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Alleviation of salt stress on *Moringa peregrina* using foliar application of nanofertilizers

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Moringa peregrina **plants were grown under four levels of saline water (0, 3000, 6000, 9000 ppm), and treated with sprayed Hoagland solution containing different concentrations of ZnO and Fe_3O_4 Nano-Particles (NP) (30, 60 and 90 mg/L); the normal Hoagland solution was used as a control. Results show that salinity levels significantly reduced growth parameters (plant height, root length, number of leaves, number of branches, shoot and root fresh and dry weights). Also, chlorophyll, carotenoids and crude protein levels decreased meanwhile proline and total carbohydrate levels, antioxidant non-enzymes (vitamins A and C) and enzymes (POD and SOD) increased.** ***Moringa*** **plants sprayed with Hoagland-containing ZnO and Fe_3O_4 NP showed an enhancement in growth parameters either under normal or saline conditions when compared to control. Also, spraying plants with Hoagland-containing ZnO and Fe_3O_4 NP resulted in significant reduction in Na^+ and Cl^- and an increase in N, P, K^+, Mg^{2+}, Mn^{2+}, Fe, Zn; total chlorophyll, carotenoids, proline, carbohydrates, crude protein levels, antioxidant non-enzymes and enzymes when compared to control, normal Hoagland sprayed-plants. Generally, this enhancement of salt tolerance was considerable in plants sprayed with 60 mg/L ZnO and Fe_3O_4 NP and grown either in saline and non-saline conditions.**

Key words: *Moringa peregrine*, nanofertilizers, salt stress, growth parameters, chemical composition.

INTRODUCTION

Moringa peregrina (Forssk.) Fiori (Moringaceae) is a tree (4 to 15 m) (Boulos, 1999). Its seeds have different economic and medical importance. Due to its unique composition, the extracted oil is highly valued for preparing cosmetics, cooking, and lubricating purposes (Somali et al., 1984). *Moringa* plants are considered a valuable source for many useful components such as vitamins A, B and C, and provide humans with minerals,

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protein and amino acids (Price, 2000). As a result of uncontrolled and indiscriminate use of this plant in many activities, the tree has decreased in numbers and become rare in Egypt (Zaghloul et al., 2008). New lands are considered as promising areas to cultivate this crop. The notable problems facing the plants cultivation in the reclaimed lands are drought, salt and heat stresses (abiotic stresses) which adversely affect the growth and productivity of the plants. Salt stress is one of the most devastating problems that limits the crop's production worldwide by imposing its effect through osmotic stress, Na^+ and Cl^- toxicity and ions uptake imbalance leading to deficiency in N, P, K^+, Ca^{2+} and micronutrients (Munns, 2005). Salt stress was reported to decrease the growth and yield of the plants as it affects the organic, ion contents and metabolic activity in the stressed plants. Accumulation of organic solutes is another mechanism that enables the plant to tolerate salt stress. Osmoprotectants (sugars, glycine betaine, proline, mannitol, etc) generally found in cytosol, plays an important role in osmotic adjustment as well as protection of enzymes and proteins (Munns and Tester, 2008). It was suggested that this osmprotecants work as scavengers of ROS (reactive oxygen species) which are induced by salt stress and negatively affect the lipid membrane and enzyme activity. Attempts have been made to increase plants' tolerance against salt stress. These efforts include classical breeding, gene transfer, seed priming, foliar application of osmoprotectants and inorganic compounds (Chen et al., 2007).

Nanoparticles (nano-scale particles; NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100 nm (Ball, 2002). Nanofertilizers have been developed and have provided a new efficient alternative to normal regular fertilizers. The properties of nano-particles (more surface area) may help in increasing the reactive points of these particles and hence increase the reactivity of these nanoparticles. This leads to changes in the physio-chemical properties of these nanoparticles which help in the absorption of fertilizers in plants (Anonymous, 2009). The promoting effect of nano-particles on seedling growth and development were reported by Zhu et al. (2008). Also, nano-iron oxide compared to other treatments such as organic materials and iron citrate facilitated photosynthesis and iron transfer in peanut leaves (Liu et al., 2005). Nanoparticles can be divided into groups; metal based materials such as nanogold, nanozinc, nanoaluminum; and nanoscale metal oxides like TiO_2, ZnO and Al_2O (Ruffini and Roberto, 2009).

Foliar application of macro and micronutrients has been reported as an effective method to increase salt tolerance in plants and have been suggested to ameliorate the adverse effect of salt stress (Hamayun et al., 2011). This promoting effect can be attributed to the increased and enhanced nutrient uptake of micronutrients through the leaf or root as a result of root improvement. Foliar application may also offer a solution to overcome root restriction caused by salt stress (El-Fouly et al., 2004). Fe is critical for chlorophyll formation and photosynthesis and is important in enzyme systems and plant respiration (Malakouti and Tehrani, 2005). For most plants, zinc is an essential component of enzymes and participates in the synthesis of chlorophyll and other proteins (Vallee and Auld, 1990). The effect of nano fertilizers on plant growth in general and specifically under salt stress by investigating the effect of Hoagland solution containing ZnO and Fe_3O_4 NPs on *M. peregrina* plants grown under different levels of salinity is therefore the aim of this study.

MATERIALS AND METHODS

This study was conducted at the Experimental Laboratories of the Natural Resources Department, Institute of African Research and National Institute of Laser Enhanced Sciences (NILES) Cairo University, Giza, Egypt during the two seasons of 2013 and 2014.

One year old seedlings of *M. bergrina* were obtained on the 1st of May in the first and second seasons, respectively, from Orman Botanical Garden, Cairo, Egypt. Then the seedlings were transplanted into 25 cm diameter-plastic bags filled with 6 kg sandy soil, and watered every 3 days with Hoagland's nutrient solution (Hoagland and Arnon, 1950) for plant maintenance.

Soil analysis

The soil texture was sandy having the following characteristics: 30.82% coarse sand, 62.61% fine sand, 1.22% silt, 5.35% clay, pH 7.75, EC 1.15 dS/m, organic matter 0.08%, available N 6.9 ppm, available P 6.2 ppm, available K 64 ppm, $CaCO_3$ 0.26%, and water holding capacity 14.5%.

Salinity treatments

Two weeks after transplanting (in both seasons), the salinity treatments were initiated after 10 days. Four levels of salinity (Control, 3000, 6000, and 9000 ppm) were used for testing salt stress. The different saline water concentrations were prepared using a mixture of synthetic seawater salt obtained from Sigma Company. At each irrigation, the plants were watered till 100% of soil field capacity (F.C.). To maintain the required soil medium salt levels, the soil EC was measured periodically by portable EC meter.

Nano treatments

***Synthesis of ZnO and Fe_3O_4 magnetic nanoparticles (NPs)*:** Zinc acetate [Zn (H_3COO) $2H_2O$.], NaOH and isopropyl alcohol (2-propanol) with 99.5% reagent grade were obtained from Sigma Aldrich (99.9%). 0.073 mmol Zn (OAc). $2H_2O$ was dissolved at 60°C in 50 ml 2-propanol under stirring. In a second flask, 1.5 mmol NaOH was dissolved under vigorous stirring in 25 ml^2 propanol at 60°C. NaOH solution was added drop wise under stirring to the acetate solution. The product was stirred for an hour at 60°C and then cooled to room temperature. The precipitate was washed twice with 2-propanol and centrifuged at 4500 rpm for 30 min (Bardhan et al., 2007). In addition, the Fe_3O_4 magnetic nanoparticles were prepared by coprecipitation of Fe^{3+} and Fe^{2+} at a molar ratio of 2:1 with aqueous ammonia (0.3 mol/L) as precipitating agent (Laurent et al., 2008).

Characterization of ZnO and Fe_3O_4 NPs: The size and shape of

ZnO and Fe_3O_4 nanoparticles were observed directly by transmission electron microscope (TEM) (FEI, Netherland) The TEM samples were prepared by placing a few drops of the solution on a carbon-coated copper grid (Okenshoji Co., Ltd.).

Seedlings were sprayed monthly with Hoagland solution which replaced Zn and Fe with mixed ZnO and Fe_3O_4 NPs (30 (T1), 60 (T2), and 90 (T3) mg/L) after 10 days of adding salinity. Also, the normal Hoagland solution was used as a control (T0). Spraying was carried out between 09:00 and 11:00 AM.

Experimental design

The experiment was based on a Randomized Complete Block Design (RCBD) with two factors, including 16 treatments and three replicates. The first factor was control (without NPs application) and 3 levels of mixed ZnO and Fe_3O_4 NPs applications; the second factor had four irrigation water salinity treatments with each block consisting of 80 plants (five plants/ treatment). The seedlings were harvested at 90 days (in the two seasons, respectively) in order to determine the growth parameters and carry out chemical analysis.

Growth parameters

Plant height (cm), root length (cm), number of branches/plant, numbers of leaves/plant, stem diameter (cm), fresh and dry weight of shoots (leaves and stems) and roots (g/ plant) were also recorded.

Chemical analysis

Leaf pigments and total carbohydrates: Total chlorophyll and carotenoid contents were extracted using the method described by Nornai (1982). Total carbohydrates (%) in the dried leaves were also determined as described by Dubois et al. (1956).

Determination of macro and micronutrients and crude protein: Dried leaves samples were digested and the extract analyzed to determine nitrogen (N%) using the modified micro-Kjeldahl method, phosphorus (%) by Jackson (1967); K and Na% using a flame spectrophotometer (Jameel and Kahayri, 2002); while Ca, Fe, and Zn were determined by atomic absorption (Allen et al., 1984). The proline content in fresh leaves was also determined according to Bates et al. (1973). Also, protein % was determined as described by James (1995).

Antioxidant non-enzymes and enzymes determination: Antioxidant non-enzymes (Vitamins A and C) were measured according to AOAC (1999) using dried leaves. Meanwhile, enzymes extraction was carried out using fresh leaf tissues at 40°C in buffer solution (3: 1 buffer:fresh weight v/v) in a pastel. It was mortared with 100 mM potassium phosphate buffer (at pH 7.5) containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinyl pyrolidone. The homogenates were centrifuged at 10000 g for 30 min and then the supernatants were stored in separate aliquots at 8°C (Vitoria et al., 2001). Antioxidant enzymes were assayed as follows; peroxidase (POD) by spectrophotochemically according to Amako et al. (1994) and superoxide dismutase (SOD) by photochemical method as described by Giannopolitis and Ries (1977). Enzymes activities were expressed as units/min/mg protein.

Statistical analysis

The data were subjected to statistical analysis of variance and the means were compared using the least significant difference (LSD) test at the 5% level, as described by Little and Hills (1978).

RESULTS AND DISCUSSION

Characterization of photo-catalysts by TEM

The shape and diameter of the nanoparticles used were observed with TEM. TEM image emphasized that ZnO presents in spherical nanoparticle form, with a diameter range of about 10 to 15 nm (Figure 1a) while Fe_3O_4 nanoparticles diameter ranges from approximately 10 to 12 nm with an almost spherical shape (Figure 1b).

Effect of salt stress on salt stressed-plants

Table 1 shows that growth parameters (plant height, root length, stem diameter, number of leaves, number of branches) decreased in response to different concentrations of salinity and this reduction was significant in plants treated with the two levels of salinity (6000 and 9000 ppm). Furthermore, the shoot and root fresh and dry mass of the *Moringa* plants decreased significantly under salinity conditions compared to those of control plant.

Soil salinity adversely affects plant growth through several physiological and biochemical means like ion toxicity, osmotic stress, nutritional imbalance, biochemical and physiological disorders (Kao et al., 2003). Salt stress resulted in the reduction in the number of leaves and branches and stunted shoot growth in *Acacia saligna* (Soliman et al., 2012). Moreover, Bello and Igbokwe (2013) reported that salt stress reduced height of both *Acacia senegal* and *Parkia biglobosa*. The first reduction in plant growth may be attributed to the initial sudden increase in osmotic pressure as stated by Hajibagheri et al., (1989) thus suggesting that high salinity might inhibit root and shoot elongation due to slowing down of water uptake by the plant. Over time, Na^+ and Cl^- will accumulate to toxic concentrations in the shoot resulting in premature leaf senescence and death due to the ionic component of salt (Munns and Tester, 2008; Hairmansis et al., 2014). The accumulated amounts of ions enter the plant through the transpiration stream thereby causing cells injury in the transpiring leaves which may cause further reductions in photosynthesis processes thereby leading to growth reduction (El-Fouly et al., 2002; Munns et al., 2006).

Total chlorophyll (Chl a and b) and carotenoides contents were significantly lower in plants grown under salt stress conditions than those recorded in control plants (Table, 2). A reduction of 48% in total chlorophyll contents of *Moringa* leaves was recorded at the third salinity concentration (9000 ppm). An inhibition in chlorophyll biosynthesis, activation in the chlorophyllase and/or destruction of chloroplast structure (Gunes et al., 1996) could have contributed to lowering the pigment content under saline conditions.

The results also showed an increase in proline and carbohydrate concentrations in leaves of *Moriga* plants in response to different levels of salt stress (Table 2). In this

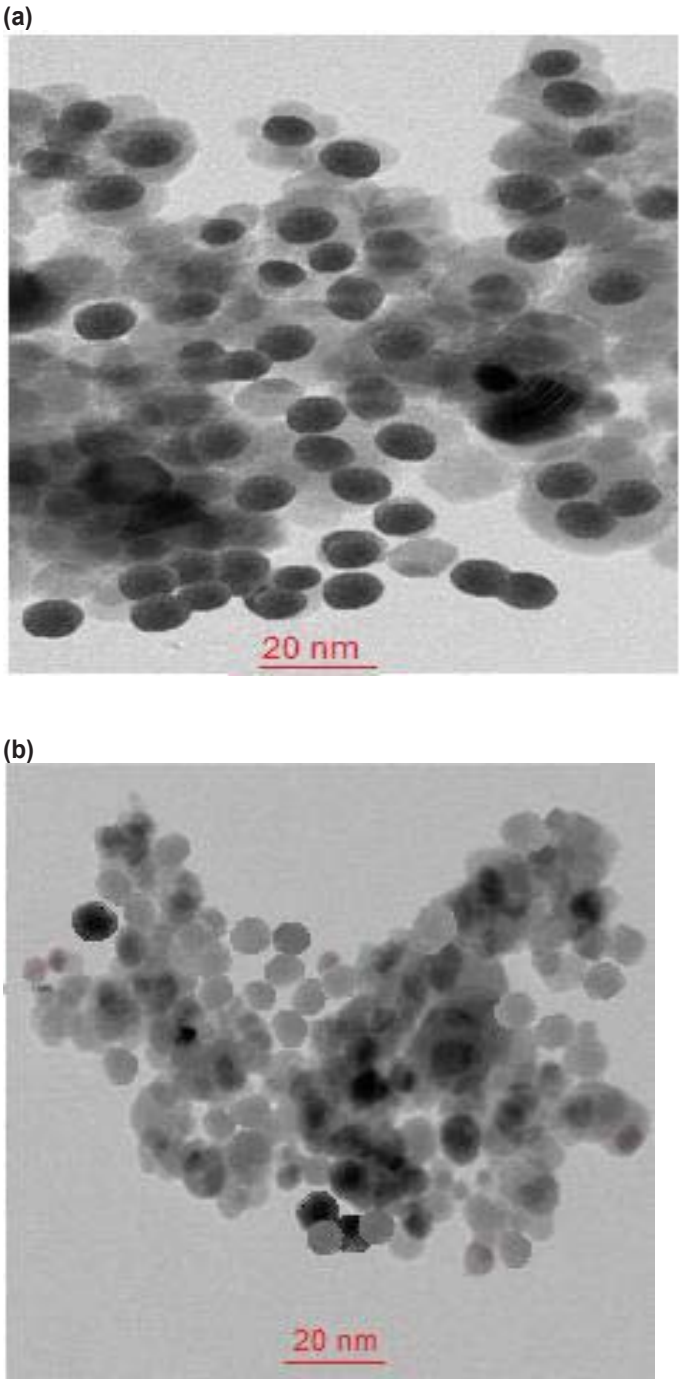

Figure 1. TEM image of the prepared nanoparticles. (a) ZnO, (b) Fe_3O_4.

this regard, increased free amino acids and proline in response to salt stress has been investigated by many researchers in many plants (Gunes et al., 1996; Sharma et al., 2010). One of the strategies that plants use to cope with salt stress is osmoprotectant synthesis of low molecular weight molecules such as sugars, proline and glycine betaine which play an important role in osmotic adjustments and protection of protein and lipids from (ROS). These further results in the protection of plasma membrane integrity and enzyme function. Also, it plays an important role as a scavenger for free radicals which protects cells from ROS actions. Proline serves as a storage sink for carbon and nitrogen and it is a free-radical. It also stabilizes subcellular structures (membranes and proteins), and buffers cellular redox potential. Hence, these organic osmolytes are known as osmoprotectants. These organic solutes may contribute to osmotic adjustment, protecting cell structure and function, and/or may serve as a metabolic or an energetic reserve (Chen and Murata, 2000).

Crude protein was found to decrease in response to salt stress. Protein synthesis has been considered as a possible primary target of salt toxicity because in vitro protein synthesis systems are dependent on physiological potassium and are inhibited by sodium and chloride (Morant-Avice et al., 1998). Considering the evidences on plant soluble protein response to salinity, there is a marked difference between the species and varieties. Thus, proteins may play a role in osmotic adjustment. According to Pareek et al. (1997), proteins may be synthesized *de novo* in response to salt stress, or may be present constitutively in low concentrations and increased when plants are exposed to salt stress.

Raising the salt concentration significantly increased antioxidant non-enzymes (vitamins A and C) and enzymes (POD, and SOD) in tissues of *Moringa* leaves (Figure 2) in both seasons. Accordingly, the lowest values of the non-enzymatic and enzymatic antioxidants were found in control plants irrigated with tap water, whereas the highest values were found in plants irrigated with water containing the highest salt concentration (9000 ppm). Such results are in harmony with Foyer and Noctor (2009), Cazzonelli and Pogson (2010) and Boguszewska and Zagdańska (2012). They found that many plants produce significant amount of a potential source of compounds such as non-enzymatic (vitamins A, and C) and enzymatic antioxidants (POD and SOD) to prevent oxidative stress caused by oxygen and photons. Piotr and Klobus (2005) and Wu et al., (2007) reported that ascorbic acid is an important antioxidant which reacts not only with H_2O_2 but also with O_2, OH and lipid hydroperoxidases. In addition, Shao et al. (2006) and Abogadallah (2010) indicated that ascorbic acid concentration significantly increases in turf grass during water deficiency. Mittler (2002) and Akram et al. (2012) reported that the enzymatic antioxidants SOD and POD are considered to be the first line of defense against ROS thus the simultaneous increase in the activity of these enzymes contributes to a decrease in the deleterious effects of H_2O_2 under stress. Also, POD activity increased in eggplant plants under saline conditions (Shaheen et al., 2013). Other studies also reported that salt stress-induced enhanced POD and SOD activities were observed in sunflower (Akram et al., 2012) and pistachio plants (Abbaspour, 2012). Thus, it becomes clearly evident that

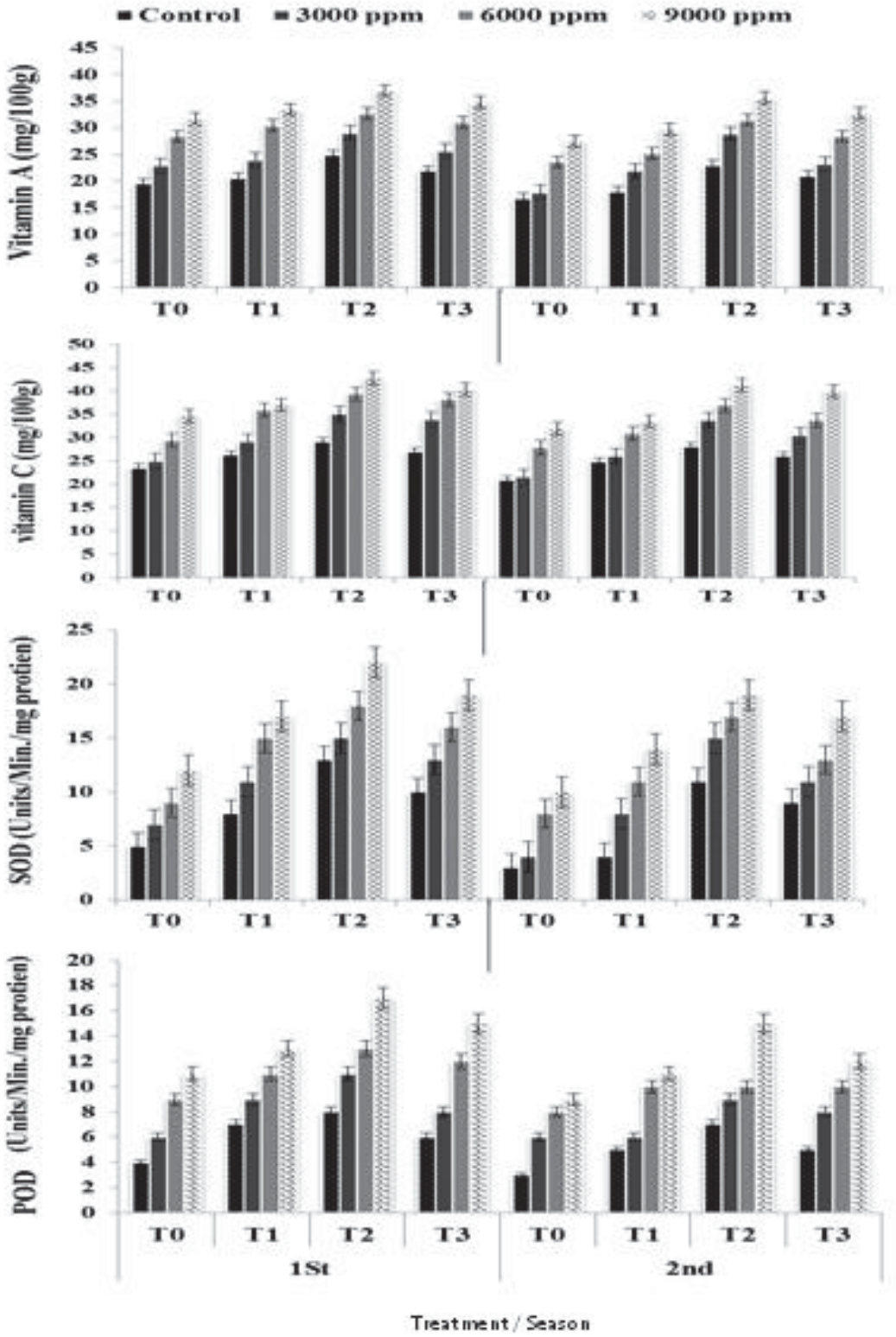

Figure 2. Effect of salt stress and nanofertilization on non-enzymatic (vitamins A, and C) and enzymatic antioxidants (POD, and SOD) in *M. pregrina* during 2013 and 2014.
T0= normal Hoagland solution (control), T1 = 30 mg/L ZnO and Fe_3O_4 NPs, T2= 60 mg/L ZnO and Fe_3O_4 NPs and T3= 90 mg/L ZnO and Fe_3O_4 NPs.

Table 1. Effect of salt stress and nanofertilization on growth parameters of *M. pregrina* during 2013 and 2014.

Treatment			Plant height (cm)	Root length (cm)	Stem diameter (cm)	No. of leaves/ plants	No. of branches/ plant	Fresh weight of leaves and stems (g/ plant)	Dry weight of leaves and stems (g/ plant)	Fresh weight of roots (g/ plant)	Dry weight of roots (g/ plant)
Control	T0	1st	45.00	24.00	2.00	29.00	11.33	21.13	8.90	95.40	46.70
	T1		59.33	29.33	2.37	39.00	23.00	24.80	11.07	128.50	63.25
	T2		70.00	38.33	3.00	52.00	27.67	32.10	16.05	149.43	74.05
	T3		67.00	33.00	2.70	48.33	26.00	28.30	13.15	142.60	70.63
	T0	2nd	41.33	19.33	0.90	24.00	9.00	18.57	7.95	91.70	45.18
	T1		50.00	24.00	1.60	35.00	16.00	21.90	9.28	125.90	61.95
	T2		67.67	32.00	2.00	47.00	22.00	29.20	14.60	140.30	69.65
	T3		60.33	29.67	1.90	43.00	20.00	28.00	12.00	136.80	67.73
3000 ppm	T0	1st	39.33	22.00	1.80	25.00	9.00	18.80	8.73	92.70	45.35
	T1		46.33	24.00	2.10	34.67	18.00	23.43	10.41	121.90	60.28
	T2		66.67	35.00	2.80	49.67	24.00	30.70	14.68	142.07	70.68
	T3		59.67	29.33	2.50	43.00	22.00	26.63	12.32	134.80	66.73
	T0	2nd	34.00	17.33	0.70	21.00	8.00	17.20	7.72	87.90	42.95
	T1		40.00	21.00	1.20	30.00	12.00	19.50	8.75	118.50	58.25
	T2		61.67	30.00	1.90	43.00	19.00	26.67	11.67	136.80	67.73
	T3		53.00	27.00	1.70	41.00	16.33	21.90	9.62	130.40	64.53
6000 ppm	T0	1st	31.33	18.67	1.20	19.67	7.00	15.30	6.95	84.10	41.38
	T1		40.00	20.00	1.73	26.00	11.33	20.09	9.38	117.60	58.80
	T2		52.67	30.33	2.10	37.00	18.33	27.37	12.96	136.70	68.02
	T3		47.33	25.00	2.00	32.00	15.67	24.47	11.91	128.90	63.73
	T0	2nd	25.00	13.00	0.53	16.67	5.00	12.79	5.59	80.40	39.53
	T1		38.33	17.33	1.17	22.33	8.00	17.90	8.02	114.9	56.45
	T2		47.33	25.00	1.60	33.00	15.00	24.44	10.89	131.60	65.30
	T3		41.67	21.00	1.30	30.00	11.00	20.13	8.73	123.70	61.35
9000 ppm	T0	1st	28.33	13.33	0.90	15.00	4.67	13.44	6.31	71.10	35.12
	T1		31.33	18.00	1.30	20.00	8.33	15.97	7.56	113.50	56.08
	T2		43.33	28.67	1.60	30.00	13.00	21.57	10.35	128.90	64.08
	T3		38.33	21.33	1.45	24.00	10.33	18.85	8.94	120.40	59.50
	T0	2nd	21.00	11.00	0.43	12.33	3.33	11.80	4.70	67.40	33.03
	T1		27.67	14.33	0.80	16.33	5.33	13.65	6.42	109.80	54.23
	T2		40.00	20.00	1.17	28.67	11.00	18.87	8.83	123.23	61.12
	T3		32.00	18.33	1.00	22.33	9.00	15.90	7.18	118.70	58.85
LSD (0.05)											
S		1st	1.69	4.07	0.30	8.01	1.53	2.92	1.13	5.92	2.36
N			5.73	6.53	0.66	5.04	2.03	5.06	2.38	3.10	1.59
N× S			11.46	13.06	1.33	10.08	4.07	10.12	4.76	6.20	3.18
S		2nd	2.44	2.64	0.26	8.33	1.24	5.59	2.38	10.88	5.26
N			5.93	5.99	0.28	6.18	2.23	3.69	1.43	6.81	3.34
N× S			11.58	11.98	0.56	12.36	4.47	7.38	2.86	13.61	6.69

T0= normal Hoagland solution (control), T1 = 30 mg/L ZnO and Fe_3O_4 NPs, T2= 60 mg/L ZnO and Fe_3O_4 NPs and T3= 90 mg/L ZnO and Fe_3O_4 NPs. S= salinity treatments, N= nano treatments. 1st = First season, 2nd = Second season.

Table 2. Effect of salt stress and nanofertilization on chemical composition of *M. pregrina* during 2013 and 2014 seasons.

Treatment			Total chlorophylls content (mg/g fresh weight)	Carotenoids content (mg/g fresh weight)	Total carbohydrate (% of dry weight)	Proline content (µ moles/g fresh weight)	Crude Protein (%)
Control	T0	1st	1.66	0.75	19.00	13.00	18.94
	T1		1.70	0.84	23.00	15.00	20.88
	T2		2.29	1.14	29.33	19.33	22.63
	T3		1.96	0.92	27.67	18.00	22.13
	T0	2nd	1.47	0.68	15.67	17.33	18.06
	T1		1.53	0.72	19.33	18.67	19.94
	T2		2.15	1.01	25.67	23.67	21.50
	T3		1.69	0.84	23.33	21.33	20.63
3000 ppm	T0	1st	1.63	0.72	22.33	16.00	17.81
	T1		1.59	0.78	28.33	19.33	18.69
	T2		2.24	1.11	33.67	22.67	20.06
	T3		1.81	0.89	30.33	20.33	19.31
	T0	2nd	1.22	0.59	17.67	19.67	16.69
	T1		1.43	0.69	24.67	21.33	17.69
	T2		2.18	1.06	29.33	28.33	19.38
	T3		1.67	0.84	27.33	25.00	18.13
6000 ppm	T0	1st	1.09	0.55	26.00	20.33	12.81
	T1		1.32	0.68	33.00	25.00	14.31
	T2		2.18	1.08	39.33	29.33	17.19
	T3		1.59	0.81	37.33	27.33	15.25
	T0	2nd	0.99	0.43	23.33	23.00	12.31
	T1		1.25	0.58	29.67	30.33	13.50
	T2		2.02	0.98	35.33	33.00	15.13
	T3		1.51	0.74	31.33	30.33	14.31
9000 ppm	T0	1st	0.86	0.41	32.33	25.67	11.00
	T1		1.05	0.50	37.67	27.67	11.88
	T2		1.49	0.79	43.33	32.33	14.19
	T3		1.13	0.57	40.00	30.67	12.63
	T0	2nd	0.75	0.32	28.00	28.00	9.69
	T1		1.01	0.48	33.00	31.33	11.69
	T2		1.30	0.66	39.33	38.33	13.63
	T3		1.02	0.51	37.67	35.33	12.38
LSD (0.05)							
S		1st	0.03	0.03	0.94	1.76	0.09
N			0.08	0.05	3.89	4.02	0.47
N×S			0.15	0.09	7.78	8.04	0.94
S		2nd	0.06	0.06	1.03	1.54	0.33
N			0.07	0.05	3.05	3.27	0.50
N×S			0.13	0.11	6.10	6.53	1.00

T0= normal Hoagland solution (control), T1 = 30 mg/L ZnO and Fe_3O_4 NPs, T2= 60 mg/L ZnO and Fe_3O_4 NPs and T3= 90 mg/L ZnO and Fe_3O_4 NPs. S= salinity treatments, N= nano treatments. 1st = First season, 2nd = Second season.

non-enzymatic and enzymatic antioxidant status of plants for ROS scavenging is an important salt tolerant trait.

Salinity stress significantly increased percentage Na, Cl and Ca and reduced percentage K, Mg and P in the leaves of *Moringa* plants (Table 3). Salinity may result in the disturbance of uptake and utilization of essential nutrients due to competition and interactions of soluble salts with mineral nutrients (Gouia et al., 1994). Ionic imbalance occurs in the cells due to over accumulation of Na^+ and Cl^- and reduced uptake of other mineral nutrients, such as K^+, Ca^{2+}, Mg^{+2} and No^- and Mn^{2+} thus leading to growth suppression (Karimi et al., 2005).

Effect of ZnO and Fe_3O_4 NPs- containing Hoagland solution on salt stressed-plants

The foliar application of nano-iron and -zinc containing-Hoagland solution caused a significant increase in previously mentioned growth parameters in comparison to control plants (Table 1). This promoting effect of these nano-applications was not only noticed in the growth of salt-stressed plants, but also did promote the growth in plants grown under normal conditions. The most interesting result is that under the highest salinity level 9000 ppm, the increment in shoots fresh weight, number of leaves and plant height reaches up to 60, 100 and 53 % respectively in plants treated with the T2 (6 mg/l ZnO and Fe_3O_4 NPs-containing Hoagland solution) over control plants (Hoagland-sprayed plants). This increment was recorded in the first and second seasons. It means that the T2 treatment has a strong promoting effect either in stress or non-stress conditions. It also noticed that T1 (3 mg/l ZnO and Fe_3O_4 NPs-containing Hoagland solution) and T3 (9 mg/l ZnO and Fe_3O_4 NPs-containing Hoagland solution) also has promoting effect on growth parameters of plants grown under both stress and non-stress conditions in comparison to Hoagland-sprayed plants but is however less than those found in the T2 treatment. The aforementioned data are in trustworthiness with Aslam et al. (1993) who mentioned that growth parameters have been used as an indicator of salt tolerance in plants e.g. shoot weight. Meanwhile, significant increase in biomass, with respect to length or diameter of stem, leaves and dry weight (DW) of plants was observed by spraying *Moringa* plants with the combination of zinc and iron nano fertilizers. This indicates that proper concentration of zinc is required for dry matter accumulation and plant growth (Dimkpa et al., 2013).

Improved salt tolerance by addition of nutrients has been reported in many plants (Zhu et al., 2004 on cucumber; Al-Aghabary et al., 2005 on tomato). In addition, application of micronutrients is reported to enhance photosynthetic activities which lead to an increase in cell division and elongation thereby increasing vegetative biomass. It was also found that foliar spray of zinc sulfate (Yildirim et al., 2008) and treatment of seedlings with zinc sulfate before transplanting (Tzortzakis, 2010) leads to relieve symptoms of salt stress.

Nano-technology can offer opportunities to enhance yield and counter environmental stress. By using nano-particles, we aim to delay releasing fertilizers. Nano-particles have high reactivity because of the larger specific surface area and increased reactivity of these areas on the particle surface. These features simplify the absorption of fertilizers and pesticides that are produced in nano scale (Anonymous, 2009). The application of nano-particles to plants can be beneficial (seedling growth and development) or non-beneficial (prevent root growth) (Zhu et al., 2008). These results are in agreement with the findings of Liu et al., (2005) who concluded that nano-iron oxide facilitated photosynthesis and iron transfer to the leaves of peanut when compared to organic materials and iron citrate. In addition, Sheykhbaglou et al. (2010) found that the nano-iron oxide had significant effects on the dry pod weight; leaf with dry pod, and yield of soybean compared to other treatments. In pumpkin, iron oxide NPs increased root elongation which was attributed to Fe dissolution (Wang et al., 2011). Thus, the positive effects of appropriate zinc and Fe concentrations on fresh and dry weight, plant height, number of leaves and branches under NaCl stress could be explained by the replacement of Fe and Zn with nano forms.

Foliar applications with nano-iron and nano-zinc containing-Hoagland solutions at different concentrations lead to increased total chlorophyll, carotenoids, proline content, total carbohydrates and crude protein percentage more than those recorded in Hoagland-sprayed plants either in non-stress or stress conditions (Table 2). At the highest level of salinity (9000 ppm), increased percentage values in chlorophyll content resulted from the application of the nano form of Fe and Zn Hoagland solution. This increase reached 73% in both seasons when treated with T2 and was noticed in increased leaf numbers. In addition, iron plays an important role in the photosynthetic reactions as it is a component of ferrodoxin, an electron transport protein associated with chloroplast (Hazra et al., 1987). Iron also activates several enzymes and contributes in RNA synthesis and improves the performance of photosystems (Malakouti and Tehrani, 2005). Moreover, iron oxide NPs have been reported as facilitators for iron and photosynthate transfer to the leaves of peanut (Liu et al., 2005). Meanwhile, Zn plays an important role in many biochemical reactions within the plants like chlorophyll and carbohydrate formation (Corredor et al., 2009), increased photochemical reduction rates (Kumar et al. 1988), chloroplast structure, photosynthetic electron transfer as well as photosynthesis (Romheld and Marschner, 1991); in enzyme structure involved in amino acid biosynthesis (Cakmak et al., 1989). These results agree with those of El-Kereti et al. (2013) and El-Feky et al. (2013). The seasons

Table 3. Effect of salt stress and nanofertilization on macro and micro nutrients in *M. pregrina* during 2013 and 2014 seasons.

Treatment		N (%)	P (%)	K (%)	Na (%)	Cl (%)	Ca (%)	Mg (%)	Fe ppm	Zn ppm
	T0	3.03	0.30	2.19	0.35	0.18	0.55	0.55	87.46	95.40
	T1 1St	3.34	0.43	2.39	0.31	0.17	0.74	0.90	95.61	103.73
	T2	3.62	0.59	2.62	0.23	0.11	1.02	1.32	121.25	140.50
	T3	3.54	0.47	2.45	0.29	0.13	0.88	1.18	110.50	128.90
Control										
	T0	2.89	0.26	1.89	0.41	0.23	0.47	0.48	82.72	88.50
	T1 2nd	3.19	0.37	2.14	0.37	0.21	0.60	0.78	90.85	99.87
	T2	3.44	0.48	2.33	0.29	0.16	0.93	1.19	117.54	123.80
	T3	3.30	0.40	2.20	0.35	0.19	0.79	0.93	100.25	110.60
	T0	2.85	0.27	1.77	0.30	0.27	0.76	0.41	80.26	86.77
	T1 1St	2.99	0.32	2.00	0.35	0.25	1.10	0.82	87.92	95.63
	T2	3.21	0.48	2.29	0.29	0.18	1.25	1.02	100.53	119.48
	T3	3.09	0.37	2.07	0.31	0.23	1.16	0.96	93.51	105.56
3000 ppm										
	T0	2.67	0.24	1.63	0.42	0.31	0.63	0.40	76.77	70.29
	T1 2nd	2.83	0.28	1.83	0.44	0.28	0.99	0.68	80.39	89.80
	T2	3.10	0.39	2.00	0.34	0.21	1.17	0.95	93.34	106.58
	T3	2.90	0.32	1.90	0.41	0.25	1.10	0.79	88.36	97.28
	T0	2.05	0.22	1.59	0.47	0.40	1.01	0.32	72.70	69.34
	T1 1St	2.29	0.27	1.79	0.41	0.33	1.30	0.64	80.26	80.48
	T2	2.75	0.36	1.93	0.33	0.26	1.46	0.95	98.42	102.80
	T3	2.44	0.30	1.88	0.37	0.29	1.39	0.77	90.53	88.77
6000 ppm										
	T0	1.97	0.19	1.44	0.54	0.47	0.85	0.28	64.36	57.14
	T1 2nd	2.16	0.22	1.63	0.49	0.39	1.19	0.57	74.61	77.83
	T2	2.42	0.31	1.77	0.38	0.29	1.35	0.90	95.43	93.32
	T3	2.29	0.26	1.70	0.45	0.34	1.26	0.73	87.33	80.87
	T0	1.76	0.17	1.37	0.64	0.45	1.15	0.24	39.30	44.81
	T1 1St	1.90	0.23	1.45	0.48	0.40	1.54	0.48	55.21	76.92
	T2	2.27	0.29	1.67	0.40	0.30	1.73	0.87	67.85	90.41
	T3	2.02	0.25	1.58	0.45	0.36	1.60	0.59	60.44	87.33
9000 ppm										
	T0	1.55	0.12	1.30	0.70	0.50	1.10	0.21	32.45	30.20
	T1 2nd	1.87	0.16	1.37	0.55	0.46	1.30	0.35	49.47	69.47
	T2	2.18	0.25	1.59	0.47	0.35	1.50	0.81	63.32	87.83
	T3	1.98	0.21	1.46	0.51	0.41	1.39	0.53	57.48	80.30
LSD (0.05)										
S		0.001	0.12	0.22	0.001	0.05	0.08	0.20	3.98	5.57
N	1St	0.08	0.07	0.23	0.04	0.03	0.08	0.06	6.78	5.87
N× S		0.15	0.14	0.47	0.08	0.05	0.17	0.12	13.57	11.73
S		0.05	0.03	0.33	0.03	0.05	0.08	0.07	4.99	6.28
N	2nd	0.08	0.06	0.22	0.04	0.04	0.07	0.10	4.98	9.23
N×S		0.16	0.12	0.44	0.08	0.08	0.14	0.19	9.97	18.46

T0= normal Hoagland solution (control), T1 = 30 mg/L ZnO and Fe_3O_4 NPs, T2= 60 mg/L ZnO and Fe_3O_4 NPs and T3= 90 mg/L ZnO and Fe_3O_4 NPs.
S= salinity treatments, N= nano treatments. 1st = First season, 2nd = Second season.

seasons, the foliar application of a combination of ZnO and Fe_3O_4 NPs in Hoagland solution significantly increased non-enzymatic (vitamins A, and C) and enzymatic antioxidants (POD and SOD) in *Moringa* seedlings in comparison with control plants. The elevated amount in non-enzymatic and enzymatic antioxidants may be attributed to the beneficial effects of Fe and Zn represented in the increasing liberation of more nutrients from the unavailable reserves through correcting iron and zinc deficiency thus resulting in photosynthesis efficiency, increasing amino acids and vitamins to be absorbed by plant roots. This may be attributed to the importance of iron as a cofactor for many enzymes that catalyze unique biochemical reactions that are essential plant development such as chlorophyll and thylakoid syntheses and chloroplast development (Miller et al., 1995). Meanwhile, zinc is an essential element for plants that act as a metal component of various enzymes or as a functional structure or regulatory cofactor for protein synthesis and photosynthesis (Marschner, 1995). Also, Chang and Sung (1998) concluded that priming with antioxidant compounds such as ascorbic acid could increase free radical scavenging enzymes such as superoxide dismutase (SOD), and peroxidase in seeds.

Salt-stressed *Moringa* plants accumulated lower amounts of Na^+, Cl^- and higher amount of N, K^+, P, Ca^{2+}, Mg^{2+}, Fe and Zn upon foliar application of ZnO and Fe_3O_4 NPs-containing Hoagland solution when compared to those of the salt-stressed plants that received only foliar application of Hoagland solution (Table 3). The accumulation of less Na^+ is an important indicator of salt tolerance in plants as those subjected to foliar applications with ZnO and Fe_3O_4 NPs-containing Hoagland solution showed less accumulation of Na^+ in their shoots either in stress or non-stress conditions. The reduction of Na^+ in shoots of *Moringa* plants grown under the highest salinity level and sprayed with T2 reached 37 and 32 % in first and second seasons, respectively, in comparison to plants that received only Hoagland solution and grown under the same salinity level (9000 pm). At highest salinity level, the increase of K^+ in T2- sprayed plants reached 21 and 22% in both the first and second seasons, respectively, over Hoagland-sprayed plants. The importance of determining percentage Na^+ and K^+ in the plants is because they reflect salt tolerance in plants (Tunçtürk et al., 2011). Foliar feeding with micronutrients could partially alleviate the adverse effect of NaCl on nutrients uptake through improving root growth and preventing nutritional disorders and consequently resulting in an increase in nutrients uptake by the roots (El-Fouly et al., 2002). Also, zinc may help nutrient translocation from aged cells to newborn cells (Rockenfeller and Madeo, 2008). Zinc may, therefore, play an important role in membrane permeability, phospholipids (P) accumulation, and free oxygen radical scavenging. These results correlate with the findings of Qu et al. (2009) who reported that zinc application could alleviate possible Na^+ and Cl^- injury in plants.

Our results reveal that salt toxicity in *Moringa* plants can be alleviated by foliar spray of nano- zinc and iron. The results are consistent with Cakmak and Marschner (1988) who reported that zinc could play an important role in the maintenance of the structural integrity of the plasma membrane and thus control Na and other toxic ions uptake. Similarly, Saleh and Maftoun (2008) observed that zinc application reduced Na^+ concentration in rice shoot. Cakmak and Marschner (1988) reported that under zinc application, the activity of membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase producing reactive oxygen species (ROS), decreased.

In this study, alleviation of salt stress can be attributed to two reasons: first, promoting effect of spraying nutrients in Hoagland solution on *Moringa* plants grown under salt stress conditions and control conditions; second, the properties of ZnO and Fe_3O_4 NPs (larger specific surface area and moew reactive areas) that help in enhanced enzyme activity related to salt tolerance. Thus the Fe_3O_4 NPs were found to induce oxidative stress and higher antioxidative enzyme activity than the bulk Fe_3O_4 particles.

Conclusion

In this study, we succeeded in showing that salt stress can be alleviated in *Moringa* plants using foliar applications of ZnO and Fe_3O_4 NPs-containing Hoagland solution in comparison to spraying only with normal solution. Growth parameters and chemical composition related to salt tolerance were enhanced when nano-forms of Fe and Zn were used in Hoagland solution (60 mg/L).

Conflict of Interest

The authors have not declared any conflict of interest.

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