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Impact of ZnO Nanoparticles on Growth of Cowpea and Okra Plants under Salt Stress Conditions

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Salt stress causes a serious threat to agricultural productivity and global food security. It is one of the most pervasive crops limiting factor. This study examined the effect of six salinity concentrations (0, 10, 25, 50, 75 and 100% of seawater); on the growth of two crop species, cowpea (Vigna unguiculata L. var.california blackeye NO.46) and okra (Abelmoschus esculentus L. Moench var.Hasawi) in the presence or absence of (10 mg/L) of the green synthesized zinc oxide nanoparticles (ZnO NPs) or zinc oxide (bulk ZnO), as a foliar spray after (20, 40 and 60 days) from sowing. The results showed a gradual decrease in shoot and root lengths, fresh and dry weights of shoot, leaf area and relative growth rate (RGR) with the increase of seawater concentrations in both plants. However, application of ZnO enhanced the growth parameters compared to the control plants, but better results were observed in the plants treated with (ZnO NPs). Thus, nanoparticles of (ZnO) environmentally friendly, cheap cost, and can be considered as a promising application to alleviate the effects of salt stress on plants.

Keywords: Foliar Spray; Plant Growth; Nanotechnology; ZnO Nanoparticles.

Salt stress affecting almost 20–33% of cultivated areas, 50% of irrigated areas and affects almost one billion hectares of global land[1,2]. More than 397 million hectares of lands worldwide is affected by salinity and/or more than 434 million hectares affected by salinity[3], which causing desertification around the world4. While the agricultural land, which is exposed to salinity, minimize, the food is demanded with the increase of the population[5]. By the year 2050, even more than 50% of the global agriculture land will be vulnerable to salt stress[6]. There is a general understanding, that salinity only occurs in arid and semi-arid regions, but there is no climatic area free from this problem[7]. Around 97.5% of the planet's water is saline. Seawater is the most available source of water in the world. Thus, there are growing interested to use it in the agricultural sector to irrigate the plants[8,9]. The major constituent of seawater is sodium chloride (NaCl)[10].

Nanotechnology is a description of synthesis, fabrication, characterization and utilization of Nano-sized materials[11]. The use of the applications of nanotechnology is increasing in different fields[12] such as industry, information technology, medicine, energy and agriculture which in turn impacts the environment, society and economy[13,14]. There are different properties in

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the nanoparticles of metal oxides that are not found in their bulks counterparts like their shapes, size, surface reactivity, chemical stability and their large surface area to their volume ratio[15].

Zinc (Zn) is a micronutrients and one of the essential nutrients for humans, animals and has an influential role in plant growth, development and protection. Generally, the plants uptake the Zn as a cation ($Zn2+$)[16]. The appropriate concentrations of zinc oxide nanoparticles (ZnO NPs) improved the growth and protection of different plant species[15]. Using the nanoparticles, which are synthesized by green methods like (ZnO NPs) as a foliar application on the plants is one of the promising methods to reduce water and soil pollutions by putting less input and producing less waste than ordinary approaches[17]. Fertilizers at the nano size improve the plant's growth because of their diminutive size, which in turn could enhance the uptake of micronutrients in a controlled and gradual manner in the plants compared to the regular fertilizers[18].

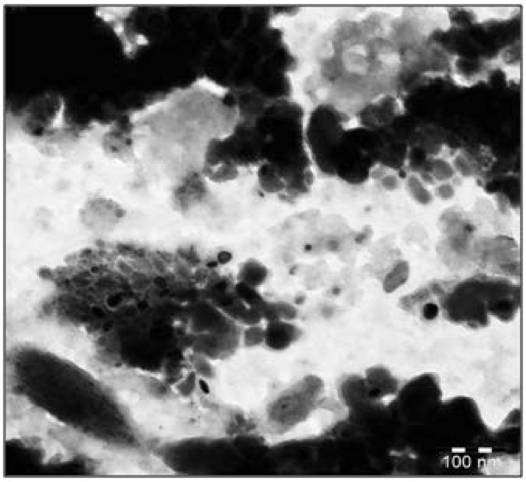

Fig. 1. TEM image of the biosynthesized (ZnO NPs) from [*Phoenix dactylifera* L. cv. Khalas] leaflets extract

Cowpea considers as one of the most important economically cultivated legumes worldwide which provides many economic, agronomic and environmental advantages to millions of people worldwide. It is a feed, food and forage crop[19]. This species is a herbaceous warm-season annual plant grown in tropical and subtropical regions and in the semiarid regions[20,21].

Okra is one of the most popular vegetables annually renewable crops cultivated during the hot summer seasons. It is a multipurpose crop which have been used in industrial and health applications, and it has nutritional quality[22]. It grows commercially in many countries[23].

Table 1. Effect of different concentrations of seawater (SW) in the presence or absence of (bulk ZnO) or (ZnO NPs) on shoot length and root length (cm), fresh and dry weights of shoot and root (g) of Vigna unguiculata plants after 20 days of age

		After (20 days)					
Treatments	SW (%)	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)
control	0	15.73	17.27	3.48	0.49	0.76	0.31
	10	14.63^b	15.31^c	3.17^c	0.34^b	0.57^c	0.26^c
	25	13.50^c	13.77^c	2.63^c	0.28^b	0.44^c	0.20^b
	50	11.97^b	10.26^a	1.96^a	0.20^a	0.27^b	0.13^a
	75	9.37^a	8.70^a	1.07^a	0.12^a	0.21^b	0.07^a
	100	7.50^a	6.77^a	0.86^a	0.07^a	0.16^b	0.02^a
bulk ZnO	0	18.33^c	19.11^c	3.97^c	0.59^b	0.83^c	0.37^c
	10	17.40^b	16.63^c	3.27^c	0.45^b	0.66^c	0.32^c
	25	15.53^c	14.53^c	3.11^c	0.35^c	0.58^c	0.26^c
	50	12.77^c	12.10^c	2.12^c	0.29^b	0.32^c	0.19^c
	75	10.87^c	9.17^c	1.36^c	0.20^b	0.27^c	0.16^b

Significance of values at $p<0.05$, a= (highly significant), b= (significant), c= (not significant).

MATERIALS AND METHODS

All chemicals employed in this study were of high purity, purchased from Sigma-Aldrich, USA. ZnO nanoparticles prepared by using [Phoenix dactylifera L. cv. Khalas] leaflets extract and characterized their formation and size by using the UV-visible spectroscopy [UV-1800] which demonstrated that the highest absorption peak was about [370 nm] using a transmission electron microscope (TEM) [Mic JEM 1011], and the size founded [from 16 to 35nm] (Fig.1). The concentration of seawater used to irrigate the plants prepared by diluted seawater to get (0, 10, 25, 50, 75 and 100 % seawater SW). The seeds of cowpea [Vigna unguiculata L. cv.California Blackeye NO.46] and okra [Abelmoschus esculentus L. Moench cv.Hasawi] were purchased from Modesto, California U.S.A and Altuajri, K.S.A. respectively. The powders of both ZnO types were mixed with deionized water.

Pot Experiment

Seeds of V.unguiculata and A. esculentus were surface sterilized by 4% for 1 min, then rinsed thoroughly with distilled water. The seeds then germinated in 15cm pots which contain 2.5kg of sand. The experimental pots were arranged in a simple randomized design and exposed to normal day length and natural temperature 25-28°C.

All plants were irrigated 3 times per week with tap water for 15 days. After that, pots were treated with seawater concentrations (0, 10, 25, 50, 75 and 100%) with or without the foliar application of 10mg/L of bulk ZnO or ZnO NPs. The foliar treatments applied 2 times at 15 and 35 days after sowing using a hand-held sprayed separately after covering the surface of the pots with plastic film. Three vegetative stages were studied at 20, 40 and 60 days from planting dates for growth analysis.

Growth Parameters

After 20, 40 and 60days, the evaluation of shoot and root lengths had been determined by

Table 2. Effect of different concentrations of seawater (SW) in the presence or absence of (bulk ZnO) or (ZnO NPs) on shoot length and root length (cm), fresh and dry weights of shoot and root (g) of Vigna unguiculata plants after 40 days of age

		After (40 days)					
Treatments	SW (%)	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)
control	0	22.50	20.48	3.94	0.58	0.87	0.40
	10	19.97[b]	17.49[b]	3.36[c]	0.41[a]	0.68[c]	0.36[c]
	25	17.47[a]	15.16[b]	3.11[b]	0.37[a]	0.54[b]	0.28[b]
	50	14.73[a]	12.07[b]	2.12[a]	0.31[a]	0.34[b]	0.19[a]
	75	11.70[a]	10.17[a]	1.78[a]	0.22[a]	0.28[b]	0.12[a]
	100	9.33[a]	8.70[a]	1.12[a]	0.10[a]	0.21[b]	0.07[a]
bulk ZnO	0	26.17[b]	24.57[b]	4.07[c]	0.65[c]	0.98[c]	0.47[c]
	10	24.27[a]	22.37[b]	3.44[c]	0.50[b]	0.78[c]	0.39[c]
	25	19.41[b]	19.09[b]	3.15[c]	0.43[c]	0.61[c]	0.32[c]
	50	16.57[b]	14.35[b]	2.18[c]	0.37[c]	0.39[c]	0.24[c]
	75	13.73[b]	11.04[c]	1.84[c]	0.29[c]	0.34[c]	0.18[c]
	100	10.00[c]	9.48[c]	1.18[c]	0.17[c]	0.27[c]	0.09[c]
ZnO NPs	0	36.00[a]	33.40[a]	5.24[a]	0.82[a]	2.34[a]	0.63[a]
	10	34.07[a]	31.54[a]	5.07[a]	0.78[a]	2.25[a]	0.57[a]
	25	31.63[a]	30.13[a]	4.87[a]	0.64[a]	2.16[a]	0.49[a]
	50	28.23[a]	27.57[a]	4.33[a]	0.53[a]	1.87[a]	0.40[a]
	75	25.37[a]	23.66[a]	3.91[a]	0.46[a]	1.45[b]	0.34[a]
	100	21.03[a]	19.53[a]	3.30[a]	0.33[a]	1.18[b]	0.22[a]

Significance of values at $p<0.05$, a= (highly significant), b= (significant), c= (not significant).

using a metric scale and expressed in centimeter (cm). After washed the plants with double distilled water to remove the sand particles, there had been separated into shoots and roots, then fresh weights and dry weights (DW) weighted by analytical balance [HR-200]. Dry weight was recorded by drying the plants at 65°C until the weight became constant. The third leaf area measurement (cm2) was taken after 60 days since emergency by using [CI-202 AREA METER CID, INC]. The relative growth rate was measured according to the formula of Hunt[24] and Hoffmann and Poorter[25]:

R.G.R = ln W2 - ln W1 / t_2 - t1

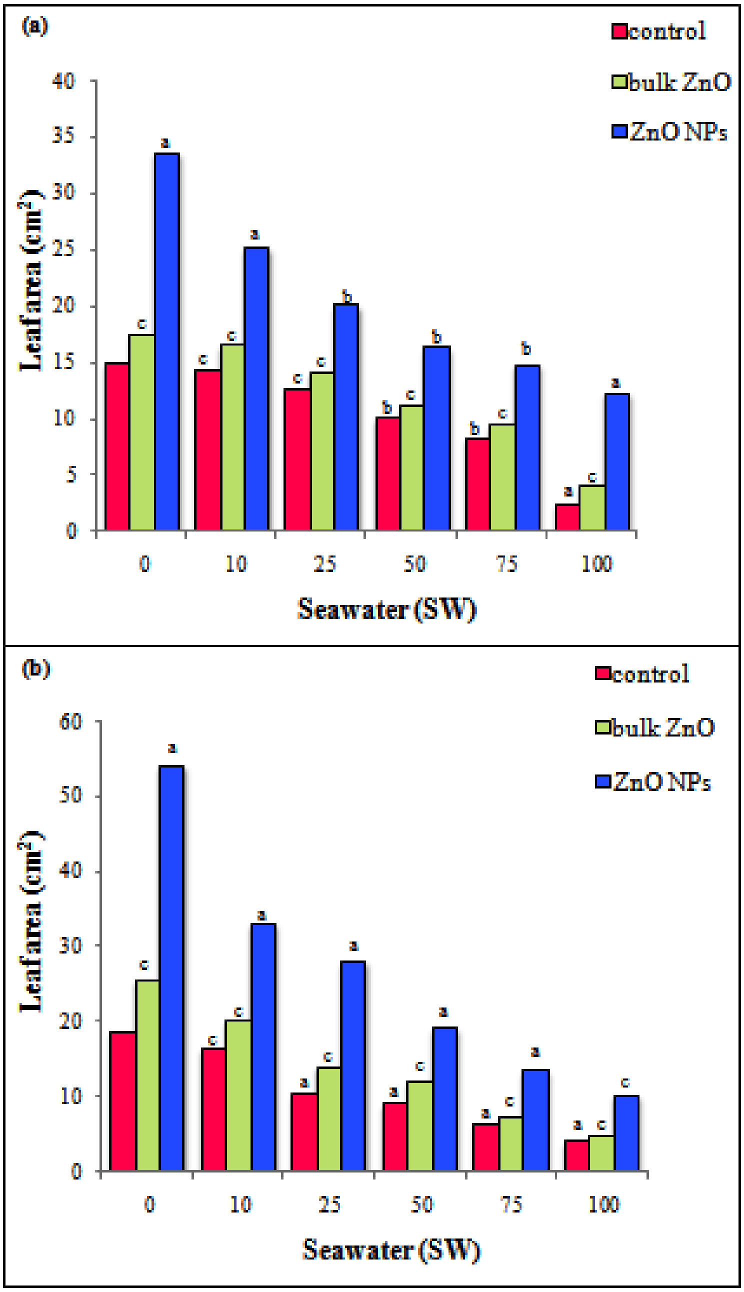

Fig. 2. Effect of different concentrations of seawater (SW) in the presence or absence of (bulk ZnO) or (ZnO NPs) on leaf area (cm^2) of (a)*Vigna unguiculata* and (b)*Abelmoschus esculentus* plants

RGR: Relative Growth Rate (g g-1 day -1)
In = natural logarithm
ln W_1 =The mean of the ln-transformed plant total dry weight
at time t1.
ln W_2 = The mean of the ln-transformed plant total dry weight at time t_2
t_1 = number of days in the first time measurement (day)
t_2 = number of days in the last time measurement (day)
W_1 and W_2 are the dry weight of the plants at time t_1 and t_2 respectively.

Statistical Analysis

All experiments were carried out using the statistical package SPSS software, version 20

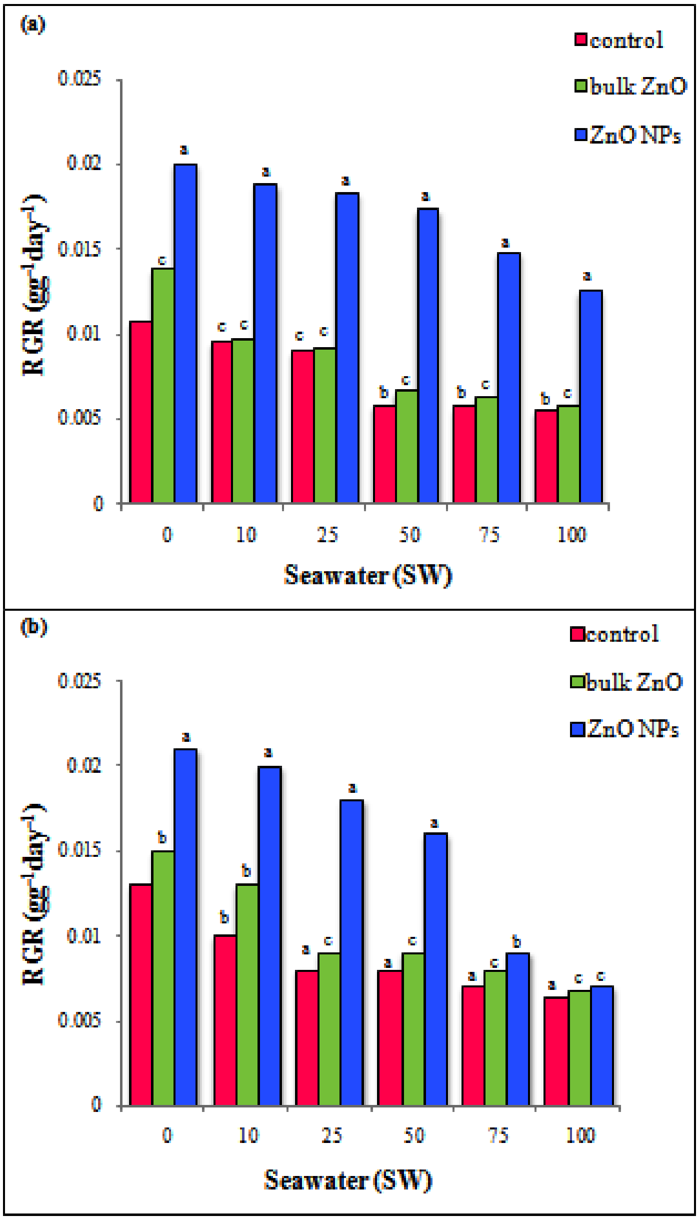

Fig. 3. Effect of different concentrations of seawater (SW) in the presence or absence of (bulk ZnO) or (ZnO NPs) on relative growth rate (RGR; g g^{-1} day $^{-1}$) of **(a)***Vigna unguiculata* and **(b)***Abelmoschus esculentus* plants

with three replicates (n=3) ±SE by a completely randomized design (CRD). Statistical analysis was carried out according to Snedecor and Cochran26, using T test. Significant differences were obtained by calculating (LSD) at $p<0.05$.

RESULTS

Growth of cowpea (Vigna Unguiculata)

The results revealed that in V.unguiculata plants the shoot and root lengths, shoot and root fresh and dry matter decreased with the increase seawater concentrations at the three vegetative stages, (bulk ZnO) improved the growth parameters non-significantly and significantly. While these parameters increased significantly and high significantly with (ZnO NPs) relative to control plants except at (20 days) the increase was non-significant in root length with (75 and 100% SW) treatments, and the fresh weight of shoot with (10, 25 and 50 % SW) treatments (Tables 1,2,3). After 60 days, V.unguiculata leaf area was measured; seawater treatment showed a non-significant decrease in leaf area with increasing salinity. When applying (bulk ZnO) non-significantly increased the leaf area in all seawater concentrations, while with (ZnO NPs) showed a better significant increase as compared to (bulk ZnO) and control treatments (Fig. 2a).

The relative growth rate (RGR) decreased gradually with the increasing seawater concentrations. The non-fertilized V.unguiculata plants (control) showed non-significant decrease in (RGR) in the lower seawater concentrations (10 and 25% SW), while the decrease was significant in (50, 75 and 100% SW). The addition of (bulk ZnO) increased the (RGR) non-significantly in all seawater concentrations. However, (ZnO NPs)

Table 3. Effect of different concentrations of seawater (SW) in the presence or absence of (bulk ZnO) or (ZnO NPs) on shoot length and root length (cm), fresh and dry weights of shoot and root (g) of Vigna unguiculata plants after 60 days of age

		After (60 days)					
Treatments	SW (%)	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)
control	0	27.73	24.38	4.20	0.72	1.09	0.56
	10	25.43c	22.60c	3.81c	0.65c	0.74b	0.47b
	25	23.58b	17.88b	3.22b	0.54b	0.61b	0.39b
	50	19.53b	15.72b	2.54a	0.40b	0.40b	0.23b
	75	14.17a	12.51b	2.12a	0.33b	0.33b	0.18b
	100	11.13a	10.94b	1.66a	0.18b	0.30b	0.10b
bulk ZnO	0	30.23c	27.41c	4.27c	0.81c	1.31c	0.62c
	10	28.62b	24.59c	3.98c	0.71c	0.81c	0.53c
	25	25.57c	21.57c	3.31c	0.60c	0.69c	0.43c
	50	20.30c	16.64c	2.61c	0.49c	0.45c	0.26c
	75	15.37c	13.23c	2.18c	0.39c	0.41c	0.21c
	100	11.97c	11.45c	1.73c	0.23c	0.34c	0.13c
ZnO NPs							
0	43.40a	40.28a	5.62a	1.51a	2.41a	1.12a	
	10	41.90a	37.63b	5.46a	1.48a	2.35a	0.98a
	25	38.53a	34.27a	5.22a	1.31b	2.28a	0.89a
	50	35.41a	29.10b	4.67a	1.24a	1.95a	0.76a
	75	29.33a	25.47b	4.11a	1.15a	1.58b	0.55b
	100	25.10a	20.07b	3.34a	0.78b	1.28b	0.47b

Significance of values at $p<0.05$, a= (highly significant), b= (significant), c= (not significant).

increased these measures high significantly as compared to their corresponding controls (Fig. 3a).

Growth of Okra (Abelmoschus Esculentus)

In A.esculentus plants, all the growth parameters decreased gradually with the increase of seawater levels. After 60 days there was high significant inhibition reached (51.70, 55.90, 67.00, 71.43, 74.7 and 75.56%) in shoot and root lengths, shoot and root fresh weights, shoot and root dry weights respectively, compared to control treatments. It is worth mentioning that the plants treated with the green synthesized (ZnO NPs) give the best results to enhance the growth measurements compared to the plants treated with (bulk ZnO), (Tables 4,5,6). Present results show that the leaf area of A.esculentus plants treated with different concentrations tend to decrease non-significantly in (10% SW), while the decrease was high significant at all the other concentrations. The leaf area increased non-significantly above the different controls when (bulk ZnO) was used, while (ZnO NPs) increased high significantly the leaf area in all concentrations except the higher concentration (100% SW), (Fig. 2b).

The decrease in RGR was significant in plants treated with (10%) of seawater and highly significant in plants treated with (25, 50, 75 and 100 % SW). Addition of (bulk ZnO) increased the relative growth rate significantly at (0 and 10%) of seawater, while it increased non-significantly at (25, 50, 75 and 100% SW). The addition of (ZnO NPs) gave positive increases than (bulk ZnO). The increase was highly significant in plants treated with all seawater concentrations (Fig. 3b).

Table 4. Effect of different concentrations of seawater (SW) in the presence or absence of (bulk ZnO) or (ZnO NPs) on shoot length and root length (cm), fresh and dry weights of shoot and root (g) of Abelmoschus esculentus plants after 20 days of age

		After (20 days)					
Treatments	SW (%)	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)
control	0	14.680	16.23	2.81	0.41	0.56	0.20
	10	14.27c	15.84c	2.30c	0.30a	0.45c	0.17b
	25	13.60c	15.02b	1.87b	0.25a	0.35b	0.13b
	50	11.48b	12.81a	1.04a	0.18a	0.24b	0.09a
	75	10.03a	8.91a	0.77a	0.11a	0.11a	0.02a
	100	8.07a	7.15a	0.38a	0.05a	0.04a	0.0077a
bulk ZnO	0	16.400c	17.50b	3.22c	0.48b	0.69c	0.28b
	10	15.73c	16.45c	2.87c	0.38b	0.56c	0.22b
	25	14.93c	16.11c	2.11c	0.31b	0.45c	0.19b
	50	12.17c	13.18c	1.36c	0.25b	0.31c	0.12b
	75	10.16c	9.12c	1.08c	0.19b	0.19c	0.10b
	100	8.97c	7.73c	0.77c	0.10c	0.11c	0.07b
ZnO NPs	0	20.176a	21.05a	4.41a	0.64a	2.07a	0.46a
	10	19.44a	20.53a	4.11a	0.56a	1.57a	0.35a
	25	18.74a	20.09a	3.86a	0.45a	1.33a	0.27a
	50	17.20a	18.45a	3.03a	0.34a	1.15a	0.21a
	75	12.61b	14.93a	2.32a	0.27a	1.02a	0.18a
	100	11.55b	14.51a	1.95a	0.14a	0.52a	0.15a

Significance of values at $p<0.05$, a= (highly significant), b= (significant), c= (not significant).

DISCUSSION

Salinity affects plant growth by ionic stress, oxidative stress, reducing cell enlargement and cell division and osmotic stress, which depends on the concentration of salts and the type of plant tissue[27]. Salt stress can strongly affect the plants morphology[28,29], it has a great inhibition influence which can lead to apparent stunting of plant growth[29,30].

The growth of roots decreases when soil salinity exceeds (40mM)[31,32], thus inhibition of root growth leads to reduction in water use efficiency, water uptake capacity, leaf water potential and transpiration rate under salt stress[33]. Also, Kaya et al[34] pointed out that stressed plants resorted to close the stomata to retain the amount of water in the leaves and thus less entry of CO2 and rate of photosynthesis, which leads directly or indirectly to a decrease the amount of photosynthetic products.

Salt stress causes a reduction in turgor pressure, which leads to a major reduction in cell growth, cell elongation, cell division[27], and consequently the whole plant growth. The decrease in leaf area is a result of cell water relations, changes in cell wall features and reduction in photosynthetic rate[35]. The reduction in fresh and dry weight is due to the formation of smaller and fewer leaves and a decrease in plant height[33].

The morphological parameters in the plants such as shoot and root lengths, shoot and root weights, leaf area as well as, relative growth rate (RGR) are indicate the plant health[36]. The measured growth parameters in cowpea (V.unguiculata) and okra (A.esculentus) plants increased with the foliar application of (ZnO bulk) and (ZnO NPs) under salinity stress. (ZnO NPs) showed better results than other treatments. These data are in agreement with other studies such as Sah et al[37] on Borago officinalis L.; Sabaghnia and Janmohammadi[38]

Table 5. Effect of different concentrations of seawater (SW) in the presence or absence of (bulk ZnO) or (ZnO NPs) on shoot length and root length (cm), fresh and dry weights of shoot and root (g) of Abelmoschus esculentus plants after 40 days of age

		After (60 days)					
Treatments	SW (%)	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)
control	0	24.603	25.01	4.00	0.70	0.83	0.45
	10	21.23a	22.14a	3.74c	0.61b	0.64c	0.39c
	25	18.84a	19.31a	3.15a	0.48b	0.56a	0.28a
	50	16.91a	16.87a	2.41a	0.38b	0.42a	0.21a
	75	15.01a	14.14a	1.86a	0.30b	0.31a	0.17a
	100	11.88a	11.03a	1.32a	0.20a	0.21a	0.11a
bulk ZnO	0	25.883c	26.11c	4.36c	0.77c	1.02c	0.53b
	10	21.93c	22.97c	4.12b	0.69b	0.79c	0.44c
	25	19.09c	19.90c	3.62b	0.54c	0.65c	0.34c
	50	17.12c	17.01c	2.91b	0.44c	0.53c	0.27c
	75	15.95c	14.88c	2.07c	0.39b	0.43c	0.22c
	100	12.08c	11.81c	1.63c	0.24c	0.28c	0.17c
ZnO NPs	0	32.507a	34.21a	5.31a	1.41a	2.32a	1.07a
	10	30.15a	32.16a	5.03a	1.39a	2.21a	0.91a
	25	27.31a	29.51a	4.42a	1.29a	2.14a	0.80a
	50	26.14a	24.96a	4.15a	1.18b	1.86a	0.70a
	75	23.14a	21.51a	3.66a	1.07a	1.51a	0.63a
	100	21.52a	18.92a	3.03a	0.72a	1.24a	0.51a

Significance of values at $p<0.05$, a= (highly significant), b= (significant), c= (not significant).

on Lens culinaris Medik.; Luksiene et al[39] on Strawberries and Shinde et al[40] on maize. Zinc applications have a positive impact on the plants salt tolerance. (ZnO NPs) can potentially alleviate the negative effects of abiotic stress on plants[41]. This enhancement influence of foliar application might be attributed to the crucial role of zinc on the biological and metabolism activity of plants such as stimulating enzymes activities, cell elongation and enlargement, nitrogen metabolism, photosynthetic pigments, maintaining the membranes structural stability of the plant cells and accumulation of the phospholipids[16,42].

The efficiency of (ZnO NPs) also, relates to their ability to penetrate in the plant cell through the natural Nano pore (stomata) in the leaves which may improve metabolic activities and consequently higher plant production[43]. The uptake of Zn through the leaves is influenced by environmental factors, type of the leaf, stress level and plants health[44]. In addition, Zn applications positively improve biosynthesis of the growth regulator IAA which promotes cell division, cell elongation and absorption of minerals, thus increased plant growth[45]. The addition of micronutrient is more economical and beneficial than soil fertilization, because of the nutrients are more actively to reach the cells and be obtainable for plant growth[46].

Nanoparticles forms with their smaller size, have more ability and dynamic to be absorbed, translocate, assimilate and accumulate in the plant than their bulk forms. Nanoparticles can pass through the cell wall and plasma membrane[47]. Furthermore, the high specific surface area and a higher rate of uptake explain the better efficiency of the application of nanoparticles compared to bulk forms[48]. This helps to raise the rate of dissolution of zinc oxide (ZnO) which has low solubility in water[49]. The various physiological effects of the foliar supply of (ZnO NPs) may be due to the slow

Table 6. Effect of different concentrations of seawater (SW) in the presence or absence of (bulk ZnO) or (ZnO NPs) on shoot length and root length (cm), fresh and dry weights of shoot and root (g) of Abelmoschus esculentus plants after 60 days of age

		After (40 days)					
Treatments	SW (%)	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)
control	0	20.573	21.58	3.25	0.51	0.76	0.31
	10	17.43a	18.08a	3.03c	0.39b	0.56a	0.23b
	25	15.03b	16.21c	2.09a	0.32a	0.47a	0.19a
	50	14.55a	14.12b	1.56a	0.26a	0.31a	0.14a
	75	12.15b	11.77a	1.22a	0.21a	0.24a	0.10a
	100	9.92b	9.07a	0.98a	0.13a	0.13a	0.037a
bulk ZnO	0	22.120b	22.80c	4.02b	0.60c	0.88c	0.39b
	10	18.34c	18.98c	3.36c	0.48c	0.65c	0.30b
	25	16.24b	16.93c	2.54b	0.40c	0.52c	0.26b
	50	15.77b	14.89c	1.74c	0.34c	0.43c	0.20b
	75	12.73c	12.11c	1.37c	0.28c	0.31c	0.17b
	100	10.07c	9.97c	0.99c	0.19c	0.18c	0.11b
ZnO NPs	0	26.570a	28.11a	5.03a	0.73a	2.21a	0.56a
	10	24.34a	26.50a	4.51a	0.67a	2.06a	0.51a
	25	22.65a	23.88a	4.13a	0.57a	1.86a	0.43a
	50	20.55a	20.11a	3.81a	0.46a	1.68a	0.30a
	75	18.47a	18.88a	3.17a	0.34b	1.32a	0.26a
	100	16.42a	15.71a	2.29a	0.28a	1.12a	0.20a

Significance of values at $p<0.05$, a= (highly significant), b= (significant), c= (not significant).

release of Zn ion from the nanoparticles, which supplies a long-term provenance of Zn, and help to avoid toxicity by sudden uptake of Zn by plants at high concentrations[50]. The increase in plants growth with nanoparticles application might be due to rising of the efficiency of nutrient usage diminish soil toxicity which produces by over dosage of the addition of fertilizers and enhance the activities of antioxidant enzymes which help to protect the plants from injury caused by (ROS)[51]. Rising in the plant height may because of the improvements of auxin biosynthesis and synergistic relation between both nutrients nitrogen and iron[52].

CONCLUSION

The results of this study showed that both treatments of (bulk ZnO) and (ZnO NPs) enhanced the growth parameters in the salt-stressful plants cowpea (V.unguiculata) and okra (A.esculentus). Notably, both of these plants showed good tolerance to salt stress. The nanoparticles of (ZnO) gave better results by improving plant salinity tolerance than their bulk size. The foliar application of the green synthesize (ZnO NPs) can be a good alternative to their bulks because they are ecologically friendly approaches with low-priced.

REFERENCES

1. Saade, S., Maurer, A., Shahid, M., Oakey, H., Schmöckel, S. M., Negrão, S., Pillen, K. & Tester, M. J. S. R. Yield-related salinity tolerance traits identified in a nested association mapping (NAM) population of wild barley. *Scientific reports*. 2016; **6**: 32586.
2. Machado, Rui Manuel Almeida; Serralheiro, Ricardo Paulo. Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. *Horticulturae*. 2017; 3.2: 30.
3. Munns, Rana. Genes and salt tolerance: bringing them together. *New phytologist*. 2005; **167**.3: 645-663.
4. Shrivastava, Pooja; Kumar, Rajesh. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi journal of biological sciences*. 2015; **22**.2: 123-131.
5. Abreu, I. A., Farinha, A. P., Negrao, S., Goncalves, N., Fonseca, C., Rodrigues, M., Batista, R., Saibo, N. J. & Oliveira, M. M. Coping with abiotic stress: proteome changes for crop improvement. *Journal of proteomics*. 2013; **93**: 145-168.
6. Wang, W., Vinocur, B., Shoseyov, O., & Altman, A. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in plant science*, 2004; **9**.5: 244-252.þ
7. Rengasamy, P. World salinization with emphasis on Australia. *Journal of experimental botany*. 2006; **57**.5: 1017-1023.
8. Yermiyahu, U., Tal, A., Ben-Gal, A., Bar-Tal, A., Tarchitzky, J., & Lahav, O. Rethinking desalinated water quality and agriculture. *Science*. 2007; **318**.5852: 920-921.
9. Adolf, Verena Isabelle; Jacobsen, Sven-Erik; Shabala, Sergey. Salt tolerance mechanisms in quinoa (*Chenopodium quinoa* Willd.). *Environmental and Experimental Botany*. 2013; **92**: 43-54.
10. Mohamed, Kamel. Improving the tolerance of *Vicia faba* against environmental salinity resulted from the irrigation with sea water by using KNO3 and (NH_4) $2SO_4$ as chemical osmoregulators. *Acta Biológica Colombiana*. 2012; **17**.2: 295-308.
11. Mirzaei, Hamed; Darroudi, Majid. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Ceramics International. 2017; **43**.1: 907-914.
12. Mehta, C. M., Srivastava, R., Arora, S., & Sharma, A. K. Impact assessment of silver nanoparticles on plant growth and soil bacterial diversity. *3 Biotech*. 2016; **6**.2: 254.
13. Fulekar, M. H. *Nanotechnology: importance and applications*. IK International Pvt Ltd, 2010.
14. Farooqui, A. R., Tabassum, H. E., Ahmad, A. S., Mabood, A. B., Ahmad, A. D., & Ahmad, I. Z. Role of nanoparticles in growth and development of plants. *Int J Pharm Bio Sci*. 2016; **7**(4), 22-37.
15. Iziy, E., Majd, A., Vaezi-Kakhki, M. R., Nejadsattari, T., & Noureini, S. K. Effects of zinc oxide nanoparticles on enzymatic and nonenzymatic antioxidant content, germination, and biochemical and ultrastructural cell characteristics of *Portulaca oleracea* L. *Acta Societatis Botanicorum Poloniae*. 2019; **88**.4.
16. Cakmak, I. Tansley Review No. 111 Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *The New Phytologist*. 2000; **146**(2), 185-205.
17. Stewart, J., Hansen, T., Mclean, J. E., Mcmanus, P., Das, S., Britt, D. W., Anderson, A. J. & Dimkpa, C. O. Salts affect the interaction of ZnO or CuO nanoparticles with wheat. *Environmental toxicology and chemistry*. 2015; **34**(9), 2116-2125.

18. Singh, A., Singh, N. B., Afzal, S., Singh, T., & Hussain, I. Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants. *Journal of materials science*. 2018; **53**(1), 185-201.
19. Hall, A. Phenotyping cowpeas for adaptation to drought. *Frontiers in physiology*. 2012; **3**, 155.
20. Behura, R., Kumar, S., Saha, B., Panda, M. K., Dey, M., Sadhukhan, A., Mishra, S., Alam, S., Sahoo, D. P. & Sugla, T. Cowpea [*Vigna unguiculata* (L.) Walp.]. *Agrobacterium Protocols*. Springer. 2015.
21. Harouna, D. V., Venkataramana, P. B., Ndakidemi, P. A., & Matemu, A. O. Under-exploited wild *Vigna* species potentials in human and animal nutrition: a review. *Global food security*. 2018; **18**, 1-11.
22. Gemede, H. F., Ratta, N., Haki, G. D., Woldegiorgis, A. Z., & Bey, F. Nutritional quality and health benefits of okra (*Abelmoschus esculentus*) : A review. *Global Journal of Medical Research*, 2015.
23. Khan, Gazi Md Arifuzzaman; Yilmaz, Nazire Deniz; Yilmaz, Kenan. Okra Fibers: Potential Material for Green Biocomposites. In: *Green biocomposites*. Springer, Cham. 2017; p. 261-284.
24. Hunt, R. *Plant growth curves. The functional approach to plant growth analysis*. Edward Arnold Ltd. 1982.
25. Hoffmann, William A.; Poorter, Hendrik. Avoiding bias in calculations of relative growth rate. *Annals of botany*. 2002; **90**.1: 37-42.
26. Snedecor, George W.; Cochran, William G. Statistical methods. 7th. *Iowa State University USA*. 1980; 80-86.
27. Abbasi, H., Jamil, M., Haq, A., Ali, S., Ahmad, R., & Malik, Z. Salt stress manifestation on plants, mechanism of salt tolerance and potassium role in alleviating it: a review. *Zemdirbyste*. 2016;**103**, 229-238.
28. Sneha, S., Rishi, A., Dadhich, A., & Chandra, S. Effect of salinity on seed germination, accumulation of proline and free amino acid in *Pennisetum glaucum* (L.) R. Br. *Pakistan Journal of Biological Sciences*. 2013;**16**(17), 877-881.
29. Rishi, A., & Sneha, S. Antioxidative defense against reactive oxygen species in plants under salt stress. *International Journal of Current Research*. 2013; **5**,1622-1627.
30. Sneha, S., Rishi, A., & Chandra, S. Effect of short term salt stress on chlorophyll content, protein and activities of catalase and ascorbate peroxidase enzymes in pearl millet. *Am. J. Plant Physiol.* 2014; **9**(1), 32-37.
31. Tang, X., Mu, X., Shao, H., Wang, H., & Brestic, M. Global plant-responding mechanisms to salt stress: physiological and molecular levels and implications in biotechnology. *Critical reviews in biotechnology*. 2015; **35**(4), 425-437.
32. Munns, R., & Gilliham, M. Salinity tolerance of crops–what is the cost?. New phytologist. 2015; **208**(3), 668-673.
33. García-Caparrós, P., & Lao, M. T. The effects of salt stress on ornamental plants and integrative cultivation practices. *Scientia Horticulturae*. 2018; **240**, 430-439.
34. Kaya, C., Tuna, L., & Higgs, D. Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions. *Journal of Plant Nutrition*. 2006; **29**(8), 1469-1480.
35. Munns, R., & Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol*. 2008; **59**, 651-681.
36. Rizwan, M., Ali, S., Qayyum, M. F., Ok, Y. S., Adrees, M., Ibrahim, M., Zia-Ur-Rehman, M., Farid, M. & Abbas, F. J. Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review. *Journal of hazardous materials*. 2017; **322**, 2-16.
37. Sah, S., Sorooshzadeh, A., Rezazadehs, H. & Naghdibadi, H. J. Effect of nano silver and silver nitrate on seed yield of borage. *Journal of Medicinal Plants Research*. 2011; **5**, 171-175.
38. Sabaghnia, N., & Janmohammadi, M. Effect of nano-silicon particles application on salinity tolerance in early growth of some lentil genotypes. *Annales UMCS, Biologia*. 2015; **69**(2), 39-55.
39. Luksiene, Z., Rasiukeviciute, N., Zudyte, B., & Uselis, N. Innovative approach to sunlight activated biofungicides for strawberry crop protection: ZnO nanoparticles. *Journal of Photochemistry and Photobiology B: Biology*. 2020; **203**, 111656.
40. Shinde, S., Paralikar, P., Ingle, A. P. & Rai, M. Promotion of seed germination and seedling growth of *Zea mays* by magnesium hydroxide nanoparticles synthesized by the filtrate from *Aspergillus niger*. *Arabian Journal of Chemistry*. 2020;**13**, 3172-3182.
41. Sturikova, H., Krystofova, O., Huska, D., & Adam, V. Zinc, zinc nanoparticles and plants. Journal of hazardous materials. 2018; **349**, 101-110.
42. Mehrabani, L. V., Hassanpouraghdam, M. B., & Shamsi-Khotab, T. The effects of common and nano-zinc foliar application on the alleviation of salinity stress in *Rosmarinus officinalis* L. *Acta Sci Pol-Hortoru*. 2018;**17**(6), 65-73.
43. Rossi, L., Fedenia, L. N., Sharifan, H., Ma, X., & Lombardini, L. Effects of foliar application

of zinc sulfate and zinc nanoparticles in coffee (*Coffea arabica* L.) plants. *Plant physiology and biochemistry*, 2019;**135**, 160-166.

44. Fernández, V., & Brown, P. H. From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Frontiers in plant science*. 2013;**4**, 289.
45. Cakmak, I. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant and soil*. 2008;**302**(1-2), 1-17.
46. Khoshgoftarmanesh, A., Torabian, S. & Zahedi, M. Effect of foliar spray of zinc oxide on some antiox idant enzymes activity of sunflower under salt stress. *J. Agr. Sci. Tech*. 2018; **18**: 1013-1025.
47. Zhang, D., Hua, T., Xiao, F., Chen, C., Gersberg, R. M., Liu, Y., Stuckey, D., Ng, W. J. & Tan, S. K. J. C. Phytotoxicity and bioaccumulation of ZnO nanoparticles in *Schoenoplectus tabernaemontani.Chemosphere*. 2015;**120**, 211-219.
48. Mehrabani, L. V., Hassanpouraghdam, M. B., & Shamsi-Khotab, T. The effects of common and nano-zinc foliar application on the alleviation of salinity stress in *Rosmarinus officinalis* L. *Acta Sci Pol-Hortoru*. 2018;**17**(6), 65-73.
49. Mortvedt, J. J. Crop response to level of water-soluble zinc in granular zinc fertilizers. *Fertilizer research*. 1992;**33**(3), 249-255.
50. Wang, X., Sun, W., Zhang, S., Sharifan, H., & Ma, X. Elucidating the effects of cerium oxide nanoparticles and zinc oxide nanoparticles on arsenic uptake and speciation in rice (*Oryza sativa*) in a hydroponic system. *Environmental science & technology*. 2018;**52**(17), 10040-10047.
51. Hussein, M. M., & Abou-Baker, N. H. The contribution of nano-zinc to alleviate salinity stress on cotton plants. *Royal Society open science*. 2018;**5**(8), 171809.
52. El-Kereti, M., El-Feky, S., S Khater, M., Osman, Y., & El-Sherbini, E. S. ZnO nanofertilizer and He Ne laser irradiation for promoting growth and yield of sweet basil plant. *Recent patents on food, nutrition & agriculture*. 2013;**5**(3), 169-181.