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ALLEVIATION OF SALT STRESS AND IMPROVE CROP PRODUCTIVITY BY USING *ARBUSCULAR MYCORRHIZA* AND ZnO - NANO OR BULK PARTICLES IN WHEAT

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Abstract

A possible approach to alleviate salt stress and increase crop productivity is to apply *Arbuscular mycorrhiza fungus* (AMF). Wheat (*Triticuma estivum* L.) cultivar Gemmeiza 10 was grown in saline soil with or without AM and grain primary soaking in ZnO nanoparticles (NPs) or bulk particles of ZnO (BPs) (0, 5 and 10 mg/l). The field experiment was carried out at the experimental station of the Faculty of Agriculture, Wadi El-Natrun district El-Behera Governorate, Egypt, during the two winter seasons of 2016/2017 and 2017/2018. Wheat plants cultivated in the soil amended with AM showed a significant increase in growth parameters (plant height, leaves no. /tiller, tillers fresh weight and tillers dry weight), along with the increase in the level of photosynthetic pigments. AMF significantly increased yield components (plant height, tillers dry weight, spike length, spike weight, 1000 grains weight, straw yield, biological yield and grain yield, as compared with plants cultivated without AM. Moreover, AM led to increase of nutritional values of the harvested grains (carbohydrates %, proteins %, lycopene, B-carotene and total flavonoids, antioxidant activity) and macronutrients (N, P, K and Ca). Grains priming with ZnO NPs and ZnO BPs (5 and 10 mg/l) increased the growth, photosynthetic pigments, yield characteristics and nutritional values of the harvested grains in wheat. Increases in yield parameters were more pronounced in plants treated with nano-ZnO (10 mg/l). The interaction between ZnO NPs or ZnO BPs and AM was more efficient as it improved the yield components and nutritional values of the harvested grains compared with ZnO NPs or ZnO BPs sole treatments.

Keywords: *Arbuscular mycorrhiza*, Saline soil, Wheat, Zinc oxide, Nanoparticles.

Introduction

Soil salinity inhibiting crop growth and productivity are recurrent restriction to agriculture in arid and semi-arid regions. Saline soils and saline waters are common around the world, great efforts have been devoted to know physiological aspects of tolerance to salinity in plants. Salinity imposes both ionic and osmotic stresses on plants (Munns *et al*., 2006). Saline soils decreases water availability to plants; raises ion toxicity levels, decreases absorption of essential nutrients and decreases crop yields and qualities (Grattan and Grieve, 1999). Therefore, salinity influences on the physiological and biochemical of plants. Soil salinity has become a major factor limiting crop productivity worldwide. Survival, under salinity stress conditions, depends on the plant's ability to realize the stimulus, generates and transmits signals and induces biochemical alterations that regulate the metabolism (Dolatabadian & Saleh, 2009).

A possible way to enhance plant growth and productivity is the application of *Arbuscular mycorrhiza* fungi (AMF). Many studies have demonstrated that AMF protected the host plants to promote the growth of plants under environmental stress (Aroca and Ruı´z-Lozano, 2009). *Arbuscular mycorrhiza* are important in prospective agriculture because they progress plant water relations, they enhance mineral uptake by increased acquisition of phosphorus and another low mobile mineral nutrients, which decrease the use of fertilizers. They could also break down some complex minerals and organic substances in the soil and make it available to their hosts (Soliman *et al*., 2012). The *arbuscular mycorrhiza* involve regulation of stomatal conductance, an enhance in stomatal sensitivity to leaf-air vapor pressure deficit, and lowering leaf osmotic potential for turgor preservation (Sánchez-Blanco *et al*., 2004). AMF could improve plants by stimulating the production of growth regulating substances, improving photosynthesis, water use efficiency, ameliorating osmotic adjustment under salinity stress (Augé *et al*., 2015).

Nanoparticles (NPs) are microscopic particles with at least one dimension less than 1000 nm. Nano-particles are found to be very suitable in sensing and detection of biological structures and systems (Singh *et al*., 2008). Many researchers have suggested that, NPs have both positive and negative effects on plant growth and development, and the effect of nano-particles on plants depends on the composition, concentration, size, surface covering, reactivity and physical and chemical properties of NPs as well as differ from plant species. Nano-particles react with plants causing some morphological and physiological alters, depending on the properties of NPs (Khodakovskaya *et al*. 2012).

Nanotechnology is one of the options to enhance the nutritional values of crops as some engineered nanoparticles (NPs) could be used as a fertilizer. Zinc can be used in the form of zinc oxide (ZnO) NPs. Zinc is an important element essential for growth and development of plants. Abdel Latef *et al*. (2017) observed that, seed-priming with ZnNPs positively impacts the growth characters on lupine plant exposed to NaCl stress. Zn plays a essential role in building natural auxin (IAA) and consequently activating cell division and enlargement (Ali and Mahmoud, 2013), enhancement in protein synthesis (Ebrahimian and Bybordi 2011), scavenging free oxygen radicals, reducing the uptake of Na^+ and Cl^- and translocation of nutrients from the aged cells to newborn cells (Jiang *et al*., 2014). Zinc appears to influence capability for water uptake and transport in plants and decrease the negative influences of salt stress (Tavallali *et al*., 2010).

The aims of this work were: a) to investigate the effect of *Arbuscular mycorrhizal* addition to the soil and primed seeds with nano-Zinc oxide or bulk-Zinc oxide (0, 5 and 10 mg/l) on growth, yield and nutritional value, b) to find out

the best concentration of ZnO application for sustainable yield of wheat plants (*Triticum aestivum* L.) Gemmeiza 10 cultivar grown under saline conditions.

Materials and Methods

The field experiment was carried out at the experimental station of the Faculty of Agriculture, Cairo University, Wadi El-Natrun district El-Behera Governorate, Egypt, during the two winter seasons of 2016/2017and 2017/2018. Grains of wheat cultivar (Gemmeiza 10) were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. *Arbuscular mycorrhiza* fungus was obtained from the Microbiology Department, NRC. The soil texture of the experimental site was sandy. Soil analysis was performed according to the method described by Chapman and Pratt (1978). , some physical and chemical properties of a representative soil sample were listed in Table (1)**.** Analysis of irrigation water was presented in Table (2).

Table 1: Physical and chemical analyses of the experimental site soil in 2016/2017 and 2017/2018 seasons

Soil analysis	2016/2017	2017/2018
Physical properties		
Sand (%)	94.15	92.27
Silt (%)	4.35	5.20
Clay (%)	1.50	2.53
Texture class	Sandy loam	Sandy loam
Chemical properties		
$pH_{(1:1)}$	7.43	7.29
$Ec_{(1:1)}$ (dS m^{-1})	5.54	5.22
Organic matter (%)	0.51	0.62
Total Ca Co_3 (%)	3.74	5.91
Available N (mg kg^{-1})	6.4	8.9
Available P (mg kg^{-1})	1.65	2.04
Available K (mg kg^{-1})	168	187

Table 2 : Chemical analyses of irrigation water

	pH	EC		Ions concentration meq L^{-1}						
Season		**ds m^{-1}**	**ppm**	**HCO_3^-**	**CL^-**	**SO_4^-**	**Ca^{++}**	**Mg^{++}**	**Na^+**	**K^+**
2016/2017	7.7	4.1	2624	2.8	30.5	9.0	3.9	4.3	33.3	0.64
2017/2018	7.5	4.2	2688	3.2	29.1	7.9	5.3	4.6	32.5	0.55

The experimental design was split plot with four replication, where AM occupied the main plots and treatment of primed seeds of wheat (Gemmeiza 10) cultivar with ZnO (NPs) or ZnO (BPs) (0, 5 and 10 mg/l) were allocated at random to sub-plots.

Wheat (*Triticum aestivum* L.) seeds were sown at the end of November in both season in rows, 4 meters long, the distance between rows was 25 cm, plot area was 12 m (3.0 m in width and 4.0 m in length). The recommended agricultural practices of growing wheat seeds were applied; the seeding rate was (60 k seeds/fed). Pre-sowing, 150 kg/fed. of Calcium Super-phosphate (15.5 % P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of 75 k/fed. was applied at five equal doses before the 1st, 2nd ,3rd, 4th and 5th irrigation. Potassium sulfate (48.52 % K_2O) was added at two equal doses of 50 kg/fed, before the 1st and 3rd irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Plant samples were taken after 75 days from sowing for measurements of growth characters (shoot length, number of leaves /tiller, fresh and dry weight of tiller) and photosynthetic pigments. At harvest the following characters were recorded on random samples of 10 guarded plants in each plot to estimate the following characters: Plant height (cm), 1000 grains weight (g), Grain yield / spike (g), Straw yield, Biological yield (ton/fed) and Grain yield (ardab/fed). Some chemical parameters were measured on the harvested grains as proteins%, carbohydrates%, β- Carotene, lycopene flavonoids, antioxidant activity and some macro-elements (N, P, K and Ca).

Chemical Analysis

Photosynthetic Pigments

Photosynthetic pigments such as total chlorophyll a and b and carotenoid contents in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue was extracted in 80% acetone. The optical density (OD) of the solution was recorded at 662, 645 and 470 nm for chlorophyll a, b and carotenoids using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

Total carbohydrate

Determination of total carbohydrates was carried out according to Herbert *et al*. (1971). A known mass (0.2–0.5 g) of dried tissue was added in 10 ml of sulphuric acid (1 N). The tube was sealed and placed overnight in an oven at 100 °C. The total sugars were determined calorimetrically according to the method of Smith *et al*. (1956) as follows: An aliquot of 1 ml of sugar solution was mixed with 1 ml of 5% aqueous phenol solution followed by 5.0 ml of concentrated sulphuric acid. The tubes were thoroughly shaken then placed in a water bath at 23–30 °C for 20 min. The optical density of the developed color was measured at 490 nm using Shimadzu spectrophotometer.

Estimation of Total Nitrogen

Total N was determined by using micro-Kjeldahl method as described in AOAC (1970).

Determination of β- carotene and lycopene

β-Carotene and Lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100mg) was vigorously shaken with 10ml of acetone – hexane mixture (4:6) for 1min. The absorbance of the filtrate was measured at λ = 453, 505, 645 and 663 nm. Contents of β- Carotene and Lycopene were calculated according to the following equations: lycopene (mg/100ml) = - 0.0458A663 + 0.372A505 + 0.0806A453: β-Carotene (mg/100ml) = 0.216A663 – 0.304A505 + 0.452A453. The values ere expressed as µg/g of extract.

Estimation of flavonoids

The total flavonoids content were determined following the spectrophotometric method of Dewanto *et al*. (2002).

Radical Scavenging Capacity Assay (Antioxidant Activity)

The free radical scavenging activity (antioxidant activity) was determined according to (Liyana-Pathiranan and Shahidi, (2005) using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent. 1ml of methanolic solution of the extract was added to 4ml of 0.1mmoll-1 methanolic solution of DPPH. After 30 min incubation in the dark at room temperature, the absorbance was read against a blank at 517nm.

Determination of Total Phosphorus

Phosphorus was determined in the digested solution by the method described by Chapman and Pratt (1978). Vanadate molybdate and orthophosphates react to give a yellow complex in acid solutions. The acid concentration must be above 0.2 N and not over 1.6 N, the final concentration of 0.5 N is recommended, five ml of 5 N nitric acid per 50 ml of final volume are sufficient to give optimum acidity. After 10 minutes read at 436 nm against blank by using Spekol Spectrocolourimeter VEB Carl Zeiss. Blank: Colour reagent filled to 50 ml with distilled water.

Determination of Elements

Samples were dried in an oven at 80°C until constant weight. The dried matter was digested in a mixture of concentrated nitric acid and perchloric acid (4: 1 v / v). Made up to volume with deionized distilled water according to the method of Chapman and Pratt (1978) with certain modifications. The concentration of potassium, sodium and calcium in the digested material were determined by flame photometer (B 700-E).

Statistical Analysis

The data were statistically analyzed as split plot design system according to Snedecor and Cochran (1980). Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability.

Results

The effect of *Arbuscular mycorrhiza*

Growth parameters

Data in (Table 3) show that, wheat plant cultivated in the soil amended with AM led to a significant increase in the growth parameters plant height, leaves no /tiller, tiller fresh weight, tiller dry weight) when compared with plants cultivated without AM.

Photosynthetic Pigments

Photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) of wheat leaves cultivated in soils amended with AM are shown in Table (3). Wheat plant cultivated in the soil amended with AM led to significant increases in photosynthetic pigments as compared to plants grown in the absence of AM. The percentage of increases in response to presence of AM reached to 19.6, 21.7 and 21.4 for chlorophyll a, chlorophyll b and carotenoids as compared to the absence of AM, respectively.

Yield Components

Yield and yield components of the wheat variety (Gemmeiza 10) cultivated in the absence and presence of *Arbuscular mycorrhiza* (AM) are shown in Table (3). Wheat plant cultivated in the soil amended with AM led to significant increases in the yield parameters, plant height, tiller dry weight, spike length, spike weight, 1000 grains weight, straw yield/ha, biological yield and grain yield/ha when compared with plants cultivated without AM. The percentage of increase in response to presence of AM reached to 7.3, 5.9, 21, 12.0 and 15.5 for 1000 grains weight, harvest index, grain yield/ha, straw yield/ha and biological yield as compared to the absence of AM, respectively. *Arbuscular mycorrhiza* addition to soil caused significant increases in carbohydrates %, protein %, Lycopene, B-carotine, flavonoids and antioxidant activity and mineral contents (N, P, K, K/Na and Ca) in yielded grains. As the percentage of increases in mineral contents in response to presence of AM reached to 8.3, 12.5, 5.1 and 10.0 for N, P, K and Ca, respectively as compared with absence of AM. On the other hand, addition of AM to the soil decreased significantly Na content.

The effect of nano-ZnO and bulk -ZnO.

Growth Parameters

Data in (Table 4) show that, soaking wheat cultivar with nano-ZnO or bulk ZnO at (5mg/l and 10mg/l) stimulated the all growth parameters (plant height, leaves no /tiller, tiller fresh weight, tiller dry weight) as compared with the corresponding controls (Table 4).

Photosynthetic Pigments

Pre-soaking of wheat grains in nano-ZnO or bulk-ZnO at (5mg/l and 10mg/l) significantly increased photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) as compared with the corresponding controls (Table 4). More pronounced increase in chlorophyll a, chlorophyll b and total pigments was obtained with plants treated with bulk-ZnO (10 mg/l) followed by nano-ZnO (5mg/l). It is noticed that, the highest carotenoid contents was found at concentration of (5 mg/l) nano- ZnO ().

Yield and Yield components

Data in table (4) revealed, that soaking wheat seeds with nano-ZnO or bulk ZnO at (5mg/l and 10mg/l) stimulated all yield parameters (plant height, tiller dry weight, spike length, spike weight, 1000 grains weight (g), straw yield/ha, biological yield and grain yield/ha as compared with the corresponding controls. More pronounced

increase in yield parameters was obtained with plants treated with nano-ZnO (10 mg/l). The percentage of increase in response to nano-ZnO (10 mg/l) reached 16.6, 17.7, 5.3, 18.9, and 29.9, for 1000 grains weight, harvest index, grain yield, straw yield/ha and Biological yield/ha as compared to the corresponding controls, respectively.

In general, data also showed significant increases in carbohydrates %, protein %, B-carotine, and flavonoids in plants treated with nano-ZnO and bulk ZnO. Meanwhile, lycopene contents significantly decreased, when it was applied with bulk ZnO at both concentrations and antioxidant activities decreased, when grains were primed with nano-ZnO at both concentrations as compared to the control. It is noticed that, the maximum increases in flavonoids and antioxidant activities were reached when using the treatment of bulk ZnO (5 mg/l). The percentage of increases in flavonoids and antioxidant activities in response to bulk ZnO (5 mg/l) reached to 26% and 13% respectively as compared to the corresponding controls.

In response to mineral contents in harvested grains of wheat soaking with nano-ZnO and bulk ZnO significantly increased N%, P%, K%, K/Na ratio and Ca% as compared to the corresponding controls. More pronounced increase in N, K, Ca and K/Na was obtained with plants treated with nano-ZnO (10 mg/l) over all other treatments. The percentage of increases in response to nano-ZnO (10 mg/l) reached to 14, 12, 9, 17 and 13 for N, P, K, K/Na and Ca as compared to the controls, respectively. On the other hand, Na content decreased significantly by applying nano-ZnO and bulk ZnO on wheat plant as compared with the control. Maximum decrease in Na% reached 6% by treatment of nano-ZnO (10 mg/l) as compared to the control.

The interaction effect between nano-ZnO or bulk-ZnO and *Arbuscular Mycorrhiza.*

Growth parameters

Data in Table (5) show the interaction effect of nano-ZnO or bulk -ZnO in presence of AM stimulated the all growth parameters (plant height, leaves no /tiller, tiller fresh weight, tiller dry weight) as compared with the corresponding controls. More pronounced increase in growth parameters was obtained with plants treated with nano-ZnO (10 mg/l) and bulk -ZnO (10 mg/l) in soil amended with AM over all other treatments.

Photosynthetic Pigments

Pre-soaking of wheat grains in nano-ZnO or bulk-ZnO at (5mg/l and 10mg/l) in the presence and absence of AM significantly increased photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) as compared with the corresponding controls (Table 5). Mean comparisons showed that, the highest chlorophyll a, chlorophyll b and total pigments was found at concentration (10 mg/l) bulk-ZnO followed by (5mg/l) nano-ZnO in the presence of AM compared to the control and other treatments. It is also noticed that, the highest carotenoid contents was found at concentration of nano- ZnO (5 mg/l) in the presence of AM.

Yield Components

The interaction effect of nano-ZnO or bulk –ZnO in presence and absence of AM on yield and yield components are represented in (Table 5). Soaking wheat cultivar with nano-ZnO and bulk –ZnO stimulated the all yield parameters (plant height, tiller dry weight, spike length, spike weight, 1000 grains weight (g), straw yield/ha, biological yield/ha and grain yield/ha as compared with the corresponding controls in the presence or absence of AM. Data also revealed that, AM treatment of soil and soaking wheat cultivar with nano-ZnO and bulk –ZnO increased significantly all yield components as compared to the absence of AM. More pronounced increase in yield parameters was obtained with plants treated with nano-ZnO (10 mg/l) followed by bulk -ZnO (10 mg/l) in soil amended with AM over all other treatments. The percentage of increases in response to nano-ZnO) (10 mg/l) reached to 24.4, 29.7, 91.3, 22.2, 47.4, for 1000 grains weight, harvest index, grain yield, straw yield and biological yield as compared to the controls, respectively.

Data in table (5) showed that, wheat plants sowing in the soil amended with AM and different treatments with nano-ZnO and bulk -ZnO significantly increased carbohydrates and protein % on wheat harvested grains when compared with controls. Application of nano-ZnO (10mg/l) in the soil amended with AM was the most effective treatment as it improved carbohydrates by 19.5% and 13.84%, and protein by 31.1% and 22.3% as compared to control without AM and control amended with AM, respectively.

Application of *Arbuscular mycorrhiza* and /or nano-ZnO and bulk-ZnO resulted in a significant increase in lycopene and β-carotene, flavonoids and antioxidant in wheat harvested grains when compared to the controls (Table 5). More pronounced increase in Lycopene and B-carotine was obtained with plants treated with nano-ZnO (10 mg/l) in soil amended with AM over the all other treatments. Meanwhile, the most pronounced increase in flavonoids and antioxidant activity was observed in ZnO BPs (5mg/l) in the soil amended with AM.

Arbuscular mycorrhiza and/or nano-ZnO and bulk-ZnO increased the macro element contents (N, P, K, Ca and K/Na) of harvested grain as compared to the controls (Table 5). On the other hand, sodium (Na) content significantly decreased with all treatments used in the presence and absence of AM. Data also revealed that, AM treatment of soil and soaking wheat seeds with nano-ZnO and bulk-ZnO significantly increased (N, P, K, Ca and K/Na) as compared to the absence of AM. More pronounced increase in N, K, Ca and K/Na was obtained by plants treated with nano-ZnO (10 mg/l) in soil amended with AM over the all other treatments. The percentage of increases in response to nano-ZnO (10 mg/l) reached 31, 14, and 23, for N, K and Ca as compared to the controls, respectively. Meanwhile, the most pronounced increase in P% was observed in bulk ZnO (10mg/l) in the soil amended with AM by 34%.

Table 3 : Effect of absence (-ve) or presence (+ve) of *Arbuscular Mycorrhiza* on morphological characters, photosynthetic pigments, yield, yield components and the nutritional values of the wheat cultivar Gemmeiza 10 in saline soil, (Mean of two seasons).

Character	*Arbuscular Mycorrhiza*		LSD
	-ve	+ve	5%
Shoot length (cm)	43.02	46.61	0.12
leaves no /tiller	5.67	6.10	0.07
Fresh weight of tiller (g)	3.76	4.25	0.08
Dry weight of tiller (g)	1.33	1.51	0.05
Chlorophyll a (mg/g fresh weight)	15.92	19.04	0.12
Chlorophyll b (mg/g fresh weight)	4.24	5.16	0.08
Carotenoids (mg/g fresh weight)	5.76	6.99	0.08
Total pigments (mg/g fresh weight)	26.63	31.34	0.11
Plant height (cm)	54.15	58.49	0.50
Tiller dry weight (g)	1.32	1.44	0.02
Spike length (cm)	12.87	13.38	0.17
Spike weight (g)	1.56	1.75	0.02
1000 grains weight (g)	32.95	35.37	0.31
harvest index%	34.95	37.02	0.95
Grain yield/ ha (ton)	3.15	3.84	0.09
Straw yield /ha (ton)	5.81	6.51	0.09
Biological yield /ha (ton)	8.96	10.35	0.09
Carbohydrate%	48.15	51.01	0.31
Protein %	12.60	13.63	0.33
Lycopene (µg/g dry weight)	0.35	0.38	0.008
B-Carotene (µg/g dry weight)	0.40	0.42	0.005
Flavonoids (µg/g dry weight)	27.17	29.02	0.41
DPPH % (antioxidant activity)	51.09	54.79	0.37
N (mg/100g dry weight)	2014	2181	16.83
P (mg/100g dry weight)	1986	2235	35.16
K (mg/100g dry weight)	3245	3410	29.18
Na (mg/100g dry weight)	717	711	2.71
K/Na	4.58	4.76	0.04
Ca (mg/100g dry weight)	1111	1222	20.55

Table 4: Effect of nano-ZnO and bulk-ZnO (5 mg/l and 10 mg/l) on morphological characters, photosynthetic pigments, yield, yield components and the nutritional values of the wheat cultivar (Gemmeiza 10) in saline soil, (Mean of two seasons).

Character	control	nano-ZnO 5mg	nano-ZnO 10m	ZnO 5mg/l	ZnO 10mg/	LSD 0.05
Shoot length (cm)	43.57	45.08	45.08	44.00	46.35	0.20
leaves no /tiller	5.42	5.96	6.09	6.00	5.97	0.10
Fresh weight of tiller (g)	3.20	4.38	4.24	3.92	4.29	0.13
Dry weight of tiller (g)	1.18	1.54	1.44	1.45	1.50	0.08
Chlorophyll a (mg/g fresh weight)	15.12	18.03	16.79	17.08	20.39	0.19
Chlorophyll b (mg/g fresh weight)	3.10	5.61	4.26	4.95	5.58	0.13
Carotenoids (mg/g fresh weight)	5.57	7.00	6.16	6.30	6.85	0.12
Total pigments (mg/g fresh weight)	23.79	30.61	27.43	28.82	33.49	0.17
Plant height (cm)	51.34	57.47	58.92	57.55	56.33	0.79
Tiller dry weight (g)	1.13	1.33	1.56	1.51	1.39	0.04
Spike length (cm)	12.05	13.58	13.67	13.33	12.98	0.27
Spike weight (g)	1.36	1.76	1.85	1.62	1.68	0.04
1000 grains weight	31.39	35.21	36.59	34.29	33.32	0.49
harvest index%	32.20	36.82	37.90	35.66	37.35	1.50
Grain yield/ha(ton)	2.60	3. 55	3. 97	3.58	3. 78	0.14
Straw yield/ha(ton)	5.45	6. 06	6. 48	6.45	6.35	0.15
Biological yield/ha(ton)	8.05	9. 61	10. 45	10.03	10.13	0.14
Carbohydrate%	45.77	49.51	50.90	50.23	51.51	0.49
Protein %	11.55	13.23	14.10	12.78	13.91	0.53
Lycopene (µg/g dry weight)	0.37	0.37	0.39	0.34	0.34	0.01
B-Carotene (µg/g dry weight)	0.38	0.43	0.43	0.40	0.41	0.01
Flavonoids (µg/g dry weight)	25.50	26.19	27.84	32.14	28.81	0.64

DPPH % (antioxidant activity)	50.70	50.62	49.79	57.54	56.07	0.58
N (mg/100g dry weight)	1847	2117	2255	2045	2223	26.61
P (mg/100g dry weight)	1958	2067	2198	2045	2286	55.60
K (mg/100g dry weight)	3204	3343	3480	3249	3361	46.14
Na (mg/100g dry weight)	743	713	695	719	701	4.29
K/Na	4.30	4.69	5.03	4.53	4.80	0.07
Ca (mg/100g dry weight)	1095	1155	1235	1146	1203	32.50

Table 5 : Effect of nano-ZnO and bulk-ZnO (5 mg/l and 10 mg/l) in absence (-ve) or presence (+ve) of *Arbuscular mycorrhiza* on yield, yield components and the nutritional values of the wheat cultivar (Gemmeiza 10) in saline soil, (mean of two season).

Characters	**control**		**nano-ZnO 5mg/l**		**nano-ZnO 10mg/l**		**ZnO 5mg/l**		**ZnO 10mg/l**		**LSD 0.05**
Arbuscular Mycorrhiza	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	
Shoot length (cm)	41.73	45.42	44.17	46.00	42.17	48.00	42.58	45.42	44.48	48.21	0.30
leaves no /tiller	5.17	5.67	5.92	6.00	5.75	6.42	5.84	6.17	5.67	6.27	0.17
Fresh weight of tiller (g)	3.29	4.45	3.96	4.48	4.03	4.46	3.58	4.25	3.95	4.63	0.17
Dry weight of tiller (g)	1.16	1.63	1.31	1.45	1.34	1.54	1.40	1.49	1.34	1.67	0.12
Chlorophyll a (mg/g fresh weight)	13.67	16.57	15.49	19.67	16.39	18.10	15.91	18.24	18.16	22.62	0.24
Chlorophyll b (mg/g fresh weight)	3.18	4.05	3.96	5.46	4.75	4.57	4.35	5.55	4.96	6.20	0.17
Carotenoids (mg/g fresh weight)	4.86	6.27	5.72	7.70	6.30	6.59	5.60	6.99	6.34	7.37	0.17
Total pigments (mg/g fresh weight)	22.55	26.89	25.60	33.33	27.63	29.26	26.60	31.04	30.79	36.19	0.24
Plant height (cm)	48.88	53.81	55.44	59.50	57.46	60.38	55.04	60.06	53.96	58.71	1.11
Tiller dry weight (g)	1.09	1.17	1.28	1.39	1.48	1.65	1.45	1.57	1.33	1.44	0.05
Spike length (cm)	11.72	12.38	13.32	13.85	13.30	14.05	13.12	13.54	12.88	13.08	ns
Spike weight (g)	1.28	1.44	1.66	1.87	1.68	2.01	1.57	1.68	1.61	1.75	0.05
1000 grains weight	30.30	32.48	33.80	36.62	35.49	37.69	33.08	35.51	32.08	34.55	2.34
harvest index%	30.20	34.20	35.48	38.16	36.63	39.17	35.06	36.26	37.39	37.30	2.12
Grain yield/ha(ton)	2.29	2.91	3.16	3.93	3.56	4.38	3.24	3.93	3.50	4.07	0.17
Straw yield/ha(ton)	5.29	5.61	5.74	6.37	6.15	6.80	6.00	6.90	5.86	6.84	0.17
Biological yield/ha(ton)	7.58	8.52	8.90	10.30	9.71	11.18	9.24	10.83	9.39	10.91	0.17
Carbohydrate%	44.66	46.88	48.41	50.61	48.43	53.37	48.81	51.64	50.47	52.55	0.69
Protein %	11.14	11.95	12.64	13.82	13.58	14.61	12.15	13.42	13.49	14.34	0.75
Lycopene (µg/g dry weight)	0.35	0.39	0.35	0.39	0.36	0.42	0.33	0.36	0.33	0.35	ns
B-Carotene (µg/g dry weight)	0.40	0.37	0.41	0.45	0.42	0.45	0.39	0.41	0.39	0.44	0.01
Flavonoids (µg/g dry weight)	25.94	25.06	23.28	29.11	27.47	28.20	29.76	34.52	29.42	28.21	0.91
DPPH % (antioxidant activity)	49.50	51.90	50.32	50.92	45.09	54.48	56.14	58.93	54.42	57.72	0.82
N (mg/100g dry weight)	1782	1911	2022	2213	2127	2336	1942	2147	2151	2239	16.58
P (mg/100g dry weight)	1877	2038	1958	2175	2104	2291	1937	2153	2053	2519	78.63
K (mg/100g dry weight)	3096	3311	3257	3429	3431	3529	3155	3343	3286	3436	65.25
Na (mg/100g dry weight)	760	725	711	715	689	701	710	727	688	715	6.07
K/Na	4.08	4.52	4.58	4.81	5.00	5.05	4.46	4.61	4.78	4.82	0.09
Ca (mg/100g dry weight)	1042	1148	1089	1221	1186	1283	1087	1206	1153	1252	42.64

Discussion

The effect of *Arbuscular mycorrhiza*

Plants and AM react natural, as the plant gets mineral nutrients and water via the AM; the AM is provided with carbohydrates through its host (Smith and Read, 2008). Evelin *et al*. (2009) reported that AM lead to crop improvement such as growth rate, biomass, and mineral uptake under saline or drought stresses**.** AM were shown to have useful impacts in overcoming with toxic influences caused via soil salinity through preserving an physiological balance (Shokri and Maadi, 2009). This has been attributed to a more efficient nutrient uptake, particularly phosphorus by AM fungus (Al-Karaki, 2004).

AM was amended to the saline soil enhanced significantly the growth characters of wheat plant when compared with plants cultivated without AM (Table 3). These results are in agreement with those obtained by Abdallah *et al*. (2013) on sunflower plant. These increments in the growth characters can be resulted from the impacts of AM on absorbing different nutriments like nitrogen, calcium, potassium, copper, zinc, and especially phosphorus nutrition on wheat plant (Abdallah *et al*. (2015). The improved growth, in wheat plant resulted from the potential of AM inoculation to decrease the impacts of salinity stress on wheat plant. Similar results were obtained in *Cucurbita pepo* plants grown under salinity stress (Colla *et al*., 2008).

The results in (Table 3) illustrated that, wheat plant cultivated in the soil amended with AM led to significant increases in photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) as compared to plants grown in the absence of AM. In this connection, Abdallah *et al*. (2013 and 2015) reported that, treatment of sunflower and wheat plants with AM resulted in increasing photosynthetic pigments. Augé, (2001) recommended that AM enhanced the photosynthesis, and so as to increment the rates of photosynthetic storage and export at the same time**.** Moreover, in AM inoculated plants, the chlorophyll activity is restored

because of occurrence of specific enzymes necessary for its biosynthesis (Hajbagheri and Enteshari 2011). Increasing chlorophyll activity in AM-inoculated plants may be due to AM reduces Na level under salt stress. AM symbiosis enhanced the photosynthesis rate under salt stress in garlic plants (Borde *et al.,* 2010).

Addition of AM to the saline soil increased significantly yield and yield components in wheat plants (Table 3). Similar results were obtained in different plant species in response to AM application (Abdallah *et al.,* 2013; Heidari and Karami 2014). They found that, AM improved growth, yield, seeds quality and quantity of various plants under water stress. This increase in yield components may be due to the impacts of AM on absorbing different nutriments such as N, Ca, K, Cu, Zn, S and P nutrition (Sharifi *et al*., 2007). The inculcation of soil with AM efficiently improved the total carbohydrate and protein percentage of harvested wheat grains (Abdallah *et al.* 2013 and 2015) in sunflower and wheat plants grown under water stress.

Data presented in table (3) indicated that in presence of AM caused a significant and pronounced increase in total flavonoids and antioxidant activity of wheat grain as compared with control plants. The importance of these compounds were known to possess antioxidant activities and were utilized as natural plant protectants from a biotic stress. Nakabayashi *et al*. (2014) found that, flavonoids, with strong radical scavenging activity contributed to the mitigation of oxidative and drought stress in *Arabidopsis thaliana.* Dudhane *et al*. (2011) demonstrated that there was an improved growth and antioxidant activities in *Gmelina arborea* when inoculated with *Glomus fasciculatum*.

Arbuscular mycorrhiza increased the macro element contents (N, P, K and Ca) of harvested grain (Table 3). Application of AM can assist in improved assimilation of (N) in the host plant. Furthermore, co-inoculated plants with AM led to increase in nutrients uptake when compared to non-inoculated plants. The high nutrients uptake may be due to N metabolism brought concerning through alterations in the enzymes associated with N metabolism. Also, the nutrients uptake increases via the extended hyphae of the fungus which lets them to explore more soil volume (Shokri and Maadi 2009). AM showed that, it have a positive effect on the composition of mineral nutrients (especially poor mobility nutrients like phosphorus) of plants (Al-Karaki *et al*., 2004) via increasing and/or selective uptake of nutrients. Giri *et al*. (2007) demonstrated that, improvement in phosphorus plant is an important mechanism of salinity stress tolerance in mycorrhizal plants. Lee and George (2005) found that cucumber plants inoculated with mycorrhiza induced a significant increased in the uptake of phosphorus and Zinc. Al-Karaki (2017) observed that**,** pre-inoculation of green pepper transplants with AM fungi improved P and K uptake grown under non saline and saline conditions.

Mycorrhizal colonization of a plant can reverse the effect of salinity on K and Na nutrition. AM can improve K absorption under saline conditions (Zuccarini and Okurowska, 2008). Giri *et al*. (2007) found that AM plays a means role in higher K^+ accumulation and consequently higher K/Na ratio in mycorrhizal plants. AM spread the mycelium deep into the soil and absorb water and minerals for the host plant thus raised the efficiency of the plants to resist the NaCl stress. Na concentration was lower in AM than non AM plants grown under nonsaline and saline conditions (Abdel Latef and Chaoxing 2011).

Arbuscular mycorrhiza addition to soil caused significant increases in calcium in harvested grains. In this connection, Wu *et al*. (2010) stated that, mycorrhizal fungi enhanced Ca accumulation under water stress. Mycorrhizal inoculated of wheat plants were capable to keep a higher osmotic potential of cells leading to the significantly fast growth, improved N, P, K, Ca, total carbohydrates and chlorophyll contents in leaves (Soliman *et al*., 2012).

Effect of nano-ZnO and bulk-ZnO

Zinc is a necessary micronutrient needed for growth and development of plants (Pathak *et al*., 2012). Seed-priming with nano-ZnO and bulk-ZnO had a significant effect on growth under salinity stress as compared with the untreated plants (Table 4). The positive effect of Zinc oxide on growth may be due to Zn is necessary for the synthesis of tryptophan which is a precursor of IAA production and consequently activating cell division, enlargement and regulation of plant growth (Ali and Mahmoud 2013). Zinc is the important key micronutrient required for the optimum growth and development of plants which carries vital metabolic reactions (Mahajan *et al*., 2011).

Pre-soaking of wheat seeds in nano-ZnO or bulk-ZnO (5 and 10 mg/l) significantly increased of photosynthetic pigments (Table 4). More pronounced increase in chlorophyll a, chlorophyll b and total pigments was obtained with plants treated with bulk-ZnO (10 mg/l) followed by nano-ZnO (5mg/l). In this connection, Mohsenzadeh and Moosavian (2017) reported that low concentration of zinc (2 and 4g/l) and nano-zinc oxide (1 and 4 g/l) significantly increased chlorophyll and carotenoid compared to the control. While, the high concentration of nano-zinc oxide (7g/l), decreased chlorophyll and carotenoid contents as compared to the control. Moreover, seed priming of nano-ZnO linearly increased the growth characteristics, photosynthesis and biomass of wheat (Munira *et al*., 2018). Racuciu and Reanga (2006) showed that nanoparticles in low amount (50-10 ml) increase levels of chlorophyll a, as main pigments up to 13%. Zinc plays an important role in the formation of chlorophyll, protein, lipid, carbohydrate, a cofactor in enzymes (DNA and RNA polymerase) and hormones' actions synthesis (Ebrahimian and Bybordi 2011). Zinc by protection of sulfhydryl groups caused synthesized chlorophyll (Cakmak, 2000). Lebedev and Timco, (1998) observed that, Zinc is necessary for the activity of enzymes that are participatory in chlorophyll biosynthesis. Generally, metallic nanoparticles are strong influence of photosynthetic efficiency and increased the absorption of light by chlorophyll (Nadtochenko *et al*. 2008).

In this study, the highest carotenoids content was found at concentration of nano-ZnO (5 mg/l). Carotenoids are antioxidant compounds soluble in plant cells. These compounds reduced oxidative damage to the plant. Carotenoids are responsible protecting photosynthetic tissues, especially chlorophyll. It seems that certain amount of zinc induce oxidative stress and causes synthesis of carotenoids.

Data in table (4) showed that, the nano-ZnO caused greater increases in yield and yield components of wheat plant

than bulk- ZnO. In this connection, Torabian *et al*. (2016) reported that, the nano- ZnO compared to bulk-ZnO form have greater effect on biomass production of sunflower plants. Rockenfeller and Madeo (2008) stated that, zinc participates in the translocation of nutrients from the aged cells to newborn cells. Sarwar (2011) observed that, decreasing zinc availability resulted in the reduction in crop yields and qualities in rice (*Oryza sativa* L.). Khan *et al*. (2010) found that application of zinc increased grain and straw yield significantly in wheat.

In general, data also showed that, significant increases in carbohydrates % and protein % of harvested wheat grains with plants treated with nano-ZnO and bulk-ZnO. Zinc is one of the most important elements in the carbohydrates metabolism, most enzymes that play a role in carbohydrates metabolism are activated by zinc (Taheri *el al*., 2011). Also, Zinc is essential for proteins production in plants; also zinc is main composition of ribosome and is essential for their development. Pandey *et al*. (2006) found that, amino acids accumulated in plant tissues and protein synthesis improved by zinc sufficient. One of the sites of protein synthesis is pollen tube that amount of zinc in there tip is 150 micrograms per gram of dry matter. In general zinc have main role in synthesis of proteins, enzyme activating, oxidation and revival reactions and metabolism of carbohydrates (Mousavi *et al*., 2007). It can be concluded that the simulative effect of Zinc is increasing the biosynthesis of free amino acids and their incorporation into protein (Table 4) (Bassouny *et al*., 2008).

Furthermore, nano-ZnO and bulk ZnO generally stimulated the accumulation of total nitrogen, phosphorus, potassium and calcium contents in wheat plant. The increase in K^+ contents at low and high concentration of Zinc Oxide is predictable to be affected through the effect of nitrogen compounds on protein synthesis, because proteins are necessary to transport protons, inorganic ions and organic solutes across the plasma membrane and tonoplast at rates sufficient to meet the needs of the cells (Schroeder *et al*., 1999). Positively charged macronutrients such as potassium (K^+) are required in relatively large amount for plant growth and development. Thus, the above mentioned results are consistent with the results of growth parameters and also with pigments (Table 4).

Rezaei and Abbasi (2014) Zinc alleviated salinity stress by decreasing the uptake of excess of Na^+ and Cl^- on cotton (*Gossypium hirsutum* L.) Alharby *et al*. (2016) found that, the treatment of ZnO- nanoparticles under salinity stress led to significant decreases in the Na^+ content, in calli tissue of tomato varieties.

Data obtained revealed that soaking wheat cultivar with bulk-ZnO at (5mg/l and 10mg/l) stimulated the flavonoids content and antioxidant activity as compared to the controls and nano-ZnO. The results of antioxidant activity are efficiently in harmony with flavonoids. Thus, the relationship between flavonoids compounds and antioxidant capacity, in plant tissues has a close relationship with concentration of protective compounds like anthocyanin, flavonoids and total phenols (Mohsenzadeh and Moosavian, 2017).

ZnO nanoparticles with stimulating plant protected system enhanced free radical scavenging capacity and thus had a positive influence on wheat grown in salty soil. So, the utilize of nano-ZnO, with a concomitant enhancing in lycopene, B-carotene and flavonoids on harvested wheat grains.

Conclusions

Salinity stress harmfully affects growth, yield and some physiological parameters. *Arbuscular mycorrhizal* fungus (AMF) alleviate detrimental effects of salinity on growth, improve nutrition concentrations in grains (carbohydrates %, protein%, N, P, K and Ca and lower Na) and alleviate salinity impacts on cell membrane stability, by increasing lycopene, B-carotene, flavonoids and antioxidant activity at saline conditions. So, utilize of AM supply a sustainable and environmentally safe treatment to ameliorate salinity tolerance. According to the analysis performed and the comparison it is observed that the nano zinc oxide is better than bulk-zinc oxide. It is deduce from the results of the present study that grains treatment with ZnO nanoparticles, reduced the negative effects of salinity on wheat plants through promoting photosynthetic pigments. Further, protection under salinity stress was achieved via ameliorated lycopene, B-carotene, flavonoids and antioxidant. In conclusion, application of zinc oxide nanoparticles and soil amended with *Arbuscular mycorrhiza* could be a strategy to alleviate the growth, economic yield and the deleterious effects of salinity stress in wheat plants.

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