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Effects of Mycorrhizal Fungi and Nano Zinc Oxide on Seed Yield, Na^+ and K^+ Content of Wheat (*Triticum aestivum* L.) under Salinity Stress

Raouf Seyed Sharifi[1]*, Razieh Khalilzadeh[2], Soraya Soltanmoradi[3]

1- Professor, Faculty of Agricultural Sciences, University of Mohaghegh Ardabili, Ardabil, Iran.
2- Ph.D Student (Crop Physiology), University of Mohaghegh Ardabili, Ardabil, Iran.
3- M.Sc. Graduate (Agronomy), University of Mohaghegh Ardabili, Ardabil, Iran.

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ABSTRACT

This research was conducted to evaluate effects of mycorrhiza fungi and nano zinc oxide on agro physiological traits of wheat under salinity stress based on factorial experiment according complete randomized block design with three replications under greenhouse condition at 2014. Treatments included salinity in three levels [no-salt (S_0) or control, salinity 40 (S_1) and 80 (S_2) mM NaCl], two level of Arbuscular Mycorrhiza (AM) fungal [no application (M_0), application of mycorrhiza (M_1)] and Nano zinc oxide at three levels [(without nano zinc oxide as control (Zn_0), application of 0.4 (Zn_1) and 0.8 $g.lit^{-1}$) (Zn_2)]. Analysis of variance showed significant effect for the soil salinity on seed yield, chlorophyll index, relative water content, stomata conductance, K^+ content. chlorophyll index, stomata conductance, K^+ content in plant root were affected by AM fungi and nano zinc oxide application. There was a significant interaction between salinity, AM fungi and nano zinc oxide on Na^+ content, Na^+/K^+ ratio and seed yield. Salinity stress decreased seed yield, chlorophyll index, stomata conductance, and relative water content of wheat. The highest (0.44 g per plant) seed yield was obtained from plants under low salinity level, AMF (Arbuscular mycorrhiza fungal) and 0.8 $g.lit^{-1}$ nano zinc oxide. The nutrient uptake Na^+ and Na^+/K^+ ratio increased and potassium was decreased with increasing concentration of NaCl in the present study. However, the inoculated with AMF and application of nano zinc oxide significantly increased K^+ and reduced Na^+ uptake. Generally, it was concluded that AMF and nano zinc oxide can be as a proper tool for increasing wheat yield under salinity condition.

Keywords*:* *Chlorophyll, Relative water content, Stomata conductance.*

***Corresponding Author:** Raouf Seyed Sharifi ✉ raouf_ssharifi@yahoo.com

INTRODUCTION

Salinity stress is an important abiotic stress. Reducing the restrictive effects of salinity has positive influence on agriculture products (Huang *et al.*, 2009). Salinization of soil is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semiarid areas (Abdel Latef and Chaoxing, 2011). It adversely affects the growth of the most agricultural crops through its influence on certain aspects of plant metabolism such as osmotic adjustment (Bernstein, 1963), reduce the chlorophyll content, photosynthetic rate and the stomatal conductance, imbalance in the synthesis of endogenous plant growth regulators (PGR) (Iqbal and Ashraf, 2013), induce ion deficiencies, affect physiological processes such as membrane stability index and reduce relative water content (Sheng *et al.*, 2008; Talaat and Shawky, 2011). Ionic imbalance is considered as one of the main effect of salinity stress in many plants, the ionic balance has a key role in photosynthesis and other metabolic activities of the cell (Zheng *et al.*, 2008). Most plants in the salt environment accumulated a large number of Na^+ and simultaneously inhibited the K^+ absorption. Excessive accumulation of sodium in cell walls can rapidly lead to osmotic stress and cell death (Munns, 2002). Hu *et al.* (1997) believe that an increase in Na^+ and a decline in K^+ concentration by soil salinity are caused by the apparent antagonism between K^+ and Na^+. Several strategies have been developed in order to decrease the toxic effects caused by high salinity on plant growth. Tomar and Agarwal (2013) also demonstrated that maintaining higher K^+/Na^+ ratio is believed to be important strategy adopted by plants to mitigate stress-induced deleterious changes. The demand for nanotechnology based products has been increasing in recent years. Generally, nano materials refer to a colloidal particulate system, with size ranging from 10 to 1000 nm, possessing unique properties, such as size dependent qualities, high surface-to-volume ratio, and promising optical properties. It was possible that the nano materials aggregates were smaller than the pore size of the plant leaf cell, and thus some micro-sized aggregates pass through the plant cells (Al-Halafi, 2014). Among the micronutrients, Zn affects the susceptibility of plants via drought and salinity stresses (Sharma *et al.*, 2009). A number of researchers described the key role of ZnO nano materials for in crop growth (Wang *et al.*, 2004) involving processes of photosynthesis, nitrogen assimilation, respiration and activation of other biochemical and physiological processes and hence their importance in obtaining greater yields (Zozi *et al.*, 2012). Nano-particles with smaller particle size and large surface area are expected to be the ideal material for use as Zn fertilizer in plants. Application of micronutrient in the form of nanoparticles (NPs) is an important route to release required nutrients gradually and in a controlled way, which is essential to mitigate the problems of soil pollution caused by the excess use of chemical fertilizers. A number of researchers have reported the essentiality and role of zinc for plant growth and yield (Laware and Raskar, 2014). The symbiosis of plants and microorganisms plays an important role in sustainable agriculture and natural ecosystems. Interactions between plants and AM fungi results in disease and/or the mutualistic symbiosis (Garcia Garrido and Ocampo, 2002). Penetration to the root and the intracellular growth of the AM fungi involve complex sequences of biochemical and cytological events and intracellular modifications (Bonfante, 2001). It has been proven that AM fungi affect not only the plant growth but also contribute in plant tolerance to biotic and abiotic stresses (Auge, 2001). These fungi are obligatory symbiotic soil organisms that colonize roots of most crops and improve their performance (Saedmoucheshi *et al.,* 2014) by increasing nutrients supply to the plants and reducing abiotic stress effects (Qiu-Dan *et al.,* 2013). Among them the usage of (AM) fungi as a biologically based strategy to alleviate the adverse impact induced by salt is of such alternatives. Many researchers have reported that AMF could enhance the ability of plants to cope with salinity (Talaat and Shawky, 2011). AMF symbiosis promote salinity tolerance by utilizing various mechanisms, such as defending roots against soil-borne pathogens, improving rhizospheric and soil conditions, modifying microbial communities, enhancing antioxidant enzymes activity, maintaining membrane integrity, enhancing plant nutrient acquisition, maintaining K^+/Na^+ ratio and the inducing biochemical changes (accumulation of proline, betaines, polyamines, carbohydrates and the antioxidants), physiological changes (photosynthetic efficiency, relative permeability, water status and abscisic acid accumulation) (Sheng *et al*., 2008). The presence of mycorrhiza in saline soil and its symbiotic relationship with plant roots under salinity conditions show that some of these fungi are probably resistant to salinity stress and by symbiotic relationship they will increase the tolerance of plants through improvement of their growth (Yano-melo *et al*., 2003). Symbiosis with mycorrhiza leads to salinity resistance and physiological changes under salinity stress (Zhongqunlle *et al*., 2007). Ghoochani *et al*. (2015) by evaluate biochemical and physiological characteristics changes of wheat cultivars under Arbuscular mycorrhizal symbiosis and salinity stress reported results also indicated that inoculating wheat cultivars with the *Glomus intraradices* can alleviate the deleterious effects of salinity stress through improving osmotic adjustment via accumulation of more proline and increasing the activity of antioxidant enzymes. The cultivar Abari had higher antioxidant activity than other cultivar (Darab) and consequently can be used in wheat breeding programs for salinity stress. AM fungi have been shown to promote plant growth and salinity tolerance by many researchers. They promote salinity tolerance by the utilizing various mechanisms, such as (a) enhancing nutrient uptake (Evelin *et al*., 2012); (b) producing plant growth hormones; (c) improving rhizospheric and soil conditions (Asghari *et al*., 2005); (d) improvement in photosynthetic activity or water use efficiency (Hajiboland *et al*., 2010); (e) accumulation of compatible solutes (Evelin *et al*., 2013); and (f) production of higher antioxidant enzymes (Manchanda and Garg, 2011). As a result, AM fungi are considered suitable for bioamelioration of saline soils. Shekoofeh *et al*. (2012) by evaluate effect of mycorrhizal fungi, including *Glomus mosseae*, *G. intraradices*, and salicylic acid (0.2 mM) on tolerance of green basil (*Ocimum basilicum* L.) to salinity resulting from sodium chloride (75 and 150 mM) reported Mycorrhizal inoculation in plants under sodium chloride stress increases potassium content of aerial organs and decreases sodium content of aerial organs and potassium of root. Thus, it can be concluded that using this pretreatments, especially VAM (Vesicular Arbuscular Mycorrhizal) fungi, prevented the transfer of sodium to plant, especially aerial organs and decreased destructive effects from sodium chloride stress in green basil plant. Also, inoculating with mycorrhizal fungus could increase transferring potassium to aerial part of plant. It can be concluded that pretreating leaves of basil plant with salicylic acid or inoculating with mycorrhizal fungus caused to increase resistance of this plant relative to salt stress. Studies show that application of the arbuscular mycorrhiza (AM) resistant to salinity could be influential in revival and production of resistant cultivars (Rodriguez Rosales *et al*., 1999). Symbiosis with mycorrhiza leads to salinity resistance and physiological changes under the salinity stress (Zhongqunlle *et al*., 2007). Mardukhi *et al*. (2015) by evaluate mineral uptake of Mycorrhizal wheat (*Triticum aestivum* L.) under salinity stress reported the AM fungal treatments, especially the mixture treatment and *Glomus mosseae*, alleviated salt stress on wheat growth by enhancing nutrient uptake, including K, Ca, Mg, Fe, Mn, and Cu, and adjusting Na^+ and Cl^- uptake. Although line 9 genotype resulted in greater nutrient uptake under salinity stress, Chamran cultivar was more effective at adjusting Na^+ and Cl^- uptake under salt stress. With increasing salinity levels, the alleviating effects of AM species on plant growth under salt stress became more evident. Findings of this experiment are complementary to the previous findings regarding the effects of AM species on plant growth under salinity, especially the application of the mixture of AM species and the adjusting effects of AM species on Na^+ and Cl^- uptake by plant. This research was conducted to evaluate the effects of mycorrhizal fungi and nano zinc oxide on the physiological responses (i.e., Sodium and Potassium content, chlorophyll index, relative water content and stomatal conductance) of wheat (*Triticum aestivum* L., cultivar Attila 4) under salinity stress conditions.

MATERIALS AND METHODS

Field and Treatment Information

This research was conducted to evaluate the effects of mycorrhiza fungi and nano zinc oxide on agro physiological traits of wheat under salinity stress based on factorial experiment according complete randomized block design with three replications under greenhouse condition at the 2014. Treatments included salinity in three levels [no-salt (S_0) or control, salinity 40 (S_1) and 80 (S_2) mM NaCl], two level of AM fungal [no application (M_0), application of mycorrhiza (M_1)] and Nano zinc oxide at three levels [(without nano zinc oxide as control (Zn_0), 0.4 (Zn_1) and 0.8 g.lit^{-1}) (Zn_2)]. Mycorrhiza fungi (*Glomus mosseae*) were purchased from the Zist Fanavar Turan institute and soils were treated based on the manufacturer's protocol 10 g of inoculums per 1 kg soil, each pot containing approximately 790 spores. The soil was silty loam, with pH 6.9. Air temperature ranged from 22 to 27°C during the day and 18 to 21°C during night. Humidity ranged from 60 to 65%. Wheat cultivar Attila 4 was used in experiment. Optimal density of cultivar Attila 4 is 400 seeds.m^{-2}, so 40 seeds were sown in each pot with 4 cm deep, filled approximately with 20 kg above mentioned soil. Pots were immediately irrigated after planting. Salt stress treatments were applied two weeks after planting (at 3-4 leaf stage). Foliar application ZnO nano was done in two growth stages (4-6 leaf stage and before booting stage).

Measured Traits

Root and shoot Na^+/K^+ ratio assay

Root and shoot Na^+/K^+ ratio was estimated according to the method of Izadi *et al*. (2014). In this method leaf samples were collected washed in distilled water to remove any external salt and oven dried at 60°C for 48 hours. The dried samples were ground into a fine powder using a mortar and pestle. Samples (1 g) were ashed by putting them into crucibles and placed in 600°C electric furnace, for 4 h, 5 mL of 2 N hydrochloric acid (HCl) were added to cooled ash samples, dissolved in boiling deionized water filtered and made final volume to 50 mL. The Na^+ and K^+ were measured using standard flame photometer procedure and reported as $mg.g^{-1}$ dry weight.

Chlorophyll content, Relative water content (RWC) and stomatal conductance

The fully developed flag leaf of the main tillers was randomly selected from five plants of the each plot for determination of stomata conductance with leaf prometer (Model SC J Eijkelkamp, Netherlands). The chlorophyll content of the leaves was determined with a SPAD-502 (Konica Minolta Sensing, Osaka, Japan) (Jifon *et al*., 2005). RWC was estimated gravimetrically according to the method of Tambussi *et al*. (2005).

Statistical Analysis

Analysis of variance and means comparison were performed with using SAS software. The mean comparison was done via least significant difference (LSD) test at 5% probability level.

RESULTS AND DISCUSSION

Chlorophyll index

According result of analysis of variance effect of different level of salinity, Nano zinc oxide and Arbuscular Mycorrhiza (AM) on chlorophyll index was significant at 1% probability level but interaction effect of treatments was not significant (Table 1). Mean comparison result of different level of salinity indicated that maximum chlorophyll index (60.57 spad) was noted for control and minimum of that (54.4 spad) belonged to 80 mM NaCl treatment (Table 2). As for LSD classification made with respect to different level of Nano zinc oxide maximum and minimum amount of chlorophyll index belonged to 0.8 $g.lit^{-1}$ (60.16 spad) and control (54.57 spad) (Table 2). According result of mean comparison maximum of chlorophyll index (58.76 spad) was obtained for application of mycorrhiza and minimum of that (54.67 spad) was for control treatment (Table 2). Nano zinc oxide, mycorrhiza and saline condition had strong effects on leaf chlorophyll content. Soil salinity drastically lowered leaf chlorophyll content. However, nano zinc oxide and mycorrhiza inoculation considerably increased the pigment content. Salinity causes a reduction in chlorophyll content due to reduction in RWC, increasing stomata resistance the antagonistic effects of NaCl on N absorption (Table 3), also which is the essential component of the chlorophyll structure (Kadian *et al*., 2013), suppressing the activity of specific enzymes required for the synthesis of photosynthetic pigments and decreased uptake of nutrients (e.g., Zu) needed for chlorophyll biosynthesis (Abdel Latef and Chaoxing, 2011). A decrease in chlorophyll index of wheat under salinity stress (Table 3) would be a typical symptom of oxidative stress (Reddy *et al*., 2004). Higher contents of chlorophyll pigments in the AMF inoculated plants contribute to greater photosynthetic activity leading to maintained growth. Our results of enhanced chlorophyll contents in AMF colonized plants are in support of the findings of Hajiboland *et al*. (2010) for *Solanum lycopersicum* L. and Aroca *et al*. (2013) for lettuce. Enhancement in chlorophyll pigments due to AMF is because of enhanced mineral uptake especially magnesium, an important component of chlorophyll molecule (Sheng *et al*., 2008). Kadian *et al*. (2013) also reported that chlorophyll activity is restored in mycorrhiza plants grown due to increased activity of the specific enzymes required for its biosynthesis. Balashouri and Prameeladevi (1995) reported that the increased chlorophyll content was obviously due to zinc at low level act as a structural and catalytic components of proteins, enzymes and as cofactors for normal development of pigment biosynthesis. Sharma *et al*. (1994) reported that added zinc enhanced the growth of cabbage and improved the chlorophyll content and photosynthetic activity in leaves. Zarrouk *et al*. (2005) indicated a positive correlation of the Zn concentrations with leaf chlorophyll content in plants.

Relative water content (RWC)

Result of analysis of variance revealed effect of different level of salinity on RWC was significant at 1% probability level, but effect of nano zinc oxide, Arbuscular Mycorrhiza (AM) and interaction effect of treatments was not significant (Table 1). Mean comparison result of different level of salinity indicated that maximum relative water content (77.83%) was noted for control and minimum of that (65.74%) belonged to 80 mM NaCl treatment (Table 2). However, high salinity stress reduced RWC by 15.53% in plants. The RWC value were decreased in wheat plants exposed to saline conditions, which has been partly attributed to the impact of the salt on the electrical potential of the plasma membrane that affected not only the absorption of ions but also that of water, generating water stress (Munns, 2002). Katerji *et al*. (1997) reported that the decrease in RWC indicated a loss of turgor that resulted in limited water availability for the cell extension process. Hussain *et al*. (2008) also reported that under different salinity levels, increased ionic flux can damage the plant cellular membranes and affect water potential of the plant's cell.

Table 1. Result of analysis of variance of measured traits

S.O.V	df	Chlorophyll index	Relative water content	Stomata conductance
Replication	2	51.30^{*}	17.89^{ns}	29.87^{**}
Salinity (S)	2	236^{**}	88.53^{**}	109.37^{**}
Nano zinc oxide (Zn)	2	163.5^{**}	4.72^{ns}	108.44^{**}
Mycorrhiza (M)	1	224.89^{**}	28.19^{ns}	47.98^{**}
S × Zn	4	13.452^{ns}	18.47^{ns}	2.84^{ns}
S × M	2	2.16^{ns}	0.51^{ns}	4.97^{ns}
Zn × M	2	3.61^{ns}	21.72^{ns}	3.27^{ns}
S × Zn × M	4	2.58^{ns}	4.43^{ns}	1.05^{ns}
Error	34	10.25	10.12	2.56
C.V (%)	-	9.8	4.66	10.53

ns, * and **: non-significant, significant at 5% and 1% probability level, respectively.

Stomatal conductance

According the result of analysis of variance effect of different level of the salinity, Nano zinc oxide and Arbuscular Mycorrhiza (AM) on stomatal conductance was significant at 1% probability level but interaction effect of treatments was not significant (Table 1). Mean comparison result of different level of salinity showed that the maximum stomatal conductance (35.49 $mmol.m^{-2}.s^{-1}$) was noted for control and minimum of that (30.79 $mmol.m^{-2}.s^{-1}$) belonged to 80 mM NaCl treatment (Table 2). As for LSD classification made with respect to different level of Nano zinc oxide maximum and minimum amount of stomatal conductance belonged to 0.8 $g.lit^{-1}$ (36.29 $mmol.m^{-2}.s^{-1}$) and control (31.51 $mmol.m^{-2}.s^{-1}$) (Table 2). According result of mean comparison maximum of stomatal conductance (34.51 $mmol.m^{-2}.s^{-1}$) was obtained for application of mycorrhiza and minimum of that (32.62 $mmol.m^{-2}.s^{-1}$) was for control treatment (Table 2). Stomatal conductance to water vapor is of critical agronomic and ecological importance because it determines rates at which CO_2 enters and water vapor exits leaves, exerting a controlling influence on photosynthesis, hydration and ultimately biomass accumulation, crop yield, and carbon sequestration (Aroca *et al*., 2013). The stomatal conductance was significantly decreased under salinity stress. The stomatal conductance of 40 mM salinity did not differ from that of the non-salinity ones. In addition, 80 mM NaCl reduced stomatal conductance in wheat by about 13.24% (Table 2). Mycorrhiza inoculation as M_1 and nano zinc oxide as Zn_2 increased stomatal conductance by 8.44% and 15.16%, respectively (Table 2). Very *et al*. (1998) found that long-term exposure to salinity affects growth through closure of stomata, limiting transpiration and thus the transport of salts. In the present study, decrease in RWC due to salt stress would result in stomatal closure (Table 3) in order to maintain their water status (Sheng *et al*., 2008). It seems that higher RWC in mycorrhiza than in non-mycorrhiza plants may be beneficial for moving water through the plants to the evaporating surfaces and maintaining opened stomata in leaves (Nelsen and Safir, 1982). The mechanisms involved in water uptake by the AMF symbiosis include regulation of stomatal conductance, an increase in stomatal sensitivity to leaf air vapor pressure deficit, and lowering leaf osmotic potential for turgor maintenance (Sanchez-Blanco *et al*., 2004).We could say that AM fungal colonization can elevate the photosynthetic ability through improving the gas exchange capacity of *triticale* plants under salt stress. Zn is thought to be involved in stomatal regulation due to its role in maintaining membrane integrity (Khan *et al*., 2004).

Continue Table 1.

S.O.V	df	K^+ concentration	Na^+ concentration	Na^+/K^+ ratio	Seed yield
Replication	2	0.27^{ns}	0.007^{ns}	0.069^{ns}	0.036^{ns}
Salinity (S)	2	6.65**	0.24**	9.52**	0.163**
Nano zinc oxide (Zn)	2	2.92**	1.89**	11.86**	0.166**
Mycorrhiza (M)	1	0.18**	0.58**	3.97**	0.107**
S × Zn	4	0.09^{ns}	0.069*	4.16*	0.028^{ns}
S × M	2	0.045^{ns}	0.133*	1.8*	0.034^{ns}
Zn × M	2	0.03^{ns}	0.047*	1.6*	0.050^{ns}
S × Zn × M	4	0.028^{ns}	0.035*	1.13*	0.033*
Error	34	0.043	0.015	0.085	0.020
C.V (%)	-	13.5	14.47	12.81	2.032

ns, * and **: non-significant, significant at 5% and 1% probability level, respectively.

Table 2. Means comparison of measured traits affected by salinity, nano zinc oxide and mycorrhizal fungi

Treatment	Chlorophyll index (Spad)	Relative water content (%)	Stomata conductance ($mmol.m^{-2}.s^{-1}$)	K^+ concentration ($mg.g^{-1}$)
Salinity stress				
S_0	60.57^{a*}	77.83^{a}	35.49^{a}	6.02^{a}
S_1	55.17^{ab}	73.81^{b}	34.42^{ab}	1.84^{ab}
S_2	54.4^{b}	65.74^{c}	30.79^{b}	1.10^{b}
Nano zinc oxide				
Zn_0	54.57^{b}	72.89^{b}	31.51^{c}	0.82^{c}
Zn_1	55.41^{ab}	76.81^{b}	32.88^{b}	1.13^{b}
Zn_2	60.16^{a}	85.69^{a}	36.29^{a}	1.62^{a}
Arbuscular mycorrhiza				
M_0	54.67^{b}	70.08^{b}	32.62^{b}	1.13^{b}
M_1	58.76^{a}	76^{a}	34.51^{a}	1.25^{a}

*Means with similar letters in each column are not significantly different.
Different salinity level; $\mathbf{S_0}$: no-salt or control, $\mathbf{S_1}$: salinity 40 mM, $\mathbf{S_2}$: 80 mM NaCl.
Different level of Arbuscular Mycorrhiza (AM) fungal; $\mathbf{M_0}$: no application, $\mathbf{M_1}$: application of mycorrhiza
Different level of Nano zinc oxide; $\mathbf{Zn_0}$: non use nano zinc oxide as control, $\mathbf{Zn_1}$: application of 0.4, $\mathbf{Zn_2}$: 0.8 $g.lit^{-1}$.

K^+ concentration

Result of analysis of variance showed the effect of different level of salinity, Nano zinc oxide and Arbuscular Mycorrhiza (AM) on K^+ concentration was significant at 1% probability level but interaction effect of treatments was not significant (Table 1). Mean comparison result of different level of salinity indicated that maximum K^+ concentration (6.02 $mg.g^{-1}$) was noted for control and minimum of that (1.10 $mg.g^{-1}$) belonged to 80 mM NaCl treatment (Table 2). As for LSD classification made with respect to different level of Nano zinc oxide maximum and minimum amount of K^+ concentration belonged to 0.8 $g.lit^{-1}$ (1.62 $mg.g^{-1}$) and control (0.82 $mg.g^{-1}$) (Table 2). According result of mean comparison maximum of potassium concentration (1.25 $mg.g^{-1}$) was obtained for application of mycorrhiza and minimum of that (1.13 $mg.g^{-1}$) was for control treatment (Table 2). Nano zinc oxide AM and saline condition had strong effects on K^+ concentration. Treatments of 40 and 80 mM salinity caused significant decrease of 81.72% and 69.43% in potassium content respectively, as compared to control (Table 2). Elevated Na^+ in the soil solution inhibits the uptake of other nutrients by interfering with various transporters in the root plasma membrane, such as K^+ selective ion channels, and inhibiting root growth by the adverse effects of Na^+ on soil structure (Porcel *et al*., 2012). Sodium shares antagonistic relationship with the potassium. This can explain why the Na^+/K^+ ratio was increased in our study which reflects the growth reduction in our results. However, AM and nano zinc oxide application significantly increased K^+ content. Percent increase in potassium content due to MF was 11% (Table 3). However, foliar application of zinc oxide at higher level (Zn_3), percent increase potassium was 97.5%. The effect of potassium is considered to its ease in the penetration into the plant cell which results in higher protoplasmic change to increase water retaining capacity and resistance to stress (Tammam *et al.,* 2008). Bartels and Sunkar (2005) have also reported the role of potassium in raising salt tolerance of rice, cotton, wheat, and barley plants, respectively. However, the higher reduction in root K^+ content of plants that salt induced shoot growth inhibition is mainly due to metabolic changes resulting from ion imbalance or ion toxicity occurring in root system (Munns, 2002). Higher K^+ accumulation by mycorrhizal plants could be beneficial by maintaining a high K^+/Na^+ ratio and by influencing the ionic balance of the cytoplasm or Na efflux from plants (Abdel-Fattah and Asrar, 2012). Mycorrhizal fungi can enhance K^+ absorption under saline conditions (Abdel Latef and Chaoxing, 2011) and prevent the translocation of Na to shoot tissues. Gobarah *et al.* (2006) reported that foliar application with zinc levels had a significant effect on plant growth under soil conditions.

Na^+ concentration and Na^+/K^+ ratio

Result of analysis of variance revealed effect of different level of the salinity, Nano zinc oxide and Arbuscular Mycorrhiza (AM) on the Na^+ concentration and Na^+/K^+ ratio was significant at 1% probability level also interaction effect of treatments was significant at 5% probability level (Table 1). Salinity causes an imbalance in the ion flux inside plants. In addition, due to the Na^+ content increased sharply with the increasing salts, the Na^+/K^+ had the similar change trends with the Na^+ content (Table 3). A higher Na^+/K^+ ratio resulted by salinity interrupts the cytoplasm ionic balance, and the consequently inhibit various metabolic pathways (Giri *et al.*, 2007; Hajiboland, 2009). The Na^+/K^+ ratio may serve as an indicator of crop tolerance to stress as the increase of K^+ in the nano zinc oxide and AM treated plants is generally associated with a decrease in its Na^+ content (Upadhyay *et al.,* 2012). The present results showed that during salinity, the control plants had higher Na^+ and decreased K^+, while AM inoculation and application nano zinc oxide resulted in significantly decreased Na^+ and increased K^+ concentration. This is also according to the results of (Rojas-Tapias *et al*., 2012). The highest content of Na^+ concentration (8.41 $mg.g^{-1}$ DW) and Na^+ /K^+ ratio (105.13 $mg.g^{-1}$ DW) was obtained in salinity of 80 mM, and non- application of mycorrhizae and nano zinc oxide in control treatment (M_0 and Zn_0) (Table 3).

Table 3. Means comparison interaction effect of salinity, nano zinc oxide and mycorrhiza fungi on measured traits

Treatment		Na^+ concentration ($mg.g^{-1}$)		Na^+/K^+ ratio		Seed yield (g per plant)	
Salinity Stress	Nano zinc oxide	M_0	M_1	M_0	M_1	M_0	M_1
S_0	Zn_0	0.89^{bc}	0.62^{de}	0.68^{efg}	0.407^{f-i}	0.34^{bd}	0.36^{ab}
	Zn_1	0.60^{de}	0.44^{e-h}	0.403^{f-i}	0.23^{ghi}	0.36^{cd}	0.40^{bc}
	Zn_2	0.28^{hij}	0.17^{j}	0.116^{i}	0.066^{i}	0.45^{a}	0.44^{a}
S_1	Zn_0	0.92^{b}	0.86^{b}	1.29^{c}	1.066^{bcde}	0.32^{f-j}	0.35^{f-i}
	Zn_1	0.58^{def}	0.53^{ef}	0.64^{e-h}	0.47^{f-i}	0.37^{f-i}	0.38^{cde}
	Zn_2	0.41^{f-i}	0.32^{g-j}	0.280^{ghi}	0.190^{hi}	0.40^{d-g}	0.43^{def}
S_2	Zn_0	1.56^{a}	0.92^{a}	2.27^{a}	2.15^{a}	0.27^{c-f}	0.34^{j}
	Zn_1	0.73^{cd}	0.49^{egh}	1.24^{cd}	0.78^{def}	0.35^{j}	0.35^{g-j}
	Zn_2	0.48^{efg}	0.25^{ij}	0.533^{e-i}	0.276^{ghi}	0.39^{d-g}	0.41^{cd}

*Means with similar letters in each column are not significantly different.

Different salinity level; S_0: no-salt or control, S_1: salinity 40 mM, S_2: 80 mM NaCl.

Different level of Arbuscular Mycorrhiza (AM) fungal; M_0: no application, M_1: application of mycorrhiza.

Different level of Nano zinc oxide; Zn_0: non use nano zinc oxide as control, Zn_1: application of 0.4, Zn_2: 0.8 $g.lit^{-1}$.

But the minimum of the value (0.17 and 0.066 mg.g^{-1} DW, respectively) observed in application of AM and nano zinc oxide as M_1Zn_2 in no salinity stress (Table 3). AMF-treated plants in combination with 0.8 g.lit^{-1} nano zinc oxide showed decrease of 83.97% in Na^+ content and 87.84% in Na^+/K^+ ratio as compared to high salinity treated plants alone and control. The treatment of MF and 0.8 g.lit^{-1} nano zinc oxide was very effective in alleviating the deleterious effects of salinity stress by decreasing Na^+ and Na^+/K^+ ratio in plants. Hussain *et al*. (2008) have been proposed, increased ionic flux can damage the plant cellular membranes and effect water potential of the plant's cell. Plant growth is dependent on water status of leaf, as salt and drought stress can create a water deficit inside plant tissues. Measuring the RWC indicates stress response of plant (Sheng *et al*., 2008). Mycorrhiza colonization of a plant can reverse the effect of salinity on K and Na nutrition. Mycorrhiza fungi can enhance K absorption under saline conditions (Abdel Latef and Chaoxing, 2011) and prevent the translocation of Na to shoot tissues.

Seed yield

Result of analysis of variance showed effect of different level of salinity, Nano zinc oxide and Arbuscular Mycorrhiza (AM) on seed yield was significant at 1% probability level, also interaction effect of salinity × Nano zinc oxide and Arbuscular Mycorrhiza (AM) was significant at 5% probability level, interaction effect of another treatments was not significant (Table 1). Interaction effect between salinity, mycorrhiza and nano zinc oxide showed that the highest seed yield (0.45 g per plant) was obtained at no salinity condition, application of mycorrhiza and nano zinc oxide as M_0Zn_2 (Table 3). But the minimum of seed yield (0.27 g per plant) observed in salinity of 80 mM and Zn_0M_0 (Table 3). Salt stress affects plant metabolism, which results in decreased growth and yields. Rhizosphere micro organisms, particularly beneficial bacteria and fungi, can improve plant performance under the stress environments and, consequently, enhance yield both directly and indirectly (Dimkpa *et al*., 2009). Azcón and Barea (2010) has been proposed co-inoculation with bio fertilizer as an efficient procedure to increase the plant growth. Vivas *et al*. (2003) suggested that there are synergistic effects on plant growth when the mycorrhiza is inoculated, particularly under growth limited conditions. Gobarah *et al*. (2006) reported that the foliar application with zinc levels had a significant effect on plant growth, yield and its components as well as seed quality under the salinity conditions. Significant increases in seed yield with foliar Zn application have been reported in other crops such as rice (Cakmak, 2008), triticale (Cakmak *et al*., 1997) and maize (Potarzycki and Grzebisz, 2009).

CONCLUSION

The results showed that salinity stress reduced seed yield, chlorophyll index of wheat, stomata conductance, relative water content and potassium concentration in plant shoots and roots. But sodium concentration in plant shoots and roots, Na^+/K^+ ratio in plant shoots and roots increased. Also AMF (Arbuscular mycorrhiza fungal) and nano zinc oxide improved Na^+ concentration and Na^+/K^+ ratio under salinity condition. It seems that application of mycorrhiza and nano zinc oxide can be recommended to improve wheat production under salinity condition.

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