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RESEARCH ARTICLE

ENVIRONMENTAL PHYSIOLOGICAL STUDIES ON THE PHASEOLUS VULGARIS L. PLANT UNDER SALINITY AND THE TREATMENT OF IRON OXIDE NANOPARTICLES

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ABSTRACT

Salinity has adversely affected the soil properties and plant productivity. *Phaseolus vulgaris* (Pole bean) is one of the important crops for human consumption that is considered very sensitive to salinity stress. However, nanoparticle application under severe stress conditions has mitigated salinity impact. In this experiment, the effect of spraying of iron oxide nanoparticles at (0, 10, 20 and 30 µM Fe_2O_3) has been examined on the Pole bean seed germination, soil properties and Pole bean growth parameters under 200 mM NaCl. Salinity at 200 mM reduced significantly the seed germination, shoot length, root length, root volume, shoot fresh and dry weight, root fresh and dry weight, leaf number, leaf area, leaf relative water content, chlorophyll a and b, carotenoids, total and soluble carbohydrate in the shoot and root, total and soluble proteins in the shoot and root, the free amino acid in the shoot and minerals content (N, Fe, P and k) in the shoot and root, whereas, salinity increased the Na content in the shoot and root compared to control. On the other hand, the foliar method of Fe_2O_3 at different doses under salinity increased all plant growth and enhanced the metabolism functions. The foliar application of 10 µM Fe_2O_3 + 200 mM NaCl improved the seed germination percentage, shoot length, root length, root volume, shoot fresh weight, leaves number, soluble carbohydrate in the shoot and root, soluble proteins in the shoot and root, P the shoot and root and K in the root, but Na reduced in the shoot. Furthermore, spraying the 20 µM Fe_2O_3 + 200 mM NaCl increased the leaf area, root fresh weight, relative water content, chlorophyll b, carotenoids, total carbohydrate in the shoot, and total proteins in the shoot and root, the free amino acid in the shoot and K in the shoot. In addition, foliar application at 30 µM Fe_2O_3 + 200 mM NaCl raised the chlorophyll a, total carbohydrate in the root, N in the shoot and root, while the Na declined in the root. Both concentrations of Fe_2O_3 NPs at 20 and 30 µM under 200 mM NaCl enhanced the Fe in the shoot and root.

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INTRODUCTION

Salinity in the soil is defined according to Tang *et al*. (2015) that the soil containing an increased level of soluble salt on it. Salinity which belongs to abiotic stress influences significantly the arid and semi-arid environments that suffer from water scarcity and land degradation (Hasanuzzaman *et al.,* 2013 a, b and Geissler *et al.,* 2010). Shrivastava and Kumar (2015) mentioned that in the whole world, salinity adversely effects about 20% of all agricultural areas and 33% of irrigated agricultural areas, and according to Jamil *et al*. (2011) and Wang *et al*. (2003) the percentage of agricultural areas affected by salinity will rise by 50% in the year 2025. Saudi Arabia environment is considered a semi-arid area with a decrease in the annual precipitation amount, unstable temperature and restricted groundwater resources (Chowdhury and Al-Zahrani, 2013).

In the agricultural soils, salts accumulate in the soil after applying irrigation water leading to an adverse effect on the plant productivity (Aldakheel, 2011). According to Munns (2005) soil becomes salinized when the salts in the soil solution reach 4 $dS.m^{-1}$ (40 mM of NaCl) or higher which impacts most the crops. However, recently many scientists have examined the positive impact of applying nanoparticles in plants under much abiotic stress such as salinity. Duhan *et al*. (2017) announced that nanotechnology is one of a newborn science that has successes in the solve the impact of salinity on the agriculture sector (soil and plant). The dimension size of the nanoparticles is between 1nm to100 nm (nm = nanometer) (Jeevanandam *et al.,* 2018) and "1 nm equal one-billionth of a meter (10^{-9} m)" (Usha *et al.,* 2017). Utilizing the nanoparticles positively influences the whole plant life starting in the seed germination phase and continuing through plant development (Kamle *et al.,* 2020).

One example of nanoparticles with have been applied in ecology science is iron oxide nanoparticles. Iron nano-sized raises the ability of a plant to tolerate the saline media by elevating the permeability of the selective plasma membrane (Taiz *et al.,* 2015). Regarding to enzymatic plant system, iron has been proved as a co-factor for around 140 enzymes (Brittenham, 1994). Pole bean plant (*Phaseolus vulgaris* L.) belonging to the legume family is among most of the plants that are influenced by salinity (Rangel *et al.,* 2005). According to Geil and Anderson (1994), *Phaseolus vulgaris* has benefits to the human body providing the body with sugar, proteins, iron element, and zinc nutrient andit is cholesterol-free. This experiment aims to confirm the positive effect of the foliar method of iron oxide nanoparticles on the plant growth parameters and plant physiological processes under high salinity levels.

METHODS AND MATERIALS

Iron oxide nanoparticles (Fe_2O_3 NPs) characteristics and preparation: Iron oxide NPs were purchased from https://www.Aliexpress.com. The size and purity of iron oxide NPs were 30 nm and 97%. According to Moradbeygi *et al.* (2020),the ultrasonic homogenizer device (Cole-Parmer, 750-watt an ultrasonic processor, 115 VAC) was used to prepare four concentrations of Fe_2O_3 (0, 10, 20 and 30 µM).

Plant material: Pole bean seeds (*Phaseolus vulgaris* L.) were obtained from the local market Alyaseen Agri shop in the Qassim district.

Pole bean growth conditions and treatments: This experiment was conducted in the physiology plant laboratory in Science Faculty at King Abdulaiziz University and Qassim University, KSA from July 23rd, 2021, to August 24th, 2021. The unified concentration of NaCl at 200 mM was chosen according to Al-huraby and Bafeel (2022) and four levels of Fe_2O_3 at (0, 10, 20 and 30 µM) were sprayed to examine the effect of nano- Fe_2O_3 under high salinity (200 mM) on the 15 days old Pole bean in a completely randomized design with three replicates.

Determination of the percentage of seed germination: 10 seeds of Pole bean were placed in a Petri dish between two filter papers in the darkroom and at 25°C from July 13th, 2021 for six days. This study has five groups of Petri dishes: the first group of seeds irrigated with 10 ml of tap water (control), the second group of seeds irrigated with 10 ml of 200 mM NaCl, the third group of seeds irrigated with 10 ml of 200 mM NaCl and 10 µM Fe_2O_3 NPs, the fourth group of seeds irrigated with 10 ml of 200 mM NaCl and 20 µM Fe_2O_3 NPs and the fifth group of seeds irrigated with 10 ml of 200 mM NaCl and 30 µM Fe_2O_3 NPs. The filter prepare shave to renew after 48 hours to avoid the NaCl accumulation (Rahman *et al.,* 2008). According to (Mena *et al.,* 2015), the germinated seeds are considered when the radical length reaches 2 mm. The germinated seeds percentage was calculated according to the formula:

Germination percentage (G%) = (number of germinated seeds /number of the total seeds) × 100

Pots experiment: Pole bean plants were grown in a greenhouse under controlled environment [temperature (38.45 / 26.06 °C) and humidity (30.83/ 8.91%)]. Pots (with 29 × 33 cm) were filled with homogeneously mixed sand and meat moss soil (2:1) with 9 seeds in each pot. The seeds were watered with tap water 3 times in a week for 15 days until the second real leaf appeared. Then, the six healthiest seedlings in each pot were kept. The pots were divided into five groups for treatment one time only(El-Fouly and El-Nour, 2021) as follows: The first group was irrigated with tap water as control, the second group was irrigated with 200 mM NaCL for saline treatment and the rest groups were irrigated with 200 mM NaCl and separately were sprayed with Fe_2O_3one 10 µM, the other one20 µM and the last 30 µM, according to (Hassanpouraghdam *et al.,* 2019) in the plant leaf only one time (in the 15th day after germinated seeds) early in the morning. Soil and plant samples have collected after two weeks of Fe_2O_3 NPs treatments (Askary *et al.,* 2017).

Plant analysis

Growth parameter

Shoot length, root length and volume: The shoot and roots length were determined by using a ruler metric (cm). Root volume was measured by the "displacement technique" according to (Obeng-Asamoah, 1984 and Garg, 2012) methods. A graduated glass cylinder was filled with distilled water at a certain point (cm^3). Then the fresh roots in each treatment were dipped in this water. The increase in the water level in the graduated glass cylinder was recorded using this formula: RV (cm^3) = volume of the water after immersing the roots into the cylinder – the volume of the water before immersing the roots.

Fresh and dry mass of shoot and root: The clean fresh stem and root were recorded as fresh weight by utilizing electrical analytical balance expressed by grams (g). Later the stem and root were stored in labeled oven bags to dry oven at 70 °C for two days to reach the constant weight. The dry weight for all samples was determined by using an electrical analytical balance expressed by grams (g).

Leaves number (LN) and leaf area measurement (LA): The total number of fully, fresh and green expand leaves was counted in all samples. Also, the leaves area (cm^2) for each treated sample was measured according to (Bhatt and Chanda, 2003) method using the current formula:

LA = 11.01 + 0.07 LW

Where LA = leaf area, L = length, and W = width

Leaves relative water content: The leave relative water content was estimated according to Cornic (1994) method. Three fresh green leaves from each replicate were measured and recorded (FW). Then each replicate was left in a petri dish filled with distilled water for 24 hours in dark (Howladar, 2014) to get turgid weight (TW). later they were placed in the oven to dry at 65 °C for 72 hours to record the dry weight (DW). Finally, the leave relative water content was calculated according to the formula:

RWC % = [(FW-DW) / (TW-DW)] × 100

Determination of chlorophylls and carotenoid contents: The chlorophylls (a and b) and carotenoid contents were extracted and determined by Metzner *et al*. (1965) method. 0.5 g of the fresh green leaf was homogenized with 10 ml of 85% acetone for 5 min under normal conditions. The extract was transferred into a flask and completed the volume with acetone (85%) to 50 ml. The Agilent Spectrophotometer (UV/ Visible) Cary 60 was used at lengths of 663, 644 and 440 nm to estimate chlorophyll (a), chlorophyll (b), and carotene, respectively. The liquid of acetone 85% was used as a blank. The content of chlorophylls (a and b) and carotenoid was calculated by using this formula:

Chlorophyll a ($mg.g^{-1}$ FW) = 10.3 E663 – 0.918 E644
Chlorophyll b ($mg.g^{-1}$ FW) = 19.7 E644 – 3.87 E663
Carotenoids ($mg.g^{-1}$ FW) = 4.2 E440 – [(0.0264 Chl a + 0.426 Chl b)]

Preparation of shoot and root plant extraction

Extraction for soluble carbohydrates, soluble proteins and free amino acid in shoot and root plant: 0.1 g of dried powder sample was placed into test tube containing 15 ml of distilled water and was left in a water bath for 1 hour at 90^{o}C. After that, the sample was filtered and completed the volume until 100 ml with distilled water. These water extractions must be kept in the refrigerator for further analysis.

Preparation of shoot and root plant extraction for total carbohydrates: 0.1 g of dried and powder tissue was mixed with 10 ml of distilled water and 15 ml (4N) HCl in a test tube. This tube was heated in a water bath for 1 hour at 90 ^{o}C. Later the solution was filtered, and the volume was completed to 100 ml distilled water, and the plant extracts must be kept in the refrigerator. 0.2 ml of solution was used to estimate the total carbohydrate (Fales, 1951 and Schlegel, 1956).

Preparation of shoot and root plant extraction for total proteins: 0.1 g of dried powder sample and 10 ml of distilled water and 5 ml (1N) NaOH were transferred to a test tube to heat in the water bath for 30 minutes at 90 ^{o}C. later, the solution was filtered and the volume was completed to 100 ml. 1 ml of solution was used to estimate the total protein (Lowry *et al.,* 1951).

Determination of total and soluble proteins in shoot and root: The total and soluble proteins in shoot and root were measured according to Lowry *et al*. (1951). 1 ml of the shoot tissue extract mixed well with 5 ml of the alkaline reagent solution. Later, the tube was left for 10 min at room temperature to stand. Then 0.5 ml of Folins-Ciocalteau's reagent was added, mixed, and placed in the dark for 30 min. After that, the blue colour was measured at 750 nm by using an Agilent spectrophotometer (UV/ Visible) cary 60 against a blank containing all the above reagents and distilled water instead of extract of the shoot and root samples. The protein content of the extract was calculated from the standard graph of Bovine Serum Albumin.

Determination of total and soluble proteins in shoot and root: The total and soluble proteins in shoot and root were measured according to Lowry *et al*. (1951). 1 ml of the shoot tissue extract mixed well with 5 ml of the alkaline reagent solution. Later, the tube was left for 10 min at room temperature to stand. Then 0.5 ml of Folins-Ciocalteau's reagent was added, mixed, and placed in the dark for 30 min. After that, the blue colour was measured at 750 nm by using an Agilent spectrophotometer (UV/ Visible) cary 60 against a blank containing all the above reagents and distilled water instead of extract of the shoot and rootsamples. The protein content of the extract was calculated from the standard graph of Bovine Serum Albumin.

Determination of free amino acid in the shoot: The free amino acid content in the shoots was determined by the method of Moore and Stein (1948). First, the Stannous chloride reagent was prepared in a conical flask, by dissolving 0.01 mg of stannous chloride with 10 ml citrate buffer and 10 ml ninhydrin reagent. 0.5 ml of the water extract shoot sample was mixed with 1 ml of stannous chloride reagent in a test tube. The test tube was placed in a water bath for 20 min to boil and then cooled. The extinction of violet colour was recorded by using a Spectrophotometer at 570 nm against a blank containing all the above reagents and distilled water instead of extracting of shoot sample.

Determination the percentage of shoot and root minerals (N, Fe, P, K and Na): The shoot and root minerals have measured as follows: total plant nitrogen was determined by using kjeldahl method (AOAC 973.06). Whereas other plant minerals such as iron, phosphorus, potassium and sodium were assessed by utilizing (ICP_OES instrument) (Determination of heavy metals and minerals in food and feed matrices by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) After Microwave Digestion AS-CC-012.04). All the minerals tests had done at IDAC Merieux NutriSciences, Exit 9 Al-kharj Road, Riyadh, KSA.

Statistical analysis: In this work, analyses of variance (ANOVA) for seed germination and plant data are presented by using open source software R (ver. 3.5.2, Vienna Austria, https://www.R-project.org/). Data in the tables and figures are means ± SE (n=3) and the LSD test indicates non-significant differences for the same letters at $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Seeds germination percentage: The results of seed germination percentage of pole bean showed in Table 1. It is clear to see that NaCl and Fe_2O_3 ions at specific concentrations had significant influences on the pole bean seed germination. Over control, all the seeds germinated at the combination of salts and Fe_2O_3 except at 200 mM NaCl and 30 µM Fe_2O_3NPs + 200 mM NaCl which reduced the germination significantly by 10% and 3%, respectively. Many studies have similar results such as Demir and Ermis, (2003), Almodares *et al*. (2007) and Siddiqui *et al*. (2014). Kuriakose and Prasad (2008) and El-Hendawy *et al*. (2011) considered that the germination phase is the most crucial phase in the plant cycle life. There were several studies that explained germination declination. It was noticed that saline particles at high levels led to embryo toxicity (Almodares *et al.,* 2007 and Kaymakanova 2009). According to Parida and Das (2005), Panda and Khan (2009) and Daszkowska-Golec (2011) the toxicity influence caused disorder in the structures of macromolecules and many physiological functions. Moreover, the adverse consequence of the saline ions is cellular osmotic pressure through decreasing the cellular sugar level and lowering the uptake of water and

Table1: Seed germination percentage under 200 mM of NaCl and different Fe 2 O 3 nanoparticles concentrations.

Treatments	Seed Germination (%)
0.0	100.00 ± 0.00^{a}
200 mM NaCl	90.00 ± 0.10^{c}
200 mM NaCl + 10 µM Fe_2O_3	100.00 ± 0.00^{a}
200 mM NaCl + 20 µM Fe_2O_3	100.00 ± 0.0^{a}
200 mM NaCl + 30 µM Fe_2O_3	97.00 ± 0.05^{b}

Table 2: Shoot length, root length (cm) and root volume (cm^3) under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

Treatments	Shoot length (cm)	Root length (cm)	Root volume (cm^3)
0.0	176.00 ± 13.30^{a}	53.53 ± 4.30^{a}	2.62 ± 0.21^{c}
200 mM NaCl	135.70 ± 15.30^{d}	40.05 ± 3.80^{d}	2.07 ± 0.81^{d}
200 mM NaCl + 10 µM Fe_2O_3	158.40 ± 13.80^{b}	50.09 ± 4.20^{b}	3.09 ± 0.35^{b}
200 mM NaCl + 20 µM Fe_2O_3	141.00 ± 11.10^{c}	46.36 ± 3.50^{c}	3.33 ± 0.39^{a}
200 mM NaCl + 30 µM Fe_2O_3	153.30 ± 18.70^{b}	45.51 ± 3.30^{c}	3.09 ± 0.34^{b}

Table 3. Shoot and root fresh weight and dry weight (g $plant^{-1}$) under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

Treatments	Fresh weight (g $plant^{-1}$) in shoot	Dry weight (g $plant^{-1}$) in shoot	Fresh weight (g $plant^{-1}$) in root	Dry weight (g$plant^{-1}$) in root
0.0	12.81 ± 2.38^{b}	1.56 ± 0.18^{a}	2.63 ± 0.23^{c}	0.22 ± 0.03^{a}
200 mM NaCl	10.37 ± 1.18^{c}	1.05 ± 0.11^{b}	1.56 ± 0.16^{d}	0.13 ± 0.01^{d}
200 mM NaCl + 10 µM Fe_2O_3	13.96 ± 3.08^{a}	1.51 ± 0.20^{a}	2.90 ± 0.32^{b}	0.21 ± 0.02^{b}
200 mM NaCl + 20 µM Fe_2O_3	12.22 ± 2.13^{b}	1.20 ± 0.12^{b}	4.59 ± 0.37^{a}	0.21 ± 0.02^{b}
200 mM NaCl + 30 µM Fe_2O_3	13.23 ± 2.21^{a}	1.47 ± 1.10^{a}	2.62 ± 0.24^{c}	0.81 ± 0.02^{c}

Table 4. Leaf number and leaf area (cm^2) under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

Treatments	Leaves number	Leaves area (cm^2)
0.0	6.14 ± 0.65^{a}	1093.35 ± 75.57^{a}
200 mM NaCl	5.33 ± 0.22^{c}	727.85 ± 103.72^{d}
200 mM NaCl + 10 µM Fe_2O_3	6.24 ± 0.41^{a}	938.48 ± 113.26^{c}
200 mM NaCl + 20 µM Fe_2O_3	5.86 ± 0.24^{b}	987.10 ± 80.59^{b}
200 mM NaCl + 30 µM Fe_2O_3	5.76 ± 0.46^{b}	946.79 ± 116.65^{c}

Table 5: Relative water content % in leaf under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

Treatments	Leaf Relative water content %
0.0	82.58 ± 0.03^{b}
200 mM NaCl	77.71 ± 0.02^{c}
200 mM NaCl + 10 µM Fe_2O_3	82.30 ± 0.04^{b}
200 mM NaCl + 20 µM Fe_2O_3	87.34 ± 0.07^{a}
200 mM NaCl + 30 µM Fe_2O_3	80.75 ± 0.01^{b}

Table 6. Chlorophyll a, b and carotenoids (mg.g^{-1} fresh weight) under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

Treatments	Chlorophyll a (mg.g^{-1} fresh weight)	Chlorophyll b (mg.g^{-1} fresh weight)	Carotenoids (mg.g^{-1} fresh weight)
0.0	9.78 ± 0.01^{a}	3.28 ± 0.01^{a}	6.32 ± 0.01^{a}
200 mM NaCl	5.26 ± 0.03^{e}	1.81 ± 0.01^{d}	3.86 ± 0.02^{d}
200 mM NaCl + 10 µM Fe_2O_3	6.89 ± 0.01^{c}	2.10 ± 0.01^{c}	5.64 ± 0.02^{b}
200 mM NaCl + 20 µM Fe_2O_3	6.28 ± 0.01^{d}	2.93 ± 0.02^{b}	5.62 ± 0.02^{b}
200 mM NaCl + 30 µM Fe_2O_3	7.47 ± 0.01^{b}	2.86 ± 0.01^{b}	5.21 ± 0.01^{c}

Table 7. Carbohydrate (mg.g^{-1}DW) in shoot under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

	Shoot Carbohydrate (mg.g^{-1} DW)		Root Carbohydrate (mg.g^{-1} DW)	
Treatments	Total	Soluble	Total	Soluble
0.0	351.86 ± 35.20^{a}	295.47 ± 17.50^{a}	340.00 ± 32.20^{c}	94.20 ± 9.10^{a}
200 mM NaCl	224.68 ± 20.20^{d}	206.19 ± 18.69^{d}	234.00 ± 18.70^{e}	76.60 ± 8.30^{c}
200 mM NaCl + 10 µM Fe_2O_3	308.93 ± 39.71^{c}	296.47 ± 22.20^{a}	295.00 ± 31.20^{d}	97.40 ± 8.20^{a}
200 mM NaCl + 20 µM Fe_2O_3	346.87 ± 50.41^{b}	287.49 ± 16.40^{b}	356.00 ± 27.40^{b}	95.00 ± 7.40^{a}
200 mM NaCl + 30 µM Fe_2O_3	311.59 ± 62.30^{c}	282.22 ± 25.40^{c}	398.00 ± 31.40^{a}	90.00 ± 8.40^{b}

Table 8: Protein (mg.g^{-1} DW) in shoot and root under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

	Shoot Protein (mg.g^{-1} DW)		Root Protein (mg.g^{-1} DW)	
Treatments	Total	Soluble	Total	Soluble
0.0	127.83 ± 6.35^{a}	90.11 ± 8.10^{a}	272.00 ± 23.90^{d}	142.09 ± 13.20^{c}
200 mM NaCl	118.38 ± 8.93^{e}	88.61 ± 6.80^{c}	109.00 ± 10.20^{e}	101. 00 ± 20.20^{d}
200 mM NaCl + 10 µM Fe_2O_3	121.00 ± 11.20^{d}	92.56 ± 4.20^{b}	324.00 ± 28.60^{c}	150.10 ±12.38^{b}
200 mM NaCl + 20 µM Fe_2O_3	125.00 ± 5.20^{b}	90.80 ± 7.10^{a}	388.00 ± 32.30^{a}	164.13 ± 13.20^{a}
200 mM NaCl + 30 µM Fe_2O_3	123.00 ± 8.90^{c}	91.10 ± 6.50^{a}	360.00 ± 31.20^{b}	152.40 ± 17.14^{b}

Table 9: Free amino acid (mg.g^{-1} DW) in shoot under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

Treatments	Shoot Free amino acid (mg.g^{-1}DW)
0.0	33.86 ± 0.01^{a}
200 mM NaCl	17.87 ± 0.01^{d}
200 mM NaCl + 10 µM Fe_2O_3	29.55 ± 0.01^{b}
200 mM NaCl + 20 µM Fe_2O_3	30.88 ± 0.01^{b}
200 mM NaCl + 30 µM Fe_2O_3	24.69 ± 0.02^{c}

Table 10: Shoot minerals percentage of Pole beans under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

	Shoot minerals (%)				
Treatments	Nitrogen	Iron	Phosphorus	Potassium	Sodium
0.0	3.91±0.35^{b}	0.23 ±0.02^{a}	0.63 ±0.06^{c}	8.51±0.76^{c}	0.13 ±0.76^{c}
200 mM NaCl	3.68±0.22^{c}	0.14 ±0.01^{b}	0.34 ±0.02^{d}	5.74±0.40^{d}	0.41 ±0.40^{a}
200 mM NaCl + 10 µM Fe_2O_3	4.14±0.39^{a}	0.22 ±0.02^{a}	0.78 ±0.02^{b}	9.43±0.94^{b}	0.27 ±0.94^{b}
200 mM NaCl + 20 µM Fe_2O_3	4.09±0.45^{a}	0.25±0.02^{a}	0.81 ±0.06^{a}	10.94±1.20^{a}	0.29 ±1.20^{b}
200 mM NaCl + 30 µM Fe_2O_3	4.58±0.41^{a}	0.25 ±0.02^{a}	0.77 ±0.08^{b}	10.12±0.91^{a}	0.30 ±0.91^{b}

Table 11: Root minerals percentage of Pole beans under 200 mM of NaCl and different Fe_2O_3 nanoparticles concentrations

	Root minerals (%)				
Treatments	Nitrogen	Iron	Phosphorus	Potassium	Sodium
0.0	4.17±0.37^{a}	0.13 ± 0.01^{a}	0.56 ± 0.05^{b}	3.54 ±0.24^{a}	0.86 ±0.06^{c}
200 mM NaCl	3.89±0.29^{b}	0.07 ± 0.01^{b}	0.50 ± 0.05^{b}	1.43 ±0.15^{c}	3.40 ±0.30^{a}
200 mM NaCl + 10 µM Fe_2O_3	4.25±0.40^{a}	0.12 ± 0.02^{a}	0.79 ± 0.08^{a}	3.84 ±0.26^{a}	3.28 ±0.22^{a}
200 mM NaCl + 20 µM Fe_2O_3	4.47±0.38^{a}	0.14 ± 0.02^{a}	0.78 ± 0.07^{a}	1.35 ±0.19^{c}	2.67 ±0.29^{b}
200 mM NaCl + 30 µM Fe_2O_3	4.48±0.35^{a}	0.14 ± 0.02^{a}	0.77 ± 0.06^{a}	1.78 ±0.20^{b}	2.48 ±0.19^{b}

soluble nutrients (Ashraf *et al.,* 2002, Nizam, 2011, Hua-long *et al.,* 2014, Zhang *et al.,* 2017 and Liu *et al.,* 2018). Salinity stress inhibited the function of many enzymes like an α-Amylase enzyme that is in the endosperm providing the embryo with energy and essential minerals through the degradation of starch to sugar (Liu *et al.,* 2018 and El-Hendawy *et al.,* 2019). However, the application of Fe_2O_3 NPs increased the germination percentage under high salinity stress. Applying Fe_2O_3 NPs at low doses (at 10 and 20 µM) with 200 mM NaCl significantly enhanced the percentage of seed germination, but the germination reduced at 30 µM (high concentration).

This result agreed with Feizi *et al*. (2013) study. In the Raskar and Laware (2014) study, they mentioned that the onion germinated seeds increased at 20 µg.ml^{-1}ZnO NPs and then reduced obviously at 40 µg.ml^{-1}. Similarly, Bayramzadeh *et al*. (2019) found that AgNP at 80 mg.kg^{-1} soil decreased the germination of P. *sylvestris*. Zheng *et al*. (2005) and Feizi *et al*. (2013) mentioned the ability of nano-materials to penetrate into the seeds. The positive effect of nanoparticles on the seed was nanoparticles promote the antioxidant system as well as some enzymes such as nitrate reductase enzyme which improve the seed's ability to uptake water and minerals, catalase activities and ascorbate peroxidase enzymes. Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at P ≤ 0.05.

plant results

Pole bean plant growth parameters

Shoot, root length and root volume: The shoot length, root length and root volume were shown in Table 2. Applying NaCl irrigation water at 200 mM and foliar application of iron oxide nanoparticles at various levels on Pole bean plant altered ($p \leq 0.05$) the shoot length, root length and root volume. Compared to control, there was a significant reduction in the shoot length by 22.89%, root length by 25.18% and root volume by 20.99% after having treated Pole bean with only 200 mM NaCl. Our data agreed with Siddiqui *et al*. (2014) and Ali *et al*. (2021) studies. Moreover, the scientists Bakhoum and Sadak (2016), Dawood (2017) and Sadak (2019) reported a decrease in the sunflower, quinoa and wheat growth parameters under abiotic stresses like salinity and drought which caused metabolic alteration and synthesis of ROS at high levels. The declination in the length is correlated to the increasein osmotic pressure and ion toxicity resulting in short cellular length, volume and turgor Franco *et al*. (2011) and Hasanuzzaman *et al*. (2013a). In the plant environment, the toxic ions are considered when the salts increased at an adverse amount and the osmotic stress resulted from the reduction in the water potential (El-Dengawy *et al.,* 2021). In our experiment, salinity reduced the root volume which supported the results of Sánchez-Blanco *et al*. (2014) and Li *et al*. (2019) studies. The concentration of NaCl water between 50 to 150 mM adversely

affected the functions of the cellular wall of the root by synthesis of H_2O_2 at a high level (Lin and Kao, 2001). On the other hand, the length of shoot, root and root volume enhanced obviously ($p \leq 0.05$) after applying different levels of Fe_2O_3 NPs under 200 mM NaCl over 200 mM NaCl only. At 10 µM Fe_2O_3NPs + 200 mM NaCl there was an increase in the shoot and root length by 14.33% and 20.04%, respectively. Whereas root volume enhanced by 37.83% at 20 µM Fe_2O_3 NPs + 200 mM NaCl. Our results are in agreement with Rui *et al*. (2016) and Suriyaprabha *et al*. (2012) studies who reported that the shoot and root length of Peanut (*Arachis hypogaea*) and maize (*Zea mays* L.) increased after applying Fe_2O_3 NPs and silica NPs, respectively. According to Dhoke *et al*. (2013), after 12 days of the foliar method of the ZnO NPs and FeO NPs the mung length of shoot and root enhanced. Silicon reduced the effect of salinity stress by enhancing the permeability of the plasma membranes in the plant cells through increasing the anti-oxidative enzymes (SOD and CAT) and raising the plant water content (Al-Aghabary *et al.,* 2005 and Haghighi and Pessarakli, 2013). Many previous reports had mentioned the positive effect of different nanoparticles (ZnO, Fe_2O_3, Al_2O_3 and CuO) on the length of both shoot and root of rice, wheat, maize, tomato, and barley plants (Rizwan *et al.,* 2017). The application of a high concentration of nanoparticles may cause a negative impact on some plant species and this is agreed with Zuverza-Mena *et al*. (2016) study. They mentioned that nano-Ag at 500 $mg.L^{-1}$ reduced the shoot and root length of radish (*Raphanus sativus*). Rizwan *et al*. (2017) documented that the toxicity of the nanoparticles on plant specious causes declining in the gas-exchang3e and photosynthesis rates, changing in the nutrient status and highly synthesizing ROS. Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.

Shoot and root fresh and dry weight: The results in Table 3 proved that under irrigation with salty water at the level of 200 mM of NaCl and sprayed with nano-Fe_2O_3 altered significantly ($p \leq 0.05$) the shoot and root fresh (FW) and dry weight (DW) of Pole bean plant. Over control, the treatment of 200 mM of NaCl significantly declined the shoot and root FW and DW by 19.04% (shoot FW), 40.69% (root FW), 32.69% (shoot DW) and 40.90% (root DW). Our data agreed with Sreelakshmy *et al*. (2021) study who found that the concentration of NaCl at 100 mM led to a remarkable reduction in the fresh and dry mass of tomato leaves (*Solanum lycopersicum*). In addition, Larbi *et al*. (2020) announced that the negative effect of applying NaCl at 100 and 200 mM on the shoot dry weight of the Olive plant was more than its root dry weight. Our results are supported with Al-Maskri e*t al*. (2010), Roychoudhury *et al*. (2021) and Mazumdar *et al*. (2019). It was reported that the reduction in the fresh and dry mass of banana (*Musa acuminata* cv. *Berangan*) under an increased level of applied seawater enhanced the production of ROS and decreased the function of antioxidant enzymes (Mazumdar *et al.,* 2019). However, foliar application of Fe_2O_4 NPs on Pole bean under 200 mM NaCl caused an increased ($p \leq 0.05$) in the fresh and dry mass of shoot and root over 200 mM NaCl only (Table 5). Compared to 200 mM NaCl, the maximum significant increase in the shoot fresh weight, shoot dry weight and root dry weight by 25.71%, 30.46% and 38.09%, respectively at 10 µM Fe_2O_3 NPs + 200 mM NaCl, on the other hand, root fresh weight went up significantly by 66.01% at 20 µM Fe_2O_3NPs + 200 mM NaCl. Several scientists reported the positive role of a small amount of nanoparticles on plant growth. Siddiqui *et al*. (2014) found that 6 $g.L^{-1}$ of SiO_2 NPs under NaCl enhanced the shoot and root FW and DW in the squash (*Cucurbita pepo* L.). According to Hassanpour-aghdam *et al*. (2019), 3 $mg.L^{-1}$ of nano-Fe under 75 and 225 mM of NaCl raised the shoot and root fresh mass in the *Rosmarinus officinalis* L. plant. It is important to mention that nanoparticles at a low level are capable to decline the ROS synthesis while nanoparticles at a high level incuse the generation of ROS (Rahmatizadeh *et al.,* 2019). In this experiment, the fresh and dry mass of the shoot and root declined under 30 µM Fe_2O_3NPs + 200 mM NaCl. Khan *et al*. (2021) documented the enhancement of applying (Ag NPs 10, 20 and 30 mM) on the pearl millet plant. They reported that the dry weight in the shoots and root of pearl millet plant over 20 mM Ag NPs + 150 mM NaCl increased by 88% and 54%, respectively compared to treatment of 150 mM NaCl. Our data disagreed with Kanjana (2019) study. He reported that applying iron at 4 $g.L^{-1}$ on the Cotton (*Gossipium hirsutum*) increased the shoot and root dry mass. The scientists Salem *et al*. (2016) mentioned that nano-sulfur at 300 ppm on the tomato plant increased the shoot and root fresh and dry mass, but these decreased at 400 ppm. Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.

Leaf number and leaf area (cm^2): Leaves number is considered to be the main indicator for the vegetative development stage as these leaves are the essential photosynthetic organs controlling plant growth (Ghazi, 2018). In this experiment, the leaves number (LN) and leaf area (LA) of the Pole bean plant changed significantly ($p \leq 0.05$) after spraying nano-iron oxide at various concentrations under 200 mM NaCl (Table 4). Over control, there were a significant reduction in both LN and LA by 13.19 % and 33.42%, respectively in each plant. Our data agreed with Ali *et al*. (2021) who reported that LN on Mung beans (*Vigna radiate*) declined under 150 mM NaCl. They reported that the reduction in the LN was due to uptake and accumulation of high levels of salt in the cells of the plant. In addition, Kong *et al*. (2016) noticed that salinity promotes leaf senescence and caused a reduction in the LN. Also, many studies reported a reduction in the LN and LA under salinity such as Mazumdar *et al*. (2019) in the banana plant and Simões *et al*. (2016) in the sugar cane plant. The reasons of the declination of the LA under salinity might be due to the plant reducing the loss of water and another reason could be the accumulation of salts in the plant reduced the photosynthesis process (Parida and Das, 2005 and Qados, 2011). According to our experiment, the Pole plant was exposed to the chlorosis symptom (leaves yellowing) after the plant has irrigated with 200 mM NaCl which restricted the uptake of Fe from soil to the Pole bean plant. On the other hand, the LN and LA in the pole bean increased remarkably ($p \leq 0.05$) after spraying Fe_2O_3 NPs at different concentrations under 200 mM NaCl. Compared to 200 mM NaCl, LN increased significantly by 14.58% at 10 µM Fe_2O_3 NPs + 200 mM NaCl, whereas LA raised obviously by 26.26% at 20 µM Fe_2O_3 NPs + 200 mM NaCl. Our study agreed with Ghazi (2018) report who mentioned that foliar nano-Se at a low amount enhanced vegetative growth of the plant. Our data strongly agreed with Guha *et al*. (2021) experiment who reported that 20 $mg.L^{-1}$ of nano Scale Zero Valent iron increased the LA of the *Oryza sativa* L. cv. *Gobindobhog* plant. According to our study, 30 µM Fe_2O_3NPs + 200 mM NaCl slightly increased the LN and LA. While in Tawfik *et al*. (2021) data, they noticed an increase in the LA of *Moringa*

oleifera plant after the plant has treated with 40 ppm nano- iron oxide. Also, mixing 100 $mg.kg^{-1}$ soil of nano-iron led to enhancement in the LA of the tomato (cv. *Bigdena* F1) plant (El-Desouky *et al.,* 2021). Nano-iron oxide might improve the ability of the plant to resist the salinity, which results in the development of Pole bean growth. Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.

Leaf relative water content (LRWC): Table5shows that there were alterations ($p \leq 0.05$) in the leaf relative water content (LRWC) after applying saline water at 200 mM NaCl and spraying various doses of iron oxide nanoparticles compared to untreated plants. The LRWC decreased significantly at 200 mM NaCl by 5.89% over control. Our results are supported by the Mustafa *et al*. (2021) data. Many scientists reported a reduction in the RWC under salinity. Ben Ahmed *et al*. (2010) and Hailu and Mehari, (2021) explained the reasons of this reduction, that high salinity has restricted the absorption of water and dissolved essential minerals for plant growth by roots from the soil leading to declines in the RWC and plant biomass. However, the foliar application of Fe_2O_3 at different concentrations under 200 mM NaCl obviously increased the LRWC ($p \leq 0.05$) compared to 200 mM NaCl (Table 5). Over saline treatment only, the highest value in the LRWC was at the 20 µM Fe_2O_3NPs + 200 mM NaCl by 11.02%. Several studies mentioned the positive effects of nanoparticles (NPs) on plant water content and water use (Mahmoud *et al.,* 2020 and Zulfiqar and Ashraf, 2021). According to Zulfiqar and Ashraf (2021), NPs induce stomatal conductance, transpiration as well as the leaf water content at high rates. There was an increase in the seed water content under salinity stress after exposing the seeds to the 100 and 500 $mg.L^{-1}$ Fe_2O_3 (Maswada *et al.,* 2018). They reported that Fe_2O_3 can accumulate compatible (compatible solutes)which enhanced the cellular osmotic adjustment. This compatible is increases plant ability to absorb water from the soil resulting in cell turgor. Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.

Determination of chlorophylls and carotenoids content: It is clear to see that there were variations ($p \leq 0.05$) in the chlorophylls (chl a and chl b) and carotenoids (cart) contents under salinity and at various levels of spraying Fe_2O_3 NPs over control (Table 6). Compared to untreated plants, there was a significant declination in the contents of chl a, chl b and cart by 46.21%, 44.81% and 38.92%, respectively at 200 mM NaCl. Our results agreed with Madani *et al*. (2022) and Tuna *et al*. (2008) data. The decrease in the content of the photosynthetic pigments was related to the low rate of photosynthesis, according to Gohari *et al*. (2021). They also added that salinity causes an increased rate of degradation or a decrease in the synthesis of the photosynthetic pigments that decline the chlorophyll amounts. Fang *et al*. (1998) mentioned that chl b breakdown to produce chl a explained the reduction in the chlorophylls amount. On the other hand, spraying of Fe_2O_3 at different levels under 200 mM NaCl obviously improved ($p \leq 0.05$) the chl a, chl b and cart compared to 200 mM NaCl (Table 6). According to our experiment and compared to only saline treatment, the maximum values of chl a was at 30 µM Fe_2O_3NPs + 200 mM NaCl by 29.58%, chl b and cart were at 20 µM Fe_2O_3NPs + 200 mM NaCl by 38.22% and 31.31%, respectively. The iron mineral is needed to maintain the DLA (delta-aminolevulinic acid) formation at the optimum rate for chlorophyll organ production (Yu and Miller, 1982).

In the studies of Abdel Latef *et al*. (2018) and Mustafa *et al*. (2021) the content of the pigment in the lupine (*Lupines termis*) and wheat (*Triticum aestivum* L.) leave increased at 0.01% of TiO_2 and 40 $mg.L^{-1}$ of TiO_2 respectively. The foliar application of nano-iron oxide under saline conditions may enhance the Pole bean resistance ability leading to an increase in the content of photosynthetic pigments as the development of growth increase. Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.

Determination of total and soluble carbohydrate content ($mg.g^{-1}$ DW) in the shoot and root: Table 7 shows that the content of the total and the soluble carbohydrate in the shoot and root of Pole bean plants changed remarkably ($P \leq 0.05$) after applying the foliar application of Fe_2O_3 at various doses under high salinity stress. Compared to control, 200 mM NaCl caused a significant reduction in the shoot total carbohydrate (STC) by 36.14% and the root total carbohydrate (RTC) by 31.17%. However, spraying Fe_2O_3 at several levels under 200 mM NaCl raised the levels of STC and RTC. The highest content of the STC was at 20 µM Fe_2O_3NPs + 200 mM NaCl by 35.22%, but the RTC increased highly at 30 µM Fe_2O_3NPs + 200 mM NaCl by 41.20%. These results agreed with Ali *et al*. (2021) who reported decreasing in the *Vigna radiate* LTC (leaf total carbohydrate) under salinity. On the other hand, some carbohydrates like sucrose and starch are enhanced under salinity (Yuan *et al.,* 2015). The reason behind the low TC content under 200 mM NaCl was that some non-soluble carbohydrates degraded to synthesize soluble ones.

The soluble carbohydrate in the shoot and root (SSC and RSC) remarkably decreased ($P \leq 0.05$) under 200 mM NaCl by 30.21% and 18.68%, respectively (Table 7). This result was similar to Zhang *et al*. (2016) and Kempa *et al*. (2008). According to our data, SSC and RSC amount increased after spraying the Fe_2O_3 at different quantities under 200 mM NaCl. Over 200 mM NaCl, the maximum levels of SSC and RSC were at 10 µM Fe_2O_3NPs + 200 mM NaCl by 30.45% and 21.35%, respectively. Similarly, it was mentioned that there was an increase in the maize soluble sugar after spraying the Zn (Iqbal *et al.,* 2018). Also, Singh *et al*. (2021) reported that 25 ppm IONPs + salinity stress increased the SS. SS is considered as one of the organic osmotic regulators that provide crops with the energy required for metabolism processes, on the other hand, the SS in the *Gossypium hirsutumis* reduced when the salinity increases (Chen *et al.,* 2020). Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.

Determination of total and soluble protein content ($mg.g^{-1}$ DW) in the shoot and root: The shoot and root total protein (STP and RTP) contents altered significantly ($p \leq 0.05$) under the 200 mM NaCl and the combination of this concentration with the foliar method of Fe_2O_3 as compared control plants (Table 8). In the recent data, the STP and RTP reduced significantly by 7.39% and 59.92%, respectively over control. On the other hand, the foliar application of several doses of Fe_2O_3 under 200 mM NaCl increased obviously the STP and RTP. Over the treatment of only 200 mM NaCl, the highest increase in the STP by 5.29% and RTP by 71.90% at 20 µM

Fe_2O_3 NPs + 200 mM NaCl. Ali *et al*. (2021) reported that TP (total protein) decreased in the saline conditions and this data was in agreement with our data. In this experiment, the amount of the STP and RTP raised under the high concentrations of Fe_2O_3 NPs and then decreased but this decreases stillhigher than control and 200 mM NaCl treatments. Some cell properties such as the membrane and the survival depend on the protein content (Goudarzi and Pakniyat., 2009). Table 8 represented that the shoot and root soluble protein (SSP and RSP) reduced significantly ($p \leq 0.05$) under 200 mM NaCl compared to control. Over untreated plants, SSP and RSP declined remarkably by 1.66% and 28.91%, respectively. Under the combination of nano-Fe_2O_3spray at different levels and saline conditions, The SSP and RSP were raised. At low levels, the SSP and the RSP increased by 4.26% and 38.46%, at 10 and 20 µM Fe_2O_3NPs + 200 mM NaCl respectively, compared to 200 mM NaCl. Plants under high salinity, have the ability to accumulate proteins in form of nitrogen to regulate the osmotic adjustment (Amini and Ehsanpour, 2005). Furthermore, they added that SP (soluble proteins) levels increased to produce other new proteins to regulate the wall of the cell plant. Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.

Determination of free amino acid content (mg.g^{-1} DW) in the shoot: The foliar application of Fe_2O_3at a different amount under 200 mM NaCl caused significant reductions ($p \leq 0.05$) in the content of shoot free amino acid (mg.g^{-1} DW) over control (Table 9). Compared to the control, the shoot free amino acid remarkably decreased by 47.22% at 200 mM NaCl. Our data was opposite to the Li *et al*. (2021) data who noticed a high level of free amino acid under an increased salinity. Our results were supported with Shafiq *et al*. (2019) and Saad-Allah and Ragab (2020) who documented that high salinity at 150 and 200 mM NaCl obviously declined the wheat free amino acid. Additionally, Saad-Allah and Ragab (2020) mentioned that salinity tends to degrade the free amino acid to provide plants with nitrogen and carbon minerals when these minerals exist at low levels under salinity.

Table 9 shows significant enhancement ($p \leq 0.05$) in the shoot free amino acid after spraying Fe_2O_3 at various doses under 200 mM NaCl over salt treatment only. The highest amount of free amino acid was at 20 µM Fe_2O_3NPs + 200 mM NaCl by 42.13%, on the other hand, the lowest amount was at 30 µM Fe_2O_3NPs + 200 mM NaCl by 27.62% over salt treatment only. These results indicate that theFe_2O_3NPsenhance the level of free amino acids of plants grown under salinity. Under salinity stress, the reduction in the free amino acid levels was to produce a protein (Roychoudhury *et al.,* 2021). A similar result was reported by (El-Bassiouny *et al.,* 2022) who found that low doses of TiO_2 NPs or ZnO NPs at 10 mg^{-1} enhanced the free amino acid in the wheat. However, the opposite result was reported by Mehrian *et al*. (2015). Sharifi-Rad *et al*. (2018) reported that applying 400 mg L^{-1} of SiO_2 NPs on the 6 different plants enhanced the plants free amino acids, but increase the concentration of SiO_2 NPs at 2,000 and 4,000 mg.L^{-1} reduced the free amino acid levels. Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.

Determination the percentage of the shoot and root minerals (N, Fe, P, K and Na): According to Tables 10 and 11, high salinity at 200 mM NaCl led to remark alterations ($P \leq 0.05$) in the nutrients percentage (N, Fe, P, K and Na) of shoot and root compared to control. There were significant reductions in the percentage of N, Fe, P and K in the shoot and root, but the reduction was non-significant in the root P under 200 mM NaCl only over control. In the shoot, the percentages were 5.88%, 40.17%, 46.74% and 32.54%, in the N, Fe, P and K respectively, whereas in the root were 6.71%, 42.50%, 8.95% and 59.52%, respectively. Similar data was recorded by El-Dengawy*et al*. (2021) and Doaa and shalan (2020). On the other hand, 200 mM NaCl caused raise in the percentage of the shoot and root Na by 70.28 and 74.52%, respectively compared to untreated plants. This result agreed with Abou-shlell *et al*. (2020)and El-Saadony*et al*. (2021) data and they mentioned that the high content of Na^+ in the leaves increased the ROS which has adverse impacts on the plant cells structure and function. It is obvious to see that the foliar application of iron oxide NPs at the different amounts(10, 20 and 30 µM Fe_2O_3 + 200 mM NaCl)increased ($P \leq 0.05$) the percentage of the shoot and root N, Fe, P and K under salinity (Table 10 and 11). On the other hand, Na was reduced compared to 200 mM NaCl treatment. The treatment at 10 µM Fe_2O_3 + 200 mM NaCl caused a significant increase in the root K and P percentage by 62.63% and 36.33%, respectively. Also, 20 µM Fe_2O_3 + 200 mM NaCl led to obvious enhancement in the shoot K and P percentage by 47.53% and 58.84%, respectively over 200 mM NaCl. Furthermore, 30 µM Fe_2O_3 + 200 mM NaCl improved both the shoot and root N percentage by 19.65 and 13.16%, respectively.

The concentrations of 20 and 30 µMof Fe_2O_3+ 200 mM NaCl increased the percentage of Fe in the shoot by 45.20% (at both concentrations) and root by 50% (at both concentrations) compared to saline treatment. While the presence of the Fe_2O_3 at all the concentrations reduced the percentage of Na in the shoot and root. Compared to 200 mM NaCl, the maximum reduction of Na in the shoot was at 10 µM Fe_2O_3 + 200 mM NaCl by 35.99%, whereas in the root was at 30 µM Fe_2O_3 + 200 mM NaCl by 27.14%. These findings are in line with those of Rizwan et al. (2017) and El-Dengawy *et al*. (2021). Scientists reported similar data that foliar method of Hoagland with ZnO and Fe_3O_4 NPs declined obviously the Na and Cl, however, raised the N, P, K and Fe (Ahmad and Akhtar, 2019). Naeem *et al*. (2017) announced that some minerals (N, P and Fe) are essential for plants during their whole life to synthesize macro-molecules, transfer energy and maintain enzyme activities. Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.Each value is the mean of three replicates and ± SD. The different letters illustrate the statistical significance between all treatments level at $P \leq 0.05$.

Conclusion

A recent study suggests that salinity inhibits neural development growth traits and physiological activity of pole bean plants. A further revelation was that the application of foliar iron oxide Fe_2O_3 to the leaves might be considered an effective method for mitigating the damaging effects of salinity by enhancing germination, the length of shoot, root and root volume, fresh and dry weight, leaf relative water content, chlorophyll content, carbohydrates and protein composition, free amino acid content, mineral nutrients, in addition, sodium and chloride are reduced.

Thus, it can be concluded that a spray of iron oxide nanoparticles could be a useful tool in enhancing physiological parameters and protecting plants from salinity stress.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SOB designed, monitored the work of experiments and analysis, helped in writing, and reviewed the manuscript. AIA performed the experiments, collected the sample, data analysis, and wrote the manuscript.

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