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Foliar iron and zinc nano-fertilizers enhance growth, mineral uptake, and antioxidant defense in date palm (*Phoenix dactylifera L.*) seedlings

Hussein J. Shareef[1]*, Ahmed Y. L. Hzaa[2], Nabil I. Elsheery[3]

[1]Date Palm Research Center, University of Basrah, Iraq
[2]Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, Iraq
[3]Agriculture Botany Department, Faculty of Agriculture, Tanta University, Egypt

Abstract

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Salty sandy soil usually hinders plant growth, while spraying nano-fertilizers such as iron and zinc enhances plant growth. This experiment investigated the role of iron and zinc nano-fertilizers (1 g l^{-1}) in the adaptation of date palm seedlings (cv. Barhee) subjected to salt stress (0, 75, 150 mM NaCl). Nano-fertilizer increased plant height, length of roots, number of leaves, and roots. In contrast, salt stress led to reducing these parameters. Salt stress increased hydrogen peroxide, electrolyte leakage, malondialdehyde, and antioxidants such as soluble proteins, proline, catalase, ascorbate peroxidase, and peroxidase enzyme in the leaves. Abscisic acid also increased. Nano-fertilizers increased the chlorophyll and dry matter of the plant under salt stress. Nano-iron induced better seedling growth than nano-zinc, especially in the length of the roots. Nano-iron under salt stress increased iron and potassium concentration and K/Na ratio in leaves. Nano-fertilizers help the plant adapt to environmental stresses, and seedlings succeed in growing in saline sandy soils.

Keywords

antioxidant defense, ascorbate peroxidase, electrolyte leakage, nano-fertilizers, seedlings

Introduction

Date palm (*Phoenix dactylifera L.*) is a crucial plant tolerant of salinity in advanced stages. Dates are considered one of the essential non-traditional commodities and crops that can be used for local consumption and export, providing an excellent economic return (Aseeri et al., 2021). The area cultivated with date palms is increasing day after day in the world, especially in desert areas (sandy soils). Those interested in palm trees in desert areas face limited irrigation water, and the plant's need to consume large amounts of water. Increasing competition for water assets has led to using brackish water for irrigation, despite the resource shortages associated with brackish water wells (Shareef et al., 2021). Many countries interested in date palms have established projects for planting date palms in the desert (sandy soil) either as a green belt or to obtain date production (Aldhebiani et al., 2018). One of the biggest significant problems facing these projects in sandy soils was the irrigation with salt water and the soil with poor nutrients (Haider et al., 2015). Still, numerous stresses in the first stages of development lead to reduced

*Corresponding author:
e-mail: hussein.shareef@uobasrah.edu.iq

production and sometimes death (Al-Abdoulhadi et al., 2012a). Many researchers have obtained positive results from applying foliar nano-fertilizers to date palms to improve quality and production (Amiri et al., 2016; Jubeir and Ahmed, 2019; Altemimy et al., 2019; Ressan and Al-Tememi, 2019). Nano-fertilizers can increase nutrient use efficiency, thus benefiting nutrition management; these nutrients are bound to the nano-absorbents that are applied either alone or in combination and release the nutrients at a slower rate than conventional fertilizers (Nongbet et al., 2022).

Foliar nutrients have become a reality in improving plant growth and production (Shareef et al., 2021). Foliar nanomaterials allow different nutrients to quickly reach the plant and maximize participation in plant metabolism (Mahil and Kumar, 2019). Nano-fertilizers by the spray method ensure the plant's essential nutrients during the critical and sensitive plant growth stages that the roots cannot provide (Morsy et al., 2017). The roots are responsible for absorbing nutrients in the plant; this mechanism is complicated, especially in alkaline soils. Saline soils usually hinder the absorption of elements, especially phosphate ions, iron, and zinc, leading to precipitation and the formation of complex compounds not ready for absorption by the roots (Peralta-Videa et al., 2014).

Moreover, sandy soils are low in micronutrients such as iron and zinc (Drissi et al., 2016). Iron and zinc deficiency is an abiotic stress factor influencing plants' growth and development (Rout and Sahoo, 2015). Iron and zinc have physiological significance in plant life; iron has a role in the construction of chlorophyll, oxidation, and reduction process within plant tissue, and its entry into cytochromes' composition is essential in photosynthesis and the building of proteins (Rajaie and Tavakoly, 2017). Zinc is one of the critical micronutrients in plant nutrition because it assumes a vital role in the plant's structure and growth through participation as a co-factor to the activity of 300 enzymes (Zagzog and Gad, 2017).

Salts concentrations increase in the soil or irrigation water, causing several plant dysfunctions, such as osmotic stress, accumulation of harmful elements, and oxidative stress (Shrivastava and Kumar, 2015). Several strategies improve the plant's salinity tolerance, including adapting the plant's seedlings in pre-treatments with non-lethal sodium chloride levels that prepare the plant to withstand high salt concentrations (Mbarki et al., 2020). Enhancing the salinity tolerance of date palms were discussed by researchers (Kurup et al., 2009; Tripler et al., 2011; El Rabey et al., 2015; Ait-El-Mokhtar et al., 2019). The acclimation of seedlings or seeds is easier and cheaper than the technology for genetic transformation. The osmotic and ionic stress due to salinity stimulates oxidative stress due to increasing the content of reactive oxygen species (ROS) and hydrogen peroxide (Hasanuzzaman et al., 2021). The experiment was aimed to determine the nano-fertilizer's potential to reduce oxidative stress and the long-term response of date palm seedling adaptation under salt stress to nano-fertilizers of iron and zinc.

Materials and methods

Seeds of Barhee date palm were gathered from a farmer's orchard in Abu Al-Khaseeb, Basrah, Iraq (30°26'09.2''N, 47°59'04.1''E), approved by Date Palm Research Center-Basrah University to conduct scientific experiments during the 2020–2021 seasons. For germination, the seeds were grown in unadulterated sand soil in an incubator for two months at 27 ± 2 °C on 1st March 2020. Seedlings were separately moved to 90 plastic pots (5 kg) (two pots to one replicate) filled with sand molecule size 0.7–2.0 mm, field limit 17.7 wt %, and peat moss in a 2:1 proportion. Seedlings were developed in the nursery at 30 ± 2 °C, with a relative humidity of about 20%, and photoperiod maintained at 12–16 h d^{-1} photoperiod and 1,350 µmol m^{-2} s^{-1} light intensity. The agriculture medium was irrigated with 0, 75, and 150 mM NaCl as the primary salinity source during the experiment season. On 1st August, nano-fertilizers were applied once as 1 g l^{-1} foliar spray. The NaCl is applied along with the irrigation weekly and continues to harvest plants after ten months on 1st March 2021. These concentrations represent a low and medium simulation of NaCl levels in natural and groundwater irrigation.

Treatments included (i) 0 mM NaCl + spray distilled water (control), (ii) 0 mM NaCl + spray nano-iron 1 g l^{-1}, (iii) 0 mM NaCl + spray nano-zinc1 g l^{-1}, (iv) 75 mM NaCl + spray distilled water, (v) 75 mM NaCl + spray nano-iron 1 g l^{-1}, (vi) 75 mM NaCl + spray nano-zinc 1 g l^{-1}, (vii) 150 mM NaCl + spray distilled water, (viii) 150 mM NaCl + spray nano-iron 1 g l^{-1}, (ix) 150 mM NaCl + spray nano-zinc 1 g l^{-1}. All the treatments contained the surfactant Tween 20 at 0.1%, and the droplets reached the substrate surface. The treatments were repeated five times; two pots were treated as one replicate to determine the long-term response to the adaptation of date palm seedlings under salt stress to iron zinc nano-fertilizers. Six months after applying nano-fertilizer on date palm seedlings subjected to salt stress over ten months, the seedlings were harvested (Fig. 1). All leaves of ten seedlings per treatment were used for analysis. The number of leaves, length, number of roots, and length were recorded. Each seedling's leaves and roots were separated and weighed to determine the fresh weight. The dry matter was determined by drying in an oven at 70 °C for 72 h.

Chelated nano-Iron 12% Fertilizer powder product analysis (N 4%, Fe 12%, Zn 1.5%, Mn 0.5%) and Chelated Nano-Zinc 20% Fertilizer powder product analysis (N 5%, Zn 20%) (Sepeher Parmis, Agriculture & Livestock /Agriculture /Agricultural Pesticides and fertilizers, Iran) were used.

Chlorophyll measurement

The chlorophyll contents were calculated utilizing the Konic Minolta SPAD-502 Plus in each seedling's fully expanded leaf. Five points were recorded at different sites on the same leaf, and five seedlings were recorded per treatment.

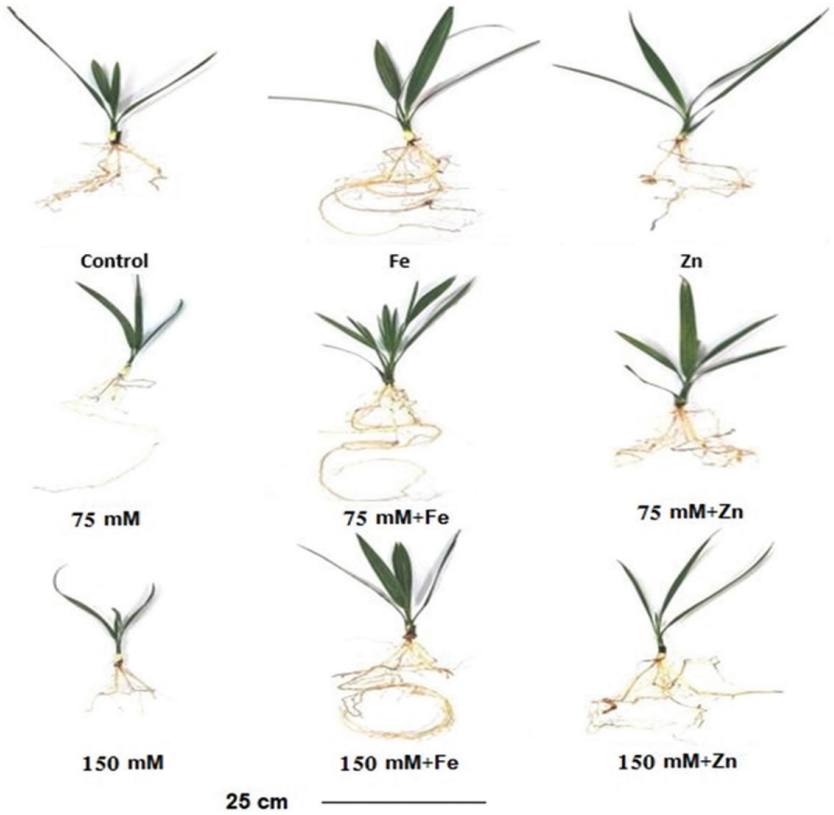

Fig. 1. The response of date palm seedlings to nano-iron and nano-zinc fertilizers under salt stress, treatments included (i) 0 mM NaCl + spray distilled water (control), (ii) 0 mM NaCl + spray nano-iron 1g l^{-1}, (iii) 0 mM NaCl + spray nano-zinc1g l^{-1}, (iv) 75 mM NaCl + spray distilled water, (v) 75 mM NaCl + spray nano-iron 1g l^{-1}, (vi) 75 mM NaCl + spray nano-zinc 1g l^{-1}, (vii) 150 mM NaCl + spray distilled water, (viii) 150 mM NaCl + spray nano-iron 1g l^{-1}, (ix) 150 mM NaCl + spray nano-zinc 1g l^{-1}.

Malondialdehyde (MDA) content

Fresh leaf segments (0.5 to 1.0 g) were homogenized in 5 ml of 5% (w/v) trichloroacetic acid, and at room temperature, the homogenate was centrifuged at 6,000 × g for 15 minutes. The supernatant was blended with an equal volume of thiobarbituric acid (0.5% in 20% (w/v) trichloroacetic acid), and the blend was bubbled for 25 min at 100 °C and centrifuged for 5 min at 7,500 × g. Absorbance was recorded at 532 nm and corrected for 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer, 1968).

Electrolyte leakage (EL)

Electrolyte leakage was utilized to evaluate membrane permeability as per Lutts et al. (1995). Samples were washed multiple times with double-distilled water to evacuate surface pollution. Leaf segments were cut from leaves and set in fixed vials containing 10 mL of double-distilled water and vibration for 24 h. The electrical conductivity of the solution (EC1) was determined. The samples were autoclaved at 120 °C for 20 min, and the electrical conductivity was estimated once more (EC2) after the solution was cooled to room temperature. The electrolyte leakage was characterized as EC1/EC2 × 100 and communicated as a rate (%).

Hydrogen peroxide (H_2O_2)

A quantity of 50 mg of leaf tissue with 3 ml of phosphate buffer (50 mM, pH 6.5) was extracted by homogenization. At that point, the extract was centrifuged at 8,000 × g for 20 min. H_2O_2 content determined by 3 ml of extracted solution was blended with 1 ml of 0.1% titanium sulfate in 20% (v/v) H_2SO_4, and the blend was centrifuged at 7,000 × g for 10 min. The intensity of the yellow colour of the supernatant was estimated at 410 nm. The H_2O_2 content was processed using the termination coefficient of 0.28 μmol^{-1} cm^{-1} (Kanwal et al., 2014).

Soluble protein contents

A quantity of 200 mg of fresh leaf tissue was homogenized in 5 ml potassium phosphate buffer (pH 7). The homogenate was centrifuged at 17,000 × g for 20 min. The supernatant was carefully transferred into test tubes. According to Bradford et al. (1976), soluble protein extract was estimated, and Bovine serum albumin (BSA) was used as a standard.

Proline concentration

The proline concentration was estimated by Bates et al. (1973) method. The leaf (250 mg dry matter) was ho-

mogenized in 3% sulfosalicylic acid as the solvent, and at 12,000 × g, the homogenate was centrifuged to estimate the proline concentration. The reaction blend consisted of 2 ml ninhydrin acid and 2 ml of glacial acetic acid, kept in a water bath at 100 ºC for 1 hr to develop the colours, with 4 ml of toluene to extract. The absorbance at 520 nm was read.

Abscisic acid analysis (ABA)

A fresh tissue sample (4 g) was homogenized in 70% methanol and stirred overnight at 4 °C; filter paper (No. 1) was used to filter the extract. The filtrate was vacuum evaporated. The vacuum-dried residue was dissolved in 10 ml of 0.1 M phosphate buffer (pH 8.5) by stirring for 30 min. The aqueous phase was partitioned twice using methanol. A rotary evaporator removed the methanol phase. In the aqueous phase, pH was adjusted to 2.5, using 1 N hydrochloric acid (HCl) and extracted four times with ethyl acetate (4 × 10 ml). The extract was lyophilized and dissolved in 5 ml of 0.5 M phosphate buffer (pH 8). The sample was then purified by passing through the Sephadex G10 column. For preparing the Sephadex column, 1 g Sephadex was swollen in 10 ml double distilled water overnight and packed in a glass column (1 cm diameter and 5 cm length). The column was equilibrated with phosphate buffer up to 2 cm. The eluted solution was again lyophilized, and the residue was finally dissolved in 1 ml of acetonitrile and analyzed by HPLC. Samples were analyzed using UV wavelength at 254 nm. The retention time of ABA was 8.78 min. The chromatographic system's reproducibility and linearity were estimated by five consecutive injecting of different concentrations of standard ABA prepared by dissolving 25 mg of ABA in 25 ml of HPLC grade acetonitrile. The calibration standards of concentrations 1, 5, 10, 50, and 100 µg ml^{-1} were prepared by successive dilutions of the above working stock solution. The analytical method was validated using a single-laboratory approach (Tang et al., 2011). The pH was kept at 4 utilizing 1 N sodium hydroxide. The temperature was kept at 25 °C, the flux rate was 0.8 ml min^{-1}, and the elution of the abscisic acid was observed at 265 nm.

Assays of antioxidant enzymes

The leaves were ground in liquid nitrogen using a mortar and pestle after being chilled with liquid nitrogen, and the frozen powder was immediately used for enzyme extractions. Enzymes were extracted at 4 °C by 0.5 g of leaves, and 1.0% PVP and 2 ml of the following optimal media (Moran et al., 1994); for catalase (CAT) and peroxidase (POD): 100 mM K-phosphate, pH 7.0, 0.1 mM EDTA, 0.1% Triton; for ascorbate peroxidase (APX): 50 mM K-phosphate buffer, pH 7.8, 50 mM ascorbate. The homogenate was centrifuged at 1,000 × g for 15 min for CAT and POD and 9,000 × g for 20 min for APX at 4 °C. The supernatants for enzymatic assays were utilized. CAT was determined by monitoring hydrogen peroxide consumption (H_2O_2) in a spectrophotometer at 240 nm (Rao et al., 1996). Samples without H_2O_2 were utilized as blanks. The activity was calculated by the extinction coefficient of 39.4 mM^{-1} cm^{-1} for H_2O_2. One unit of CAT activity corresponded to the amount of enzyme that decomposes 1 mol of H_2O_2 per minute (Havir and Mchale, 1987). POD activity was read by the Nakano and Asada (1980) method. Reaction solution contained 2.85 ml 3% guaiacol (water solution), 0.1 mL 2% H_2O_2, and 50 µl enzyme extract. The activity unit was calculated using the absorbance coefficient for guaiacol at 470 nm. Enzyme activities were expressed as enzyme units per gram of fresh weight (U g^{-1} FW). The activity of APX was determined by the absorbance of the oxidized ascorbate at 290 nm decreasing, following the method of Nakano and Asada (1980). The concentration of oxidized ascorbate was calculated using the extinction coefficient (ε = 2.8 mM^{-1} cm^{-1}). One unit of APX activity was read as the amount of enzyme that oxidizes 1 mol of ascorbate per minute.

Measurement of mineral content

The mineral content was determined by AOAC (2005) method. The samples cut from the leaves were placed in an oven at 550 °C until they became ash. After the cooling process, 500 mg was added to 5 ml of concentrated chloride acid, then evaporated on a hot plate. Drops of H_2O_2 and vinegar were added to bidistilled water and filtered into 100 ml flasks to the product. Bidistilled water was used to bring the volume up to 100 ml. This solution was used to quantify mineral elements. K and Na were determined using the flame photometric method, and Zn and Fe content was quantified by atomic absorption.

Statistical analysis

A completely randomized design was used. IBM SPSS-23 statistical software (SPSS, Chicago, IL, USA) was used to analyze the data with an ANOVA table. The means were separated using the Duncan test at the 0.01 significance level.

Results

Nano-fertilizers enhance date palm seedlings' growth under salt stress

Analysis of variance (ANOVA) (Table 1) revealed a significant effect of nano-foliar applications and salinity levels on seedlings' growth. Mean comparisons showed that nano-fertilizers treatments increased plant height, length of roots, number of leaves, and roots (Table 2). At the same time, salt stress led to reducing these parameters. Nano-fertilizers associated with 75 mM NaCl increased plant height, length of roots, number of leaves, and roots related to 150 mM NaCl treatments. Nano-iron is more effective than nano-zinc in increasing plant height, length of roots, number of leaves, and roots under normal conditions

Table 1. Mean squares from ANOVA with variance of data for growth, antioxidant defense, and mineral in date palm seedlings subjected to foliar-applied iron and zinc nano-fertilizers under salt stress

Source of variations	df	No. of leaves	Length of leaves leaves	No. of roots	Length of roots
Salt stress	2	4.172**	73.889**	5.324**	386.489**
Fertilizer	2	16.598**	89.489**	11.811**	4,095.356**
salt * fertilizer	4	0.412**	8.556*	0.282**	205.122**
Error	36	0.040	0.411	0.072	16.967
		Leaf greenness	Dry matter	MDA	Electrolyte leakage
Salt stress	2	1,322.375**	215.764**	8.338**	2,105.756**
Fertilizer	2	513.167**	90.647**	1.507**	914.289**
salt * fertilizer	4	20.163**	0.452**	0.207**	36.689**
Error	36	12.140	0.029	0.010	3.175
		H_2O_2	Soluble protein	Proline	ABA
Salt stress	2	984.422**	12.570**	405.756**	44.607**
Fertilizer	2	92.422**	1.851**	38.422**	7.046**
salt * fertilizer	4	5.922**	0.231**	9.289**	0.501**
Error	36	0.489	0.010	0.900	0.006
		Cat	APX	POD	Fe
Salt stress	2	1.872**	3.877**	22.995**	10.020**
Fertilizer	2	0.361**	1.017**	4.515**	10.795**
salt * fertilizer	4	0.002**	0.358**	1.267**	0.804**
Error	36	0.002	0.009	0.010	0.000
		Zn	Na	K	K /Na ratio
Salt stress	2	0.139**	36.936**	38.260**	38.051**
Fertilizer	2	3.882**	14.020**	1.785**	5.553**
salt * fertilizer	4	0.001**	7.107**	19.182**	2.017**
Error	36	7.722	0.000	0.000	0.000

** significant at $P \leq 0.01$.

and salt stress (Table 2). According to the SPAD technique, chlorophyll was estimated in the leaves (Fig. 2a). Nano-fertilizers increase the plant's chlorophyll and dry matter under natural conditions or salt stress. Salt stress gradually decreases the plant's chlorophyll content and dry matter by increasing the salt stress level. (Fig. 2).

Nano-fertilizers enhance antioxidant defense and reduce Na-induced oxidative damage

Analysis of variance (ANOVA) results revealed a significant effect of nano-foliar applications and salinity levels on MDA, EL, and H_2O_2 seedlings content (Table 1). Mean comparisons showed that all nano-fertilization decreased the MDA, EL, and H_2O_2 content. In contrast, NaCl treatments increased (Fig. 3). The low leaf content of MDA, electrical leakage (EL), and H_2O_2 were significant in treating nano-Fe fertilizer. Nano-Fe decreased the MDA content significantly under 75 and 150 mM NaCl. Whereas no significant differences between the treatments of nano-fertilizer under 75 mM and 150 mM NaCl treatment (Fig. 3a). Also, there were no significant differences between the treatments of nano-fertilizer under 75 mM NaCl treatment on EL percentage. At the same time, nano-Fe decreased the EL percentage significantly under 150 mM NaCl (Fig. 3b). H_2O_2 decreased with nano-fertilizers under salt stress. In contrast, H_2O_2 increased gradually with increasing salinity levels (Fig. 3c).

Analysis of variance (ANOVA) results revealed a significant effect of nano-foliar applications and salinity levels on the content of soluble protein, proline, and abscisic acid seedlings (Table 1). Mean comparisons of nano-fertilizers showed a decrease in soluble proteins (Fig. 4a). Gradually, soluble proteins increased due to the effect of NaCl. Nano-Fe decreased the soluble proteins significantly under 150 mM NaCl related to nano-Zn. There was no significant difference between nano-iron and nano-zinc treatments in increasing soluble proteins in natural conditions. Proline has gradually increased under salt stress by increasing the level of NaCl. Nano-fertilizers decreased the concentration of proline under salt stress. No significant differences existed between nano-zinc and reducing proline concentration with 75 mM NaCl. Nano-Fe decreased proline concentration significantly under 150 mM NaCl (Fig. 4b).

The increase in NaCl level from 75 to 150 mM increased abscisic acid (ABA) content. Abscisic acid de-

Table 2. Mean comparisons of date palm seedlings response to foliar nano-iron and nano-zinc fertilizers under salt stress on No. of leaves, length of leaves, No. of roots, and length of roots

Treatments	No. of leaves	Length of leaves (cm)	No. of roots	Length of roots (cm)
0 mM NaCl (control)	4.10 ± 0.10 c	26.66 ± 0.57 cd	4.13 ± 0.15 cd	25.33 ± 0.57 c
0 mM NaCl + nano-iron	6.10 ± 0.10 a	34.33 ± 0.57 a	6.11 ± 0.10 a	66.66 ± 7.63 a
0 mM NaCl + nano-zinc	5.10 ± 0.10 b	31.00 ± 1.00 b	5.15 ± 0.15 b	40.00 ± 5.00 b
75 mM NaCl	3.10 ± 0.10 d	25.66 ± 0.57 d	3.33 ± 0.57 de	24.66 ± 0.57 c
75 mM NaCl + nano-iron	5.33 ± 0.57 b	30.00 ± 1.00 b	5.11 ± 0.10 b	60.00 ± 10.0 a
75 mM NaCl + nano-zinc	5.06 ± 0.11 b	27.33 ± 0.57 c	4.88 ± 0.31 bc	30.00 ± 1.00 c
150 mM NaCl	3.06 ± 0.11 d	24.33 ± 0.57 e	3.17 ± 0.16 d	25.00 ± 1.00 c
150 mM NaCl + nano-iron	5.03 ± 0.05 b	27.33 ± 0.57 c	4.84 ± 0.27 bc	45.00 ± 5.00 b
150 mM NaCl +nano-zinc	4.03 ± 0.05 c	27.00 ± 1.00 cd	3.73 ± 0.45 de	31.00 ± 1.00 c

Means of 5 replications ± SE. Using Duncan's multiple range, means with different letters are different at $p \leq 0.01$.

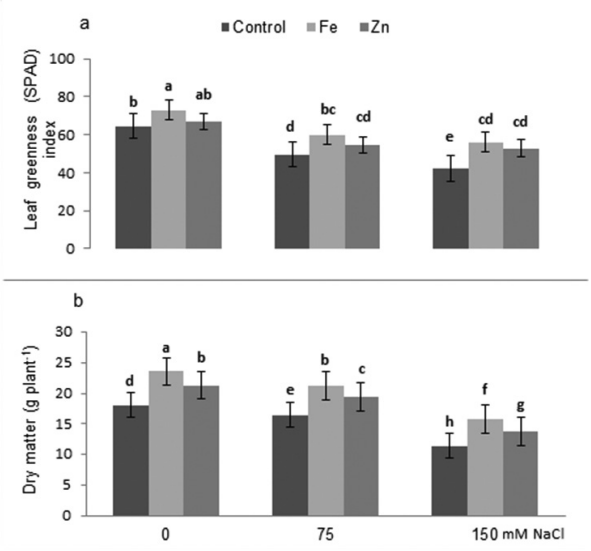

Fig. 2 Mean comparisons of date palm seedlings response to nano-iron and nano-zinc fertilizers under salt stress in leaf greenness (a) and dry matter (b). The means of 5 replicates ± SE. Bars with different letters are significantly different at $p \leq 0.01$ after a Duncan correction.

creased under the influence of nano-fertilizers in salt stress. There were no significant differences between nano-zinc and nano-iron in reducing ABA content with 75 mM NaCl. At the same time, nano-Fe decreased ABA content significantly under 150 mM NaCl (Fig. 4c).

Analysis of variance (ANOVA) results revealed a significant effect of nano-foliar applications and salinity levels on CAT, APX, and POD activity (Table 1). Mean comparisons revealed that the activity of enzymes increased with an increased salt stress level. In contrast, nano-fertilizers' effect decreased except in the case of 75 NaCl; the activity of APX is not reduced by the impact of iron and zinc nano-fertilizers (Fig. 5). Salt stress 150 mM accompanying nano-fertilizers (iron or zinc) significantly reduced CAT, APX, and POD activity. Whereas nano-Fe only under the influence of the stress level 150 mM NaCl led to a significant decrease in POD activity related to other treatments. Salt stress of 150 mM accompanied by zinc nano fertilizer did not reduce POD activity (Fig. 5c).

Nano-fertilizers enhance mineral uptake

Analysis of variance (ANOVA) results revealed a significant effect of nano-foliar applications and salinity levels on mineral uptake (Table 1). Mean comparisons showed that increasing water salinity from 75 to 150 mM NaCl significantly reduced the leaves' iron, zinc, and potassium concentrations and K/Na ratio. In contrast, sodium increased (Table 3). Nano-Fe significantly increased iron and potassium concentrations and K/Na ratio in leaves under salt stress. Nano-Fe and nano-Zn reduced potassium under normal conditions, while under salt stress, the potassium increased. Nano-zinc reduced sodium under normal conditions and salt stress. Nano-zinc significantly

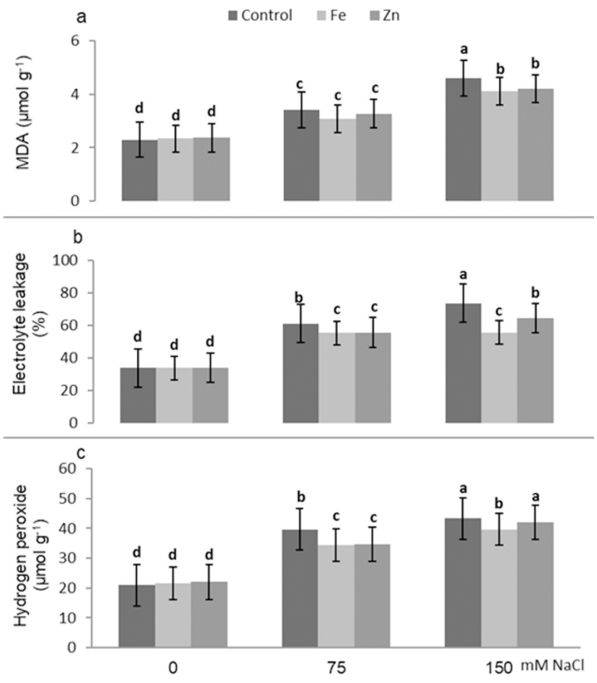

Fig. 3. Mean comparisons of date palm seedlings response to iron and zinc nano-fertilizers under salt stress on MDA (a), electrolyte leakage (b), and hydrogen peroxide (c). The means of 5 replicates ± SE. Bars with different letters are significantly different at $p \leq 0.01$ after a Duncan correction.

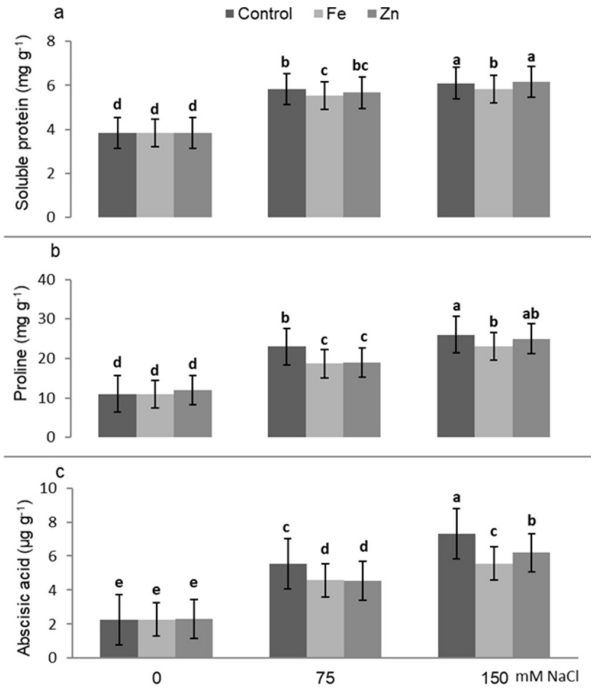

Fig. 4. Mean comparisons of date palm seedlings response to iron and zinc nano-fertilizers under salt stress on the soluble protein (a), proline (b), and abscisic acid (c). The means of 5 replicates ± SE. Bars with different letters are significantly different at $p \leq 0.01$after a Duncan correction.

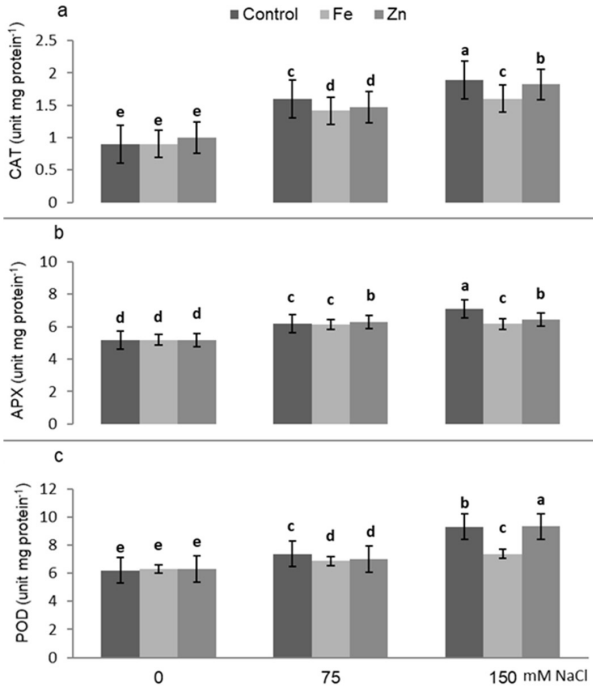

Fig. 5. Mean comparisons of date palm seedlings response to iron and zinc nano-fertilizers under salt stress in the activity of catalase (a), ascorbate peroxidase (b), and peroxidase (c). The means of 5 replicates ± SE. Bars with different letters are significantly different at $p \leq 0.01$ after a Duncan correction.

increased zinc concentration and K/Na ratio under natural conditions or salt stress.

Discussion

After ten months of NaCl applications, the results indicate a decrease in plant height, length of roots, the number of leaves and roots, dry matter, chlorophyll, potassium, iron, and zinc concentration under salt stress, and Na and K/Na ratio increased (Tables 2 and 3). The negative effect of salinity on plant growth is attributable to the osmotic effect, ionic toxicity, and nutritional imbalance, thus reducing photosynthesis and other physiological activities (Shareef, 2020). Al-Abdoulhadi et al. (2012a) found that salt tolerance is usually associated with a decrease in biomass when the date palm is exposed to salinity for a long time. The growth of the date palm seedlings is similar to other crops affected by salinity damage.

NaCl leads to an imbalance in metabolism, absorption of elements, and ion stress (Parvin et al., 2014). Many researchers (Youssef and Awad, 2008; Al Kharusi et al., 2017; Jana et al., 2019; Shareef et al., 2020) suggested a mechanism for salt tolerance in date palm at a low level of salinity limited to the exclusion of sodium and chloride from the roots by activating the selective channels such as K^+ selective ion or Ca^{+2} selective ion channels. Whereas under a high salinity level, the plant's continuous resis-

Table 3. Mean comparisons of date palm seedlings response to foliar nano-iron and nano-zinc fertilizers under salt stress on the iron, zinc, sodium, potassium concentration, and K/Na ratio

Treatments	Fe (µg g^{-1} DW)	Zn (µg g^{-1} DW)	Na (mg g^{-1} DW)	K (mg g^{-1} DW)	K/Na ratio (%)
0 mM NaCl (control)	2.71 ± 0.01 d	0.56 ± 0.01 d	3.45 ± 0.01 f	16.22 ± 0.01 a	4.69 ± 0.01 b
0 mM NaCl + nano-Fe	3.43 ± 0.02 a	0.54 ± 0.05 d	2.11 ± 0.01 h	13.25 ± 0.01 b	6.27 ± 0.04 a
0 mM NaCl + nano-Zn	2.69 ± 0.05 d	1.42 ± 0.01 a	2.84 ± 0.01 g	12.13 ± 0.05 e	4.27 ± 0.02 c
75 mM NaCl	1.35 ± 0.01 e	0.44 ± 0.07 e	4.24 ± 0.01 d	10.23 ± 0.01 H	2.41 ± 0.02 f
75 mM NaCl + nano-Fe	2.92 ± 0.02 b	0.46 ± 0.01 e	4.22 ± 0.02 d	12.46 ± 0.01 c	2.95 ± 0.01 d
75 mM NaCl + nano-Zn	1.36 ± 0.05 e	1.34 ± 0.01 b	4.14 ± 0.01 e	12.24 ± 0.01 d	2.95 ± 0.08 d
150 mM NaCl	0.63 ± 0.01 f	0.34 ± 0.01 f	8.57 ± 0.02 a	9.11 ± 0.01 i	1.06 ± 0.04 h
150 mM NaCl+nano-Fe	2.74 ± 0.01 c	0.36 ± 0.01 f	4.55 ± 0.01 c	11.75 ± 0.02 f	2.58 ± 0.05 e
150 mM NaCl+nano-Zn	0.64 ± 0.01 f	1.24 ± 0.01 c	4.67 ± 0.01 b	11.43 ± 0.01 g	2.44 ± 0.04 f

Means of 5 replications ± SE. Means with different letters are different at $p \leq 0.01$ using Duncan's multiple range test.

tance to the sodium and chloride accumulation in the photosynthesis apparatus's active cell membranes is overcome by increasing the production of compatible solutes. The accumulation of compatible solutes reduces free radicals and thus reduces the degradation of lipid peroxidation associated with salt stress (Shareef and Al-Khayri, 2021). Oxidative stress is one of the plant adaptation mechanisms under abiotic or biotic stress (Ciarmiello et al., 2011). The decrease in chlorophyll can be expected under salt stress. The survival of chlorophyll is related to the membranes' stability under salinity conditions that are difficult to maintain intact. Reduced chlorophyll in date palm under salt stress was reported (Dhawi and Al-Khayri, 2009; Al-Abdoulhadi et al., 2012b). Chlorophyll content depends on salinity tolerance in the different plant species and salt concentrations (Awad et al., 2006). Total chlorophyll decrease is noticeable due to the accumulation of ions and dysfunction during the opening and closing of stomata under salt stress (Shareef et al., 2020). The SPAD reading used in determining chlorophyll showed that nano-iron gave the highest value to chlorophyll over nano-zinc (Fig. 2a). An indication of nano-iron efficiency in regulating chlorosis and preventing low photosynthesis, chlorosis of leaves is more evident in iron deficiency due to its direct contribution to chlorophyll's biological construction (Saleh, 2008). The increase in MDA may be a signal through the cell membrane or a response to oxidative stress in plant cells (Hasanuzzaman et al., 2012). Malondialdehyde (MDA), electrolyte leakage (EL), and hydrogen peroxide (H_2O_2) decrease due to nanomaterials in the leaves (Da Costa and Sharma, 2016). High EL may result from the membranes' decomposition, especially photosynthetic membranes (Juárez-Maldonado et al., 2019).

Oxidative stress is the heart of plants' vital biotic and abiotic effects. Its occurrence indicates changes in the functions of proteins that may improve cellular metabolic function (Chen et al., 2018). There are high levels of soluble proteins under salt stress (Fig. 3a). The formation of new proteins acts epigenetically and produces new proteins such as HKT (high-affinity potassium transporter) and HSP (heat shock protein) that contribute to plants' adaptation to different stresses (Lachowiec et al., 2016). The various metabolic processes under oxidative stress are evidence of the conversion of amino acid metabolism into proline. Proline is produced in the leaf, exposed to environmental stress, such as drought, salinity, or high temperatures (Wani et al., 2019). Instead, the proline may maintain growth, stabilize vital reactions, and protect the photosynthesis system (Zouari et al., 2016). Here we observed multiple mechanisms to protect the photosynthesis system, such as proline production and abscisic acid. Abscisic acid is one of the most critical plant hormones in maintaining the signal and adapting the plant to harsh environmental conditions (Vishwakarma et al., 2017). Antioxidant enzyme activity decreased with the effect of nano-fertilizers under salt stress (Fig. 5). Nano-fertilizers' low activity of enzymes can coexist with the nanomaterial with the stress signal, which activates the plant's defense system (Elsakhawy et al., 2018). Also, enzymes activate, such as APX and POD, increase under salt stress (Naser et al., 2016). The foliar nano fertilizers' primary effect is changed in the membranes, cell building, and other molecules due to their rapid entry into the plant's active metabolism regions, alleviating oxidative stress. Moreover, Navarro et al. (2008) suggested the possibility of nano fertilizers as a store of nutrients by reserving nutrients on nano fertilizers' surfaces.

Using nano-fertilizers increased the leaf content of iron, zinc, and potassium (Table 3). Nano-fertilizers, such as Zn and Fe, are generally utilized in agricultural systems due to their physicochemical characteristics, including high reactant capacities, ability to design electron exchange, and high surface area to volume proportion (Kaushik and Djiwanti, 2019).

Nano-iron induces better seedling growth than nano-zinc, especially in the length of the roots. Iron availability promotes cell division in the root meristem cells and increases root length (Hillo et al., 2017). Iron deficiency affects the elongation of the root cells mainly by reducing the root meristem's efficacy rather than extending epidermal cells in mature areas (Sun et al., 2017).

Maintaining high K and low Na content under nano-fertilizers is consistent. The potassium concentration in date palms has the optimum maintenance capacity for photosynthesis under salt stress (Shareef, 2019). In addition to maintaining a low Na/K ratio, it is an essential factor for salt tolerance. The decrease in the dry matter of the roots and leaves and the plant's height, chlorophyll, and potassium were significant under salt stress. Whereas iron or zinc nano-fertilizers enhanced plant height, dry matter, chlorophyll, iron, and zinc concentrations increased under salt stress. Nano-fertilizers increase zinc and iron concentrations in leaves and reduce sodium despite high salt stress (Table 3). The appropriate zinc concentration accelerates the wheat plant's growth (Al-Juthery et al., 2019). Similarly, zinc positively affects wheat's salt tolerance (Fathi et al., 2017).

The positive effect of nano-fertilizers attributed to iron's appropriateness on metabolism and stimulating photosynthetic pigments and enzymes' activity encourages plant growth (Dadashzadeh et al., 2018). A greater impact of chelated iron than chelated zinc may be due to the compound's content of elements (N 4%, Zn 1.5%, Mn 0.5%) that contributed to improving the growth of seedlings.

In this investigation, salt stress mitigation can be credited to increasing the density and reactivity of nanoparticles' specific surfaces, leading to enhanced plant physiology and performance, thus increasing its ability to mitigate salinity. The long-term effect depends on improving the plant's defense system and maintenance, enhancing the energy produced to improve plant growth, and withstanding environmental stress. The use of nano fertilizers depends on the quality of the constituent elements. The plant needs these elements, and the plant gets the maximum benefit from using the nanocomposite to enhance environmental stress endurance. On account of their novel physicochemical properties, poten-

tial nanoparticles can enhance plant metabolism (Ahmad and Akhtar, 2019), nanoparticle designs can go into plant cells and leaves, and this examination region offers new expected results in plant biotechnology to target specific gene manipulation and expression in the plants' specific cells (Hussein and Abou-Baker, 2018). Nanomaterials may mimic the role of antioxidative enzymes like peroxidase, superoxide dismutase, and catalase. These antioxidants and enzymes continuously scavenge the reactive oxygen species (Mahil and Kumar, 2019).

Conclusions

Nano-iron induces better seedling growth than nano-zinc, especially in the length of the roots. Nano-iron gave the highest value to chlorophyll over nano-zinc. Nano-fertilizers increase zinc and iron concentrations in leaves and reduce sodium despite high salt stress. The success of planting seeds in a pot that contains a high percentage of sand and irrigation with saltwater confirms the possibility of growing seedlings in saline sandy soils.

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