±1.5 fg	18.33±1.5 fg	15 ppm Fe_2O_3	18.33±1.5 fg
30 ppm Fe_2O_3	16.66±0.54 g	30 ppm Fe_2O_3	16.66±0.54 g	30 ppm Fe_2O_3	30 ppm Fe_2O_3	16.66±0.54 g	16.66±0.54 g	30 ppm Fe_2O_3	16.66±0.54 g
Significance					Significance				
Nano particle (N)	0.000**				Nano particle (N)	0.000**			
Saline (S)	0.000 **				Saline (S)	0.000 **			
S×N	0.999 ns				S×N	0.999 ns			
Root K^+ ($\mu g\ g^{-1}$ DW)					**Shoot K^+ ($\mu g\ g^{-1}$ DW)**				
Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	Treatments	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl
Control	31.33±1.52 bc	26.33±4.93 de	21±1 fg	15±1.73 h	Control	31±2.64 bc	26.66±3.78 de	21±1 fg	15.66±0.57 h
15 ppm Fe_2O_3	34±3.60 ab	30±2.64 cd	23.33±2.51 ef	18.66±1.52 gh	15 ppm Fe_2O_3	33.32±0.5 ab	29±1.73 cd	22.67±1.52 e-g	18.67±1.53 gh
30 ppm Fe_2O_3	35.66±2.08 a	30.66±1.15 bc	25.66±0.58 e	19±1.22 g	30 ppm Fe_2O_3	36±1 a	30.33±4.16 b-d	24.33±2.08 ef	20.33±4.72 fg
Significance					Significance				
Nano particle (N)	0.000 **				Nano particle (N)	0.002 **			
Saline (S)	0.000 **				Saline (S)	0.000**			
N×S	0.991 ns				N×S	0.996ns			

There was a significant ($P \leq 0.05$ differences displayed in sodium in shoot in SS and foliar treatments, though, interaction was noted non-significant ($P > 0.05$) differences. Under SS, percent increase in shoot sodium was up to 66.66 to 100% as compared to normal conditions, however, the foliarly applied Fe_2O_3 increased the shoot sodium under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent decrease in shoot sodium were as 17.5 and 27.5% under 50 mM SS, 10.86 and 25% under 100 mM SS and 13.54 and 17.70% under 150 mM SS as compared to their respective controls (Table 2).

A significant ($P \leq 0.05$) difference in salinity, and nanoparticle application for calcium in root was depicted by graphical data but, interaction was found to be non-significant ($P > 0.05$) differences. Under SS, percent decrease for root calcium was up to 19.60 to 52.94% as compared to normal conditions, however, the foliarly applied Fe_2O_3 treatments increased the root calcium under all controlled and stressed conditions. However, under SS the order for root calcium was obtained higher in 15 ppm Fe_2O_3 > 30 ppm Fe_2O_3 > 50 mM SS, 15 ppm Fe_2O_3> 30 ppm Fe_2O_3 > 50 mM SS, and 15 ppm Fe_2O_3 > 30 ppm Fe_2O_3 > 50 mM SS (Table 2).

There was significant ($P \leq 0.05$) difference in salinity, and nano particle treatments for calcium in shoot but, interaction among them was found to be non-significant ($P > 0.05$) differences. Under SS, decrease trend for shoot calcium control < 50 mm SS < 100 mM SS, and 150 mM SS, however, the foliar application of iron oxide treatments increased the shoot calcium under all normal and stress condition. The trends of the percent increase in shoot calcium were as 14.63% (15 ppm Fe_2O_3) and 36.58% (30 ppm Fe_2O_3) under 50 mM SS, 8.33% (15 ppm Fe_2O_3) and 27.7% (30 ppm Fe_2O_3) under 100 mM SS and 13.79% (15 ppm Fe_2O_3) and 24.13% (30 ppm Fe_2O_3) under 150 mM SS as compared to their respective controls (Table 2).

Graphical data for potassium in root displayed significant ($P \leq 0.05$) differences in SS and foliar treatments, though, interaction among them noted non-significant ($P > 0.05$) differences. Under SS, percent decrease for root potassium was up to 15.95 to 52.12% as compared to normal conditions, however, the foliar application of Fe_2O_3 treatments increased the root potassium under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in root potassium were as 13.92 and 16.45% under 50 mM SS, 11.11 and 22.22% under 100 mM SS and 24.44 and 26.66% under 150 mM SS as compared to their respective controls (Table 2).

Significant ($P \leq 0.05$) differences in salt stress, and nano particle treatments were depicted by graphical data for potassium in shoot but, interaction among them noted non-significant ($P > 0.05$) differences. Under SS, percent decrease for shoot potassium up to 13.97 to 49.46% as compared to normal conditions, however, the foliar application of Fe_2O_3 treatments increased the shoot potassium under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in shoot potassium were as 8.75 and 13.75% under 50 mM SS, 7.93 and 15.87% under 100 mM SS and 19.14 and 29.78% under 150 mM SS as compared to their respective controls (Table 2).

Antioxidants markers

A significant ($P \leq 0.05$) difference in salt stress, and nano particle treatments for H_2O_2 was depicted by graphical data but, interaction among them was found to be non-significant ($P > 0.05$) differences. Under SS, percent increase for H_2O_2 was up to 5.40 to 44.59% as compared to normal conditions, however, the foliar application of Fe_2O_3 treatments decreased the H_2O_2 under all normal and stress condition. However, when Fe_2O_3 was applied, the order for H_2O_2 was obtained higher in 15 ppm Fe_2O_3> 30 ppm Fe_2O_3 > 50 mM SS, 15 ppm Fe_2O_3 > 30 ppm Fe_2O_3 > 50 mM SS, and 15 ppm Fe_2O_3 > 30 ppm Fe_2O_3 > 50 mM SS (Figure 2).

Graphical data for MDA depicted the significant ($P \leq 0.05$) difference, when nanoparticles were applied under salt stress but, interaction among them noted non-significant ($P > 0.05$) differences. The MDA increased from 31.70 to 63.41% when compared with control under salinity stress, however, the foliarly applied Fe_2O_3 nanoparticles decreased the MDA under both saline and control conditions. The percent decrease in MDA was 7.40 and 14.81 when 15 and 30 ppm Fe_2O_3 was applied under 50 mM SS, 10 and 13.33% under 100 mM SS and 10.44 and 13.43% under 150 mM SS when compared to their respective controls (Figure 2).

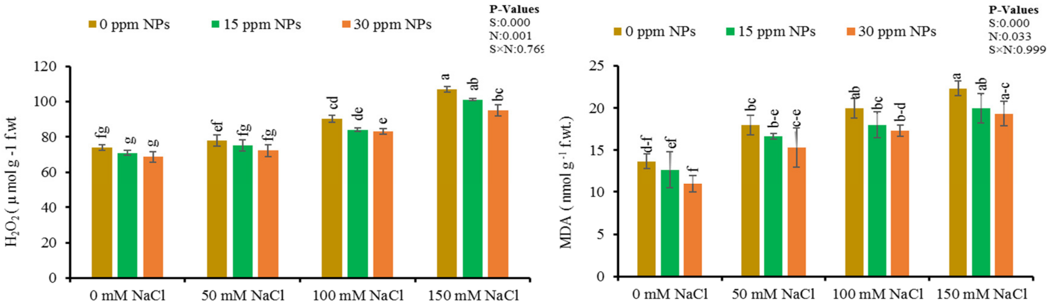

Figure 2. The influence of iron oxide nano particles on reactive oxygen species in maize crop
(Error bars = Standard errors, and bars with different letters (a, b, c, etc.) are significantly differed from each other as indicated by Tukey test (p≤ 0.05)

Enzymatic antioxidants

Graphical data for SOD displayed significant ($P \leq 0.05$) differences in SS and foliar treatments, though, interaction among them noted non-significant ($P > 0.05$) differences. Under SS, percent decrease for SOD was up to 14.18 to 36.35 % as compared to normal conditions, however, the foliar application of Fe_2O_3 treatments increased the SOD under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in SOD were as 11.94 and 21.65 % under 50 mM SS, 6.52 and 18.81% under 100 mM SS and 22.61 and 26.57 % under 150 mM SS as compared to their respective controls (Figure 3).

Significant ($P \leq 0.05$) differences in salt stress, and nano particle treatments were depicted by graphical data for POD but, interaction among them noted non-significant ($P > 0.05$) differences. Under SS, percent decrease for POD up to 31.08 to 92.74% as compared to normal conditions, however, the foliar application of Fe_2O_3 treatments increased the POD under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in POD were as 33.99 and 52.17% under 50 mM SS, 20.69 and 35.56% under 100 mM SS and 41.12 and 48.11% under 150 mM SS as compared to their respective controls (Figure 3)

There was significant ($P \leq 0.05$) difference in salt stress, and nano particle treatments for CAT but, interaction among them was found to be non-significant ($P > 0.05$) differences. Under SS, increase trend for CAT control < 50 mm SS < 100 mM SS, and 150 mM SS, however, the foliar application of iron oxide treatments increased the CAT under all normal and stress condition. The trends of the percent increase in CAT were as 31.02% (15 ppm Fe_2O_3) and 46.86 % (30 ppm Fe_2O_3) under 50 mM SS, 25.71% (15 ppm Fe_2O_3) and 37.40% (30 ppm Fe_2O_3) under 100 mM SS and 32.71% (15 ppm Fe_2O_3) and 48.49% (30 ppm Fe_2O_3) under 150 mM SS as compared to their respective controls (Figure 3).

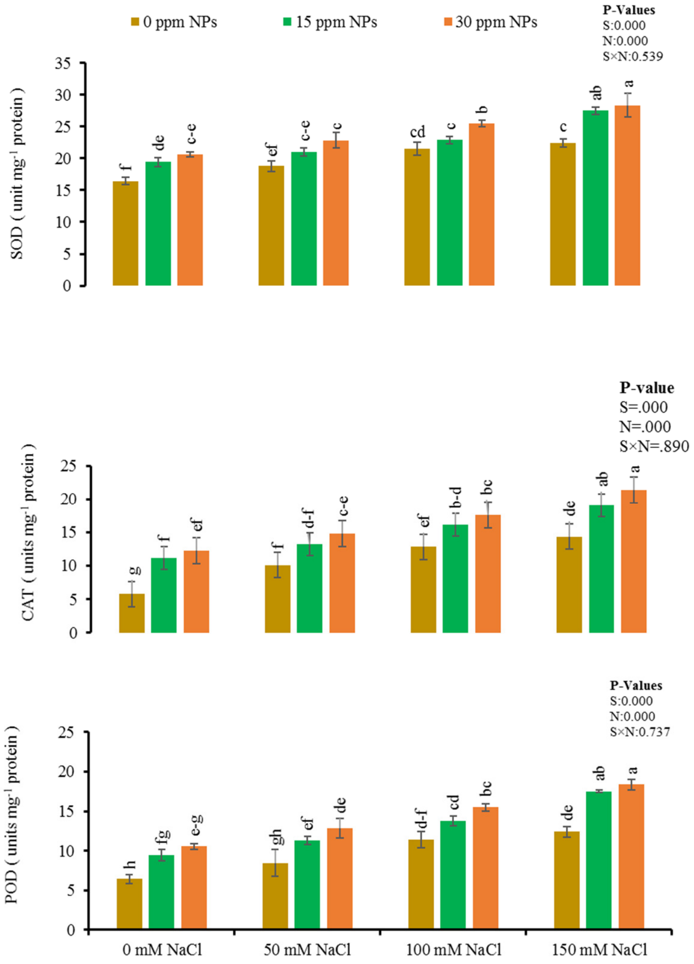

Figure 3. The influence of iron oxide nano particles on antioxidants of maize crop
(Error bars = Standard errors, and bars with different letters (a, b, c, etc.) are significantly differed from each other as indicated by Tukey test ($p \leq 0.05$)

Secondary metabolites

There was significant ($P \leq 0.05$) difference in salinity, and nanoparticle applications for total phenolics but, interaction was found to be non-significant ($P > 0.05$) differences. Under SS, decrease trend for total phenolics control < 50 mm SS < 100 mM SS, and 150 mM SS, however, the foliar application of iron oxide treatments decreased the total phenolics under all normal and stress condition. The trends of the percent increase in total phenolics were as 20.79% (15 ppm Fe_2O_3) and 41.80% (30 ppm Fe_2O_3) under 50 mM SS, 11.50% (15 ppm Fe_2O_3) and 15.92% (30 ppm Fe_2O_3) under 100 mM SS and 9.91% (15 ppm Fe_2O_3) and 20.66% (30 ppm Fe_2O_3) under 150 mM SS as compared to their respective controls (Figure 4).

Graphical data for flavonoids depicted the significant ($P \leq 0.05$) difference, when nanoparticles were applied under salt stress but, interaction among them noted non-significant ($P > 0.05$) differences. Flavonoids decreased from 7.76 to 25.95 % when compared with control under salinity stress, however, the foliarly applied Fe_2O_3 nanoparticles increased the flavonoids under both saline and control conditions. The percent increase in flavonoids was 9.85 and 16.54 when 15 and 30 ppm Fe_2O_3 was applied under 50 mM SS, 6.07 and 3.17%

under 100 mM SS and 9.46 and 11.49% under 150 mM SS when compared to their respective controls (Figure 4).

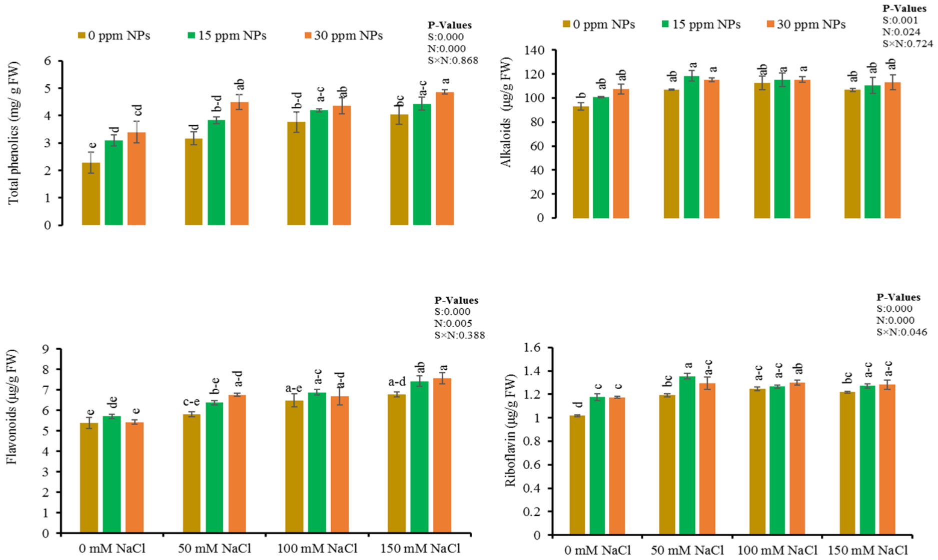

Figure 4. The influence of iron oxide nano particles on secondary metabolites of maize crop
(Error bars = Standard errors, and bars with different letters (a, b, c, etc.) are significantly differed from each other as indicated by Tukey test ($p \leq 0.05$)

Significant ($P \leq 0.05$) differences in salinity, and nanoparticle applications were depicted by graphical data for POD but, interaction noted non-significant ($P > 0.05$) differences. Under SS, percent decrease for alkaloids up to 14.89 to 21.24% as compared to normal conditions, however, the foliar application of Fe_2O_3 treatments increased the alkaloids under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent increment in alkaloids were as 10.87 and 7.67% under 50 mM SS, 2.33 and 2.35% under 100 mM SS and 3.59 and 5.80% under 150 mM SS as compared to their respective controls (Figure 4).

There were significant ($P \leq 0.05$) differences displayed in sodium in riboflavin in SS and foliar treatments, though, interaction noted as non-significant ($P > 0.05$) differences. Under SS, percent decrease in riboflavin was up to 17.67 to 22.66% as compared to normal conditions, however, the foliarly applied Fe_2O_3 treatments increased the riboflavin under all normal and stress condition. When 15 ppm Fe_2O_3 and 30 ppm Fe_2O_3 were applied then trends of the percent decrease in riboflavin were as 13.63 and 8.51% under 50 mM SS, 1.32 and 4.19% under 100 mM SS and 4.34 and 5.05% under 150 mM SS as compared to their respective controls (Figure 4).

Heatmap

The heatmap depicted (Figure 5) relationships between morpho-physiological and biochemical parameters under varying salinity levels (0 mM, 50 mM, 100 mM, and 150 mM) and foliarly applied iron oxide (Fe_2O_3) nanoparticles doses (0, 15, and 30 ppm). In the main cluster, three subclusters emerged. The first showed a positive association among Chl.a, Chl.b, and total chlorophyll at S0N0, with a negative association at S150N30. Similarly, root and shoot lengths, K+ ions, and root fresh and dry weights were positively associated at S0N15 and S0N30, but negatively at S150N0. The second subcluster indicated a positive association between root and shoot Na+ ions and MDA contents at S150N0, contrasting with a negative grouping at S0N30. The third subcluster revealed a negative association of biochemical traits like riboflavin and alkaloid at S0N0, while SOD, POD, and CAT exhibited a positive association at S150N30.

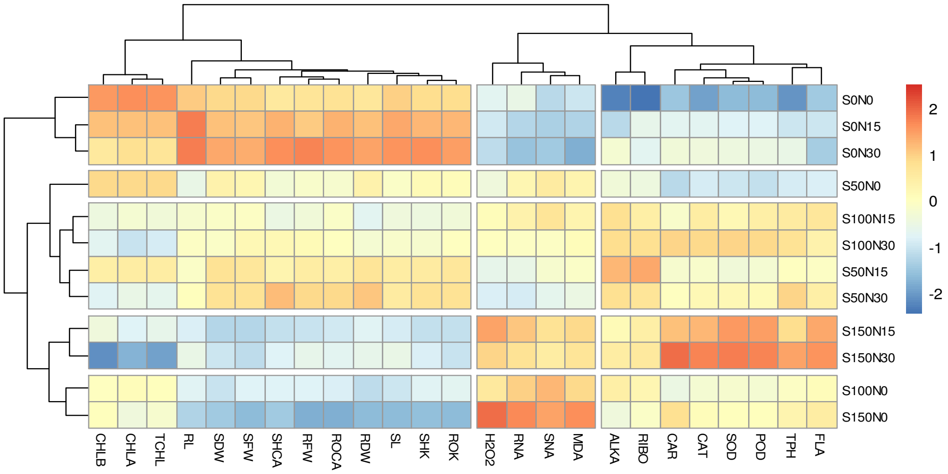

Figure 5: Heatmap between morpho-physiological and biochemical attributes with application of Fe$_2$O$_3$ nanoparticles under saline conditions

S0N0; 0 mM salinity and 0 ppm Fe$_2$O$_3$, S0N15; 0mM salinity and 15 ppm Fe$_2$O$_3$, S0N30; 0mM salinity and 30 ppm Fe$_2$O$_3$, S50N0; 50mM salinity and 0 ppm Fe$_2$O$_3$, S50N15; 0mM salinity and 15 ppm Fe$_2$O$_3$, S50N30; 50mM salinity and 30 ppm Fe$_2$O$_3$, S100 N0; 0mM salinity and 0 ppm Fe$_2$O$_3$, S100 N15; 0mM salinity and 15 ppm Fe$_2$O$_3$, S100 N30; 0mM salinity and 30 ppm Fe$_2$O$_3$, S150N0; 150 mM salinity and 0 ppm Fe$_2$O$_3$, S150N15; 150 mM salinity and 15 ppm Fe$_2$O$_3$, S150N30; 150 mM salinity and 30 ppm Fe$_2$O$_3$,CHL B; Chlorophyll b , CHL A; Chlorophyll a, TCHL; Total chlorophyll, RL; Root length, SDW; Shoot dry weight, SFW; Shoot fresh weight, ROCA; Root calcium, RDW; Root dry weight, SL; Shoot length, SHK; Shoot potassium, ROK, Root potassium, H2O2; Hydrogen peroxide, RNA; Root sodium, SNA; Shoot sodium, MDA; Malondialdehyde, ALKA; Alkaloids, RIBO; Riboflavin, CAR; Carotenoids, CAT; Catalase, SOD; Superoxide dismutase, POD; Peroxidase, TPH; Total phenolics, FLA; Flavonoids

Correlation

Correlations between morpho-physiological and biochemical traits are depicted in (Figure 6). Chlorophyll *a*, Chl *b*, chlorophyll ratio, exhibit a strong negative correlation with carotenoid, SOD, POD, CAT, total plant height, and flavonoids. Similarly, root Ca^{2+}, root dry weight, shoot length, shoot K^+, root K^+, display a positive correlation with root length, shoot fresh, shoot dry weight, root Ca+, and root dry weight, while demonstrating a strong negative correlation with H_2O_2, Root Na^+, shoot Na^+, and MDA contents. Flavonoids show a positive relation with chl.*b*, chl.*a*, total chlorophyll, root length, shoot dry weight, shoot length, root K^+ ions, while displaying a strong negative relation with carotenoids, CAT, SOD, POD, total plant height, MDA, root Na^+, and shoot Na^+ ions.

PCA

Principal component analysis between morpho-physiological and biochemical traits showed four isolated clusters (Figure 7) In the first sub-cluster, there is a weak association observed among Chl.*a*, Chl.*b*, and total chlorophyll at S50N0 and S0N0. In the second sub-cluster, root and shoot Na^+ ions exhibit a weak association with MDA and H_2O_2 contents, notably linked at S100N0. Moving to the third sub-cluster, the morphological parameters of plants, such as root length, shoot dry weight, shoot fresh weight, and root fresh weight, demonstrate a strong association with biochemical traits, including root and shoot K^+ and Ca^+ ions, particularly at S0N30. Lastly, in the fourth sub-cluster, flavonoids exhibit weak associations with alkaloids, riboflavin, and catalase (CAT), while biochemical traits such as carotenoids, superoxide dismutase (SOD), peroxidase (POD), and riboflavin show a strong association with total plant height, albeit weakly linked at S100N30 and S150N30.

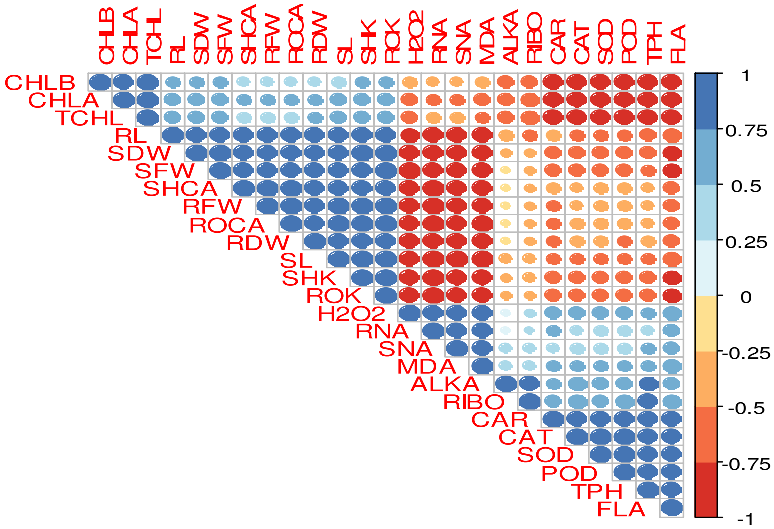

Figure 6. Correlation between morpho-physiological and biochemical attributes with application of Fe$_2$O$_3$ nanoparticles under saline conditions

CHL B; Chlorophyll b , CHL A; Chlorophyll a, TCHL; Total chlorophyll, RL; Root length, SDW; Shoot dry weight, SFW; Shoot fresh weight, ROCA; Root calcium, RDW; Root dry weight, SL; Shoot length, SHK; Shoot potassium, ROK, Root potassium, H$_2$O$_2$; Hydrogen peroxide, RNA; Root sodium, SNA; Shoot sodium, MDA; Malondialdehyde, ALKA; Alkaloids, RIBO; Riboflavin, CAR; Carotenoids, CAT; Catalase, SOD; Superoxide dismutase, POD; Peroxidase, TPH; Total phenolics, FLA; Flavonoids

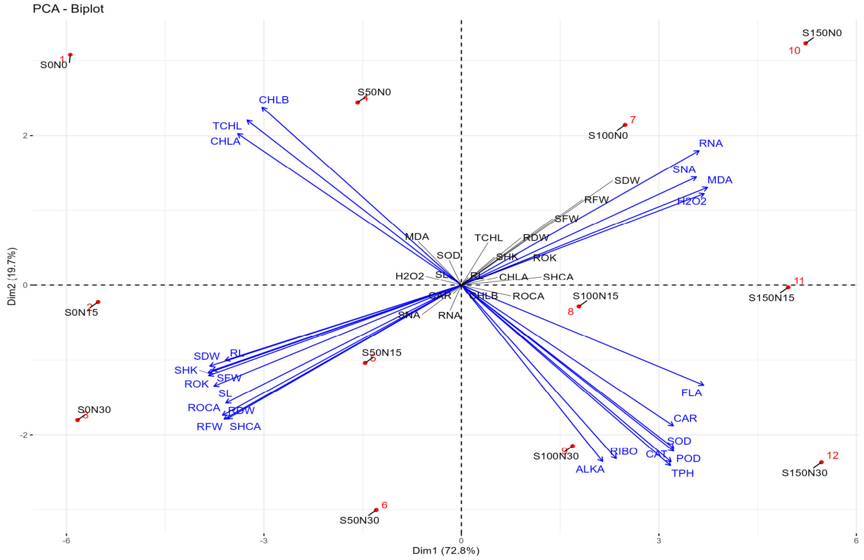

Figure 7. PCA test between morpho-physiological and biochemical attributes with application of Fe$_2$O$_3$ nanoparticles under saline conditions

S0N0; 0 mM salinity and 0 ppm Fe2O3, S0N15; 0mM salinity and 15 ppm Fe2O3, S0N30; 0mM salinity and 30 ppm Fe2O3, S50N0; 50mM salinity and 0 ppm Fe2O3, S50N15; 0mM salinity and 15 ppm Fe2O3, S50N30; 50mM salinity and 30 ppm Fe2O3, S100 N0; 0mM salinity and 0 ppm Fe2O3, S100 N15; 0mM salinity and 15 ppm Fe2O3, S100 N30; 0mM salinity and 30 ppm Fe2O3, S150N0; 150 mM salinity and 0 ppm Fe2O3, S150N15; 150 mM salinity and 15 ppm Fe2O3, S150N30; 150 mM salinity and 30 ppm Fe2O3,CHL B; Chlorophyll b , CHL A; Chlorophyll a, TCHL; Total chlorophyll, RL; Root length, SDW; Shoot dry weight, SFW; Shoot fresh weight, ROCA; Root calcium, RDW; Root dry weight, SL; Shoot length, SHK; Shoot potassium, ROK, Root potassium, H2O2; Hydrogen peroxide, RNA; Root sodium, SNA; Shoot sodium, MDA; Malondialdehyde, ALKA; Alkaloids, RIBO; Riboflavin, CAR; Carotenoids, CAT; Catalase, SOD; Superoxide dismutase, POD; Peroxidase, TPH; Total phenolics, FLA; Flavonoids

Fe_2O_3 nanoparticles

Iron oxide (Fe_2O_3) nanoparticles were prepared by precipitation method using ferric chloride ($FeCl_3.6H_2O$) and sodium hydroxide (NaOH) as precurssor and precipitator respectively. The nanoparicles were characterized for their size and morphology by transmission electron microscopy and were found to be irregular spherical in shape with an average diameter of 30 ± 5 nm (Figure 8).

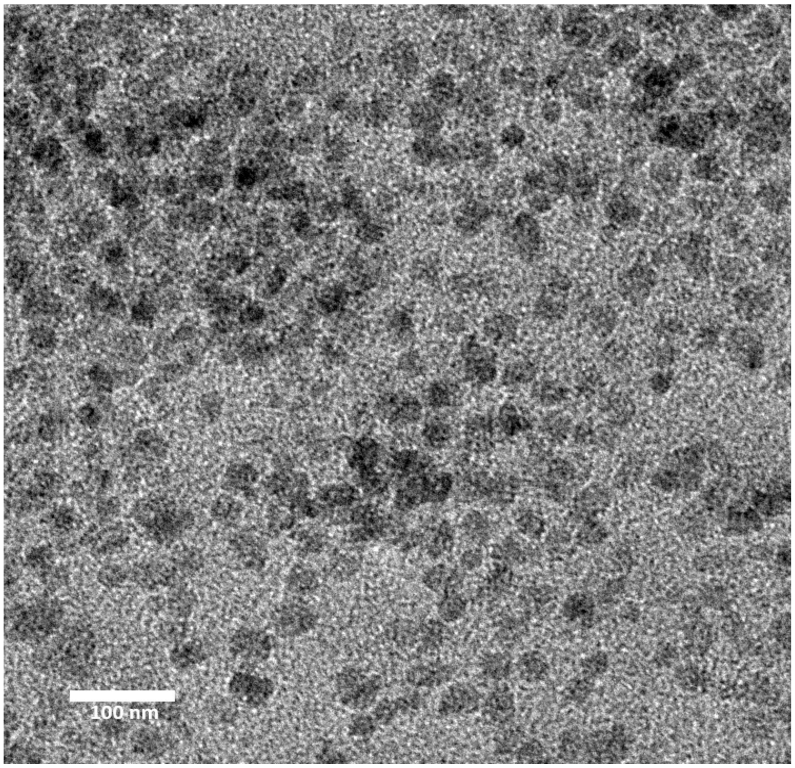

Figure 8. Transmission electron microscope (TEM) image of iron oxide nanoparticles

Discussion

Salinity stress results in the disturbance of normal plant's physiological mechanisms (Tiwari *et al.*, 2021; Victoria *et al.*, 2023). Salinity has proved itself a major hazard towards agriculture as well as it is anticipated that economically important crops yield might be reduced by 50% by continuing the current rate of salinity (El Sabagh *et al.*, 2020). Reduced growth is one of the visual symptoms of saline stress. In the current investigation, the total biomass (root and shoot dry weight) of studied maize variety declined radically with increasing salinity (Table 1). Salinity damages could be mitigated by using shot-gun techniques, which involve the exogenous administration of antioxidants. minerals, and vitamins (Ashraf *et al.*, 2022). One of these approaches is the use of iron oxide nanoparticles which can be applied to plants foliarly or by seed priming (Mahmood *et al.*, 2022). In current study, the decline in maize physiological mechanisms has been compensated by the foliar application of iron oxide NPs. This depicted that iron oxide NPs application enhanced the growth of plants due to the stimulation of signaling factors used in the regulation of plant growth. In a current study, the reduction in growth parameters of maize plants was observed under salinity stress. This might be due to stunted plant growth, reduction in photosynthetic activity, and ionic disturbance under saline conditions (Saddiq *et al.*, 2021). Photosynthetic pigments have a crucial role in the assimilation of carbon in plants which make them highly influential among all the other components comprising photosynthetic system (Zahra *et al.*, 2022). On the other hand, salinity stress disrupts the plastid structure, consequently disturbing the synthesis of different photosynthetic pigments, including chlorophyll *a*, chlorophyll *b* and carotenoid content (Saddiq et al., 2021). In the present study, salinity significantly reduced the production of all mentioned pigments in the validated maize variety (Figure 1). Iron oxide NPs foliar spray plays a significant role by enhancing the concentration of carotenoids, total chlorophyll and both chlorophyll *a* and *b* and 15 ppm spray was found to be more effective in enhancing these pigments content. Altered concentration of these pigments reduces the photosynthetic rate, because salinity stress which influences the photosynthesis process may include an impairment of the reaction center of the electron transport chain as well as photosystem II (PSII), altered pigment concentrations results in a decline in photosynthesis rate. Iron oxide NPs application might help in the protection of these pigments and enhance photosynthetic activity and oxidative bursts (Naqve *et al.*, 2021). Indeed, foliarly applied iron oxide NPs optimized the chlorophyll contents and carotenoid content under saline stress.

Salinity stress disrupts nutrient uptake, causing the membrane and the ultrastructure of cell to dissolve, resulting in both osmotic as well as ionic stress (Singh *et al.*, 2022). In addition, imbalance in nutrition is a significant issue caused due to salinity stress. In the current investigation concentration of Na^+ ion increased notably in maize tissues under saline regimes (Table 2). This elevation in Na^+ ions concentration disturbs the metabolic, antioxidant, and photosynthetic activities, ultimately leading to reduced plant productivity. Under salinity stress, the amount of K^+ and Ca^{2+} ions in tested maize variety were dramatically reduced. This nutritional imbalance causes ROS buildup and osmotic stress (Arif *et al.*, 2020). Increasing Na^+ concentration induced by elevated salinity lead up to reduction in concentration of K+, showing the ions K+ and Na+ are opposed. High salinity affects remarkably the plant processes by imposing the osmotic stress and disturbing both ionic as well as redox signaling (Singh et al., 2021). Higher salt (NaCl) concentration in the soil and the ground water constrains K^+ absorption in plants. A decrease in K^+ absorption, in turn, has a negative impact on water relations, enzyme activity, and protein production (Mahmood *et al.*, 2022).Calcium in turn plays its role in various signal transduction mechanisms and supports membrane structural integrity; however, hazardous salt level restrict Ca^{2+} uptake. Our findings showed a significant decrease in K^+ and Ca^{2+} ions and increased Na^+ ions in both shoot and root tissues. However, a considerable rise in Ca^{2+} and K^+ ion in shoots and roots of maize after foliar application of iron oxide NPs improved K^+ and Ca^{2+} absorption to maintain the ionic balance and homeostasis, therefore regulating physiological performance towards salt environment. Calcium acts as a signaling molecule and is essential for plant integrity, yet higher concentrations of toxic salts inhibit the calcium absorption. However, the foliar application of iron oxide NPs reduces NaCl ion concentration and increases K^+ absorption in both stressed and controlled conditions (Zia-ur-Rehman *et al.*, 2022). Under salinity stress, higher concentrations of reactive oxygen species (ROS) accumulate, causing tissue destruction (Lalarukh *et al*., 2023) through oxidation of macromolecules and, as a result, lowering crop productivity. Producing antioxidant compounds for ROS quenching, a frequent plant strategy for preventing or minimizing damages induced by salt stress. This drop is allegedly connected with stomatal closure, resulting in drastically reduced CO_2 absorption into mesophyll of leaf in plants grown in saline environments (Gulcin, 2020).

Additionally, enzymatic antioxidants are critical in reducing damage due to salinity caused by ROS accumulation. In our study, foliar application of IONPs enhanced POD, SOD and CAT enzymes activities in salinity (Figure 3). These enzymes are familiar for being stimulated in collaboration with IONPs and assisting plants in resisting oxidative bursts caused due to salinity (Hasanuzzaman *et al.*, 2021). The SOD activity quinches oxygen radicles, whereas POD and CAT activities are helpful for effectual quenching of hydrogen peroxide (Mujeeb-Kazi *et al.*, 2019). Therefore, in addition to the ROS-quenching antioxidant activity, IONPs supplementation is helpful to counteract oxidative bursts due to salinity through upregulating the enzymatic antioxidant activities which includes CAT, SOD, and POD (Ali *et al.*, 2019).

It is well understood that the phenolic compounds are basically secondary metabolites which protect plants from significant oxidative damage, which is induced due to salt stress (Naikoo *et al.*, 2019). Phenolic substances have antioxidant activity which results in inactivating free-lipid radicals and preventing the breakdown of hydrogen peroxides to free radicals. The enhanced antioxidant enhances the detoxification rate of reactive oxygen species, which likely increases the plant resistance to salinity (Ghanem *et al.*, 2021).

Environmental stressors, such as salt, are required for the production of secondary metabolites with high antioxidant activity (Selvam *et al.*, 2020). The secondary metabolites which include total phenolics, flavonoids and alkaloids increased salinity stress in maize plant (Shahid *et al*., 2023). This increase may be due in response to excessive accumulation of reactive oxygen species (Zahra *et al*., 2021). Secondary metabolites containing nitrogen are alkaloids with antioxidant activity and can be linked to changes in metabolites containing nitrogen in primary metabolism under salt stress (Sadeghi *et al.*, 2015).

Conclusions

Foliar application of foliar iron oxide NPs enhanced plant antioxidant potential, photosynthetic rate, osmoregulation and secondary metabolites in maize crop under normal and saline conditions. Consequently, foliar application of foliar iron oxide NPs at a rate of 30 ppm is a promising method to equip maize crop with multiple stress tolerance to reduce the pressure of changing climate conditions and meet the requirements of an augmenting population. Though, the role of foliar iron oxide NPs in eluding the production of ROS and antioxidants is not clear yet at the genetic level.

Authors' Contributions

Conceptualization: AM, MN, MAZ and MFS. Experimentation and writing original draft: AMR and AM. Reviewing and editing: HFNA, MBK, MIM, MBH, NK. Funding acquisition: MFS and EFA.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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