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COMPARISON OF THE EFFECT OF SILICON AND SILICON NANO-CHELATE IN REDUCING THE IMPACT OF SALINITY STRESS ON WHEAT SEEDLINGS

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ABSTRACT

Today, salinity stress causes extensive damage to crops, and high soil salinity is one of the limiting factors for crop yields. A practical approach to lessen the negative effect of salinity stress is to use mineral nutrition methods such as spraying plants with silicone. To investigate and compare the effect of silicon and silicon nano-chelate on the wheat plant resistance (Shiroodi cultivar) to salinity stress, a factorial experiment was designed and conducted in a completely randomized design with five replications under hydroponic conditions. Experimental treatments included concentrations of 0 and 2 mmol/L silicon, 0 and 0.424 g/L silicon nano-chelate, 0 and 150 mmol/L sodium chloride, and their interaction. The growth and physiological indices showed that salinity stress decreasing effect on shoot dry weight, root fresh weight, catalase activity, and ascorbate peroxidase. These increases indicate the activation of the plant defense system against salinity stress conditions. The results also showed that silicon nano-chelate treatment under salinity stress reduced dry and fresh weights of roots and shoots. These two compounds additionally influenced the content of catalase activity, ascorbate peroxidase, and superoxide dismutase content in shoots. Simultaneously, the silicon and silicon nano-chelate treatment under salinity stress reduced the dry and fresh weight of roots and shoots, catalase activity, and ascorbate peroxidase. Therefore, the results obtained in this study generally showed that silicon under salinity stress increased plant growth and positively affected the activity of its antioxidant system. But silicon nano-chelate not only did not improve plant performance but also reduced its growth.

Keywords: *salinity stress, silicon, wheat (Shiroodi cultivar), antioxidants, potassium*

1. INTRODUCTION:

High soil salinity is one of the factors limiting crop yields worldwide, which is one of the main problems of the agricultural sector, especially in arid and semi-arid regions. Almost all arid and semi-arid lands in the world and about fifty percent of irrigated lands deal with salinity stress (Jafarzadeh and Aliasgharzad, 2007; Dadkhah, 2011; Fateh and Alimohammadi, 2011). In Iran, saline lands are 18.5 million hectares (Qadir *et al.*, 2008). Due to the limited quality of water resources for the irrigation of crops globally, saline water for agriculture is inevitable. In such circumstances, one of the influential factors in the production and exploitation of saline soil and water is salt-tolerant plant cultivars. The literature is enriched with data on the effect of salinity on the growth and yield of crops and the identification of new plant genotypes with a high tolerance to salinity (especially bread wheat) (Sharma and Minhas, 2005; Letey and Feng, 2007).

Bread wheat is a critically important food plant globally and is the leading food source in arid and semi-arid regions. In these areas, water scarcity is the primary cause, and soil salinity is the secondary factor in reducing plant growth and grain yield (Dreisigacker *et al.*, 2008; Montenegro *et al.*, 2017). The destructive effects of salinity stress, due to the reduction of osmotic potential in the root environment and the impact on plant water balance and reduction of osmotic pressure, have been reported by many researchers at different stages of bread wheat growth (Sairam and Tyagi, 2004; Parihar *et al.*, 2015).

Crops grown in arid and semi-arid regions are often exposed to adverse environmental factors such as drought or high soil salinity. Salinity stress causes morphological, physiological, and biochemical changes in plants (Amirjani, 2010). Salinity reduces the water potential around the roots and reduces the plant water absorption capacity. Besides, with increasing salinity in the root environment, the

uptake and transfer of toxic ions to plant tissues increases, which leads to a decrease in the uptake of essential elements, disturbance of ion balance, and toxicity due to the accumulation of sodium and chlorine ions. Under saline conditions, potassium uptake by root cells is reduced by competition with sodium (Sairam and Tyagi, 2004; Rabie and Almadini, 2005; Tuteja, 2007).

The extent of these changes depends on the salinity level and its application duration (Li *et al.*, 2015). The typical effect of most saline soils is growth inhibition, which can have many causes. Still, this inhibition is often associated with high concentrations of Na+, Cl^- ions, and deficiency of K+ ions (Zhang *et al*., 2010). The indirect effect of salinity on plant growth is to reduce the plant water content. As salinity increases, the potential of soil water decreases. In general, the presence of salt in the soil solution reduces the osmotic potential of the soil. It creates water stress, impairs the uptake of sufficient water for plant growth, reducing growth rate and altering metabolic processes. Leads in plants (Hu *et al.*, 2012). Increasing sodium reduces other cations in the plant and upsets the cation balance of the plant, which in turn reduces the amount of calcium, magnesium, and potassium in the plant (Yan-de *et al*., 2007). At high salinity levels, the amount of potassium ions decreases due to replacement by sodium ions, which in addition to disturbing the ionic balance, also disrupts cellular metabolism. The potassium functions in the plant include colloidal effect, increase hydration, activation of photosynthetic enzymes, osmotic regulation, and its role in opening and closing pores. Potassium ions accumulate in the stomatal cells, causing the pores to open. As potassium escapes from the orifice, the orifices close. This role of potassium is due to the change in the protective cells due to the change in the osmotic potential in these cells (Kafi *et al.*, 2019).

Reactive oxygen species (ROS) are considered a major cell destruction source under biological and abiotic stresses (Candan and Tarhan, 2003). ROS forms oxygen reduction produced in biological processes such as light respiration, photosynthesis, and respiration (Uchida *et al*., 2002). To produce water in these processes, four electrons are needed to reduce oxygen. Still, in ROS, the transfer of one, two, and three electrons to O^2 usually results in superoxide, hydrogen peroxide, and hydroxyl radicals, respectively (Mittler, 2002). These types of oxygen are highly toxic and react with biological molecules such as lipids, proteins, and nucleic acids, causing lipid peroxidation, protein denaturation, and DNA damage (Quiles and López, 2004).

Silicon (Si) is the second most abundant soil element but is not considered an essential component of many plants. Many studies have recently shown that treating plants with Si can dramatically reduce biological and non-biological stresses such as heavy metal stress, salt, drought, cold, and frost and have beneficial effects on growth and plant production (Hu *et al.*, 2012; Kafi *et al.*, 2019; Elsheery *et al.*, 2020).

Recently, the reducing role of silicon in salinity stress has been considered. Besides, some studies have shown that silicon reduces salinity in various plant species such as barley (Ghorbanpour *et al.*, 2020), corn (Marulanda *et al.*, 2010), and wheat (Abbasi *et al.*, 2018). Silicon increases salinity stress tolerance by increasing photosynthetic activity, improving water status, stimulating the antioxidant system, and decreasing sodium uptake or potassium H + -ATPase-dependent increase in shoots (Chalmardi *et al.*, 2014). It has also been shown that silicon may activate superoxide dismutase, peroxidase, and catalase. The reduced malondialdehyde concentration in barley, tomato, and corn has been reported (Jafarzadeh and Aliasgharzad, 2007; Kafi *et al.*, 2019).

The use of nano-chelates has been considered by many agricultural researchers, although their mechanism of action is not well understood (Haghighi *et al.*, 2012). Si nano-chelates has been reported to increase nitrate reductase activity in soybean (Kafi *et al.*, 2019). Studies have also shown that 1 mM Si nano-chelates has a beneficial effect on reducing the harmful effects of salinity stress on tomato seedling growth and germination characteristics. Using 1 mM, Si nano-chelates combined with 25 mM sodium chloride increased seed germination and growth (Li *et al.*, 2015).

This study aimed to increase the resistance of wheat plants to salinity stress by evaluating the effect of silicon and silicon nano-chelate on increasing salinity stress resistance in wheat plants. In this regard, this plant salinity resistance under the application of silicon and silicon nano-chelate is examined and compared.

2. MATERIALS AND METHODS:

The plant material utilized in this was Shiroodi wheat cultivar, which was purchased from Karaj Seed and Plant Breeding Institute. Pots were filled with perlite and cocopeat and then transferred to the hydroponic environment in the

research greenhouse of the Payame Noor University of Najafabad.

2.1. Plant cultivation

First, a mixture of cocopeat and perlite was made in a one-to-one ratio to prepare the required substrate, and then soaked the seeds for 24 h. To prepare the environment, the tray was first filled with a mixture of coco peat and prepared perlite, sown the wheat seeds, then covered it with the prepared mixture and sprinkled water every day. Two weeks after planting, the plants were transferred to a hydroponic environment. Initially, four types of nutrient media were created: complete nutrient solution as a control, silicon-containing nutrient solution, silicon nano-chelate nutrient solution, and a solution containing silicon and silicon nano-chelate.

In each container, two plants were cultivated, and all the containers were connected to the air pump and aerated. Within a month, the containers were filled with the appropriate solutions, and once a week, the plants were sprayed with about 5 mL of iron chelate (to strengthen the growth). Plants were treated with 150 mM NaCl after 1 month and collected two weeks after treatment.

2.2. Measuring the fresh and dry weight of roots and shoots

After separating the roots and shoots, their weights were measured with a digital scale with an accuracy of 0.001 g. the shoots and roots were wrapped in aluminum foil and dried in an oven at 70°C for 48 hours until their weight stabilized. Then the dry weight of the samples was measured.

2.3. Superoxide dismutase (SOD)

This enzyme activity was determined based on its ability to inhibit the photochemical reduction reaction of nitroblue tetrazolium (NBT) at 560 nm based on Giannopolitis and Ries (1977). The reaction solution consisted of 50 mM sodium phosphate buffer (pH = 7.5), 13 mM methionine, 0.1 mM Na-EDTA, 75 µM nitroblue tetrazolium (NBT), 75 µM riboflavin and 100 µl of the plant extract. The reaction solution was exposed to fluorescent light for 14 minutes, and its absorbance was read at 560 nm. The basis of NBT conversion to formazan is in the presence of light and the appearance of purple dye. In the presence of the enzyme, superoxide dismutase inhibits the reaction and reduces the dye intensity. The difference between the control solution adsorption and the solution containing the extract indicates the enzyme superoxide dismutase reaction inhibition. Each unit is equal to the amount of enzyme that inhibits NBT light reduction by 50%. Therefore, this enzyme activity was calculated in units of enzyme per milligram of protein (Unitmg-1protein).

2.4. Catalase activity (CAT)

Catalase activity was assessed considering the rate of H_2O_2 decomposition by measuring the absorption changes at 240 nm (Beers and Sizer, 1952). The composition of the reaction mixture was 0.05 M phosphate buffer (pH = 7), 3% oxygenated water, and 5 µl of enzyme extract. The enzyme activity is expressed as each micromole of decomposed H_2O_2 per minute per milligram of protein.

2.5. Ascorbate peroxidase (APX)

Mhadhbi *et al.* (2004) method was used to measure the APX enzyme. Potassium phosphate buffer (0.05 M) (pH = 7), oxygenated water 0.1 mM, ascorbate 50 µM, and 10 µl of enzyme extract were mixed at 4 °C, and absorption changes at 290 nm were read. Enzyme activity was calculated in terms of each micromole of oxidized ascorbate per minute per milligram of protein.

2.6. Mineral elements

To determine the element content of the leaves, after washing and drying at 70°C for 48 hours and preparation, the samples were digested by oxidation method using 96% sulfuric acid, salicylic acid, oxygenated water, and selenium (Mozafari and Ghaderi, 2018). Weigh 0.3 g of the plant sample, and after transferring to the digestion tube, 2.5 ml of the acid mixture was added to the samples and shaken gently. After 2 hours, the digestion tubes were placed on the stove at 100°C for 2 hours. Afterward, 3 ml of oxygenated water were added to the tubes, and after each addition of oxygenated water, the tube was carefully shaken to react with the oxygenated water. Then raised the temperature to 330°C. The digestion process was completed when the color of the extract became colorless or yellow (this process lasted two h). After cooling, removed the tubes from the oven, added 48.3 mL of distilled water, and strain it after stirring. Potassium was measured by a flame photometer (Jenway C/PFPY) and iron measurement by atomic absorption spectrometer (Shimadzu AA-670) (2).

2.7. Statistical analysis

The experimental design used in this study was a completely randomized design with three replications. Statistical analysis of data was performed using one-way analysis of variance by SPSS software version 18. Duncan's test was employed to compare the mean of treatments at the error level of 0.05.

3. RESULTS AND DISCUSSIONS:

3.1.Biomass

Crops are exposed to salinity stress in many arid and semi-arid areas. The cause of this stress is in the salinity of water or soil and the method of irrigation (Parvaiz and Satyawati, 2008; Ashraf, 2010). Salinity stress severely reduces root and shoot growth in susceptible plants (Fateh and Alimohammadi, 2011). There is ample evidence that when silicon is available to plants, it plays a significant role in growth, mineral nutrition, mechanical strength, and resistance to various stresses (Hu and Schmidhalter, 2005).

The results obtained in this study showed that salinity stress had no significant effect on biomass production (fresh and dry weight). On the other hand, the use of nano-chelate significantly reduced the fresh and dry weight of the plant shoots (3.8±0.61 and 0.9±0.07 g) compared to the control, but on the contrary, silicon significantly increased the fresh and dry weight of the shoots (11.58±1.32 and 2.21±0.01 g, under 150 mmol/L salt; Figure 1a and c). Simultaneous application of silicon and silicon nano-chelate has also significantly reduced the fresh and dry weight of shoots at the level of 5% (6.25±0.93 and 2.73±0.16 g under 150 mmol/L salt) compared to the control (8.63±019 and 0.64±0.03, fresh and dry weight, respectively).

The salinity stress reduced root fresh and dry weight. The silicon nano-chelate application caused a significant reduction in root fresh and dry weight (0.263±0.58 and 4.03±0.38 g) compared to control, but silicon did not have a substantial effect on root fresh and dry weight (9.08±1.08 and 0.443±0.08; Figure 1b and d). Si and Si chelate simultaneous application significantly reduced the fresh and dry weight of roots (5.88±0.26 g). Under salinity stress, silicon nano-chelate decreased, and silicon alone increased the fresh (3.87±0.86 and 9.08±1.08 g, respectively) and dry (0.196±0.41 and 0.443±0.08 g, respectively) weight of roots. Simultaneously applying these two substances in these conditions according to the control caused a significant reduction in fresh root weight (4.78±0.84 g, P = 0.05).

In contrast, in the root dry weight (0.263±0.07 g), no significant effect was observed. Studies by Haghighi *et al.* (2012)showed a decrease in dry weight of roots and stems of four sugarcane genotypes in the presence of sodium chloride due to sodium ion toxicity. They reported that silicon reduced the harmful effects of sodium chloride by reducing absorption and adsorption of Na+ from roots to shoots and improved sugarcane growth (Chen *et al.*, 2010). In the present study, a decrease in fresh and dry weight of roots and shoots of wheat seedlings due to salinity was observed, and the treatment of wheat seedlings with silicon and improved stress conditions and relatively increased fresh and dry weight roots and shoots. In Sorghum bicolor, it was observed that the use of silicone increased the fresh weight of shoots and roots by 19.2% and 10% compared to salinity stress, which is consistent with the results of the present study (Yin et al., 2013). The positive effect of silicon on the fresh and dry weight of shoots in *Cucumis sativus* L. has also been reported (Amirossadat *et al.*, 2012). Investigation on sweet potato plants under salinity stress showed that silicon added to food improves growth inhibition caused by salinity stress (Haghighi and Pessarakli, 2013).

3.2. Antioxidants

The overall activity of catalase (CAT) in the absence of salt stress is only affected by Si nano-chelate (21.7±1.81, Figure 2a). In the salt-stressed wheat seedlings, CAT content increased markedly by 28.83±1.32 (µmol/min/gr FW), a little higher than the combination of Si and Si nano-chelate (27.65±3.15 µmol/min/gr FW, Figure 2a). Seedlings received Si only, indicated the lowest CAT content. The response of ascorbate peroxidase (APX) to Si and Si nano-chelate application was additive. That is, nano-chelate and Si treatment without salinity stress (0.221±0.03 and 0.14±0.01 µmol/min/gr FW, respectively) induced APX content significantly (>5%, Figure 2b). Under salinity stress, the APX content increased particularly in control plants (0.341±0.04, µmol/min/gr FW). Its range also increased in the Si alone level (0.245±0.02 µmol/min/gr FW). In general, superoxide dismutase (SOD) did not respond positively to either Si or salinity stress (Figure 2c). The only significant increase was observed in Si nano-chelate treatment (16.9±2.24 µmol/min/gr FW). While when seedlings were exposed to a combination of salt stress and Si nano-chelate,

SOD content decremented notably (2.61±0.41 µmol/min/gr FW). These results suggest that silicon may protect plant cell membrane structure exposed to salinity stress and prevent its degradation. Therefore, the present study results show that the addition of silicon prevents the oxidative degradation of wheat seedlings exposed to salinity stress. In general, plants have been proven to protect themselves from salinity stress by preventing lipid peroxidation with antioxidant enzymes help (Parvaiz and Satyawati, 2008; Parihar *et al.*, 2015).

Superoxide dismutase, peroxidase, and catalase are the main antioxidant enzymes for scavenging free radicals. Superoxide dismutase probably plays a central role in defending against free radical toxicity (Ashraf, 2010). According to initial reports, in the wheat plant, under salinity stress, by increasing the enzymes superoxide dismutase, peroxidase, and catalase, a certain level of protection against degradation is provided (Chen *et al.*, 2010; L. Hu *et al.*, 2012; Li *et al.*, 2015). These results suggest that salinity stress can cause significant changes in antioxidant enzyme activities in free radicals metabolism (Sui *et al.*, 2010). In the present study, an increase in catalase activity under salinity stress and a decrease in this enzyme activity were observed with the application of silicon and silicon nano-chelate. Free radicals trigger catalase production, and a reduction in the amount of catalase indicates a decrease in free radicals production and thus a reduction of oxidative degradation (Rahneshan *et al.*, 2018). These results suggest that the enzyme catalase is activated by increasing the production of reactive oxygen species (ROS) and adaptation for maize seedlings under stress conditions. The application of silicon and silicon nano-chelate in stressed wheat seedlings reduces catalase activity by reducing the effect of salinity stress and free radicals. In sunflower under salinity stress treated with silicon and silicon nano-chelate, catalase enzyme activity was significantly reduced compared to seedlings under salinity stress (Marulanda *et al.*, 2010; Mozafari and Ghaderi, 2018).

3.3. Potassium and Iron content

Silicon has been reported to improve the nutritional balance of plants under stress (Nwugo and Huerta, 2011). The uptake and transfer of sodium to the roots are greatly reduced by adding silicon to salinity stress conditions. The increase in tolerance to salinity stress by silicon is attributed to the selective uptake and transfer of potassium and sodium by plants (Amirossadat *et al.*, 2012). However, in this study, the content of K in shoots increased only in Si (0.00988 mg/g DW). Whereas, in salt exposed seedlings, the overall content of K decreased significantly (Figure 3a and b) with a combination of salinity and Si+Si nano-chelate had the lowest amount of K (0.0027 mg/g DW, at 5% level). On the other hand, the content of K in root was opposite, in which under free salt treatment, the K content was kept considerably low with no significant difference with control. In seedlings exposed to salinity, Si and a combination of Si and Si nano-chelate with 2.2 and 2.7 mg/g DW exhibited the highest content of K. the Fe content in shoots did not differ with and without salinity (Figure 3 c and d). After control with 0.25 mg/g DW only Si nano-chelate (0.175 mg/g DW) exhibited an increase. In salt-stressed seedlings. However, Si and Si nano-chelate combinations were found to have the highest Fe content in shoots (0.275 mg/g DW). The root Fe content in Si nano-chelate only treatment indicated the highest Fe in shoots (0.237 mg/g DW). In seedlings treated with a combination of chelate and salt, the root Fe content (0.512 mg/g DW) was found to be significantly increased compared to other treatments. Silicon reduces the uptake and transport of heavy metals and salts from the root to the shoot by reducing apoplastic currents (Ma and Yamaji, 2006). Studies by (Liang *et al.*, 2006); Liang *et al.* (2007) have shown that the addition of silicon increases potassium transport and improves the K + / Na + ratio by activating the H + -ATPase pump. The potassium content of silicon-treated corn seedlings increased significantly relative to salinity stress, and silicon improved the salinity effect in the present study. Similar results have been observed in *Spartina densiflora* (Mesa-Marín *et al.*, 2020) and *Saccharum officinarum* L. (Ashraf, 2010).

4. CONCLUSIONS:

In this study, first-hand scientific results were generated in favor of silicon application to mitigate the effects of salinity stress on wheat seedlings and improve seedlings growth and survival under salinity stress. Also, it was observed that the ameliorating results of silicone were considerably higher than nano- chelate silicon. Therefore, it could be suggested to use silicon as an ameliorator of salinity stress effects. However, it's highly advised to use a higher range of salinity treatments and silicone concentrations to pinpoint the optimized level of silicone applicable for agricultural practice in farmlands dealing with salinity stress.

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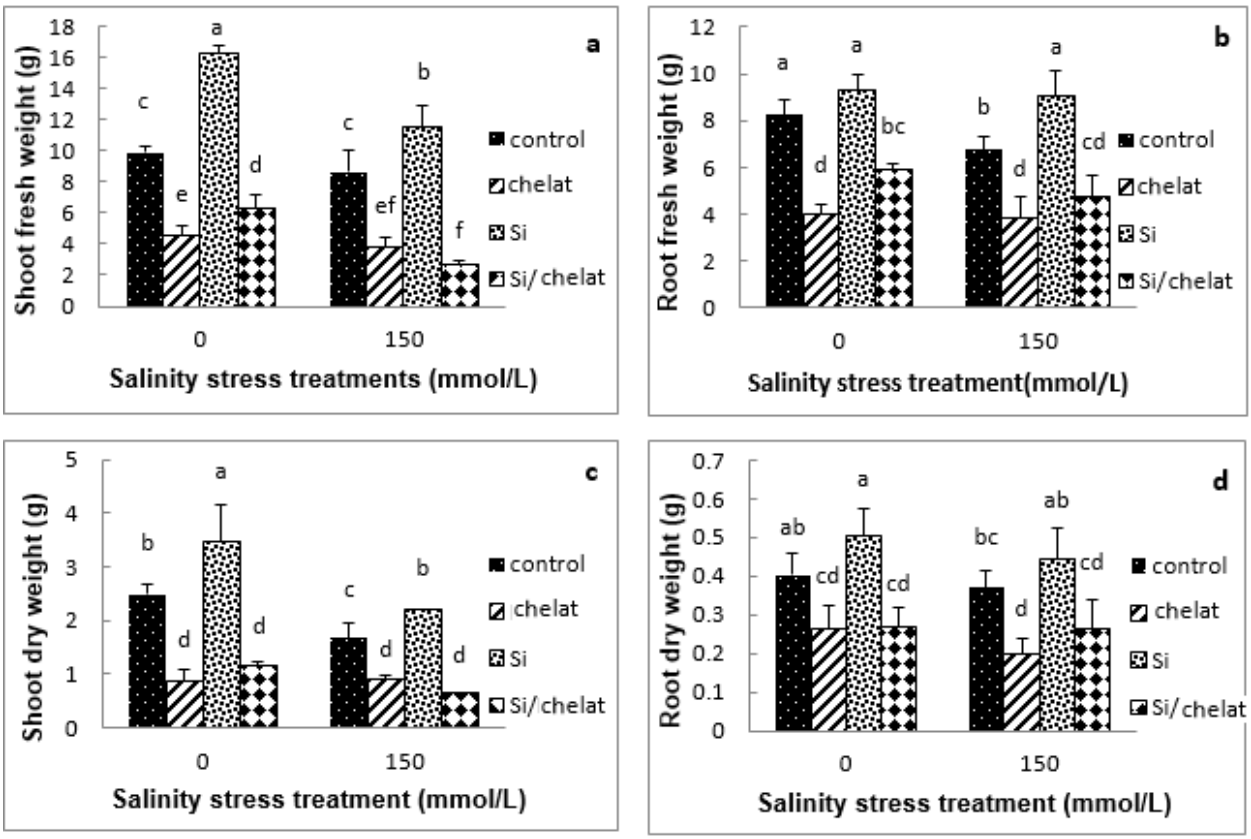

Figure 1. *Effect of Si (2 mmol/L), silicone nano-chelate (0.424 mg/L), salt (150 mmol/L) and their interaction on shoot fresh weight (a), shoot dry weight (c), root fresh weight (b), root dry weight (d). Columns with non-common letters indicate a significant difference between treatments based on Duncan's test ($p < 0.05$).*

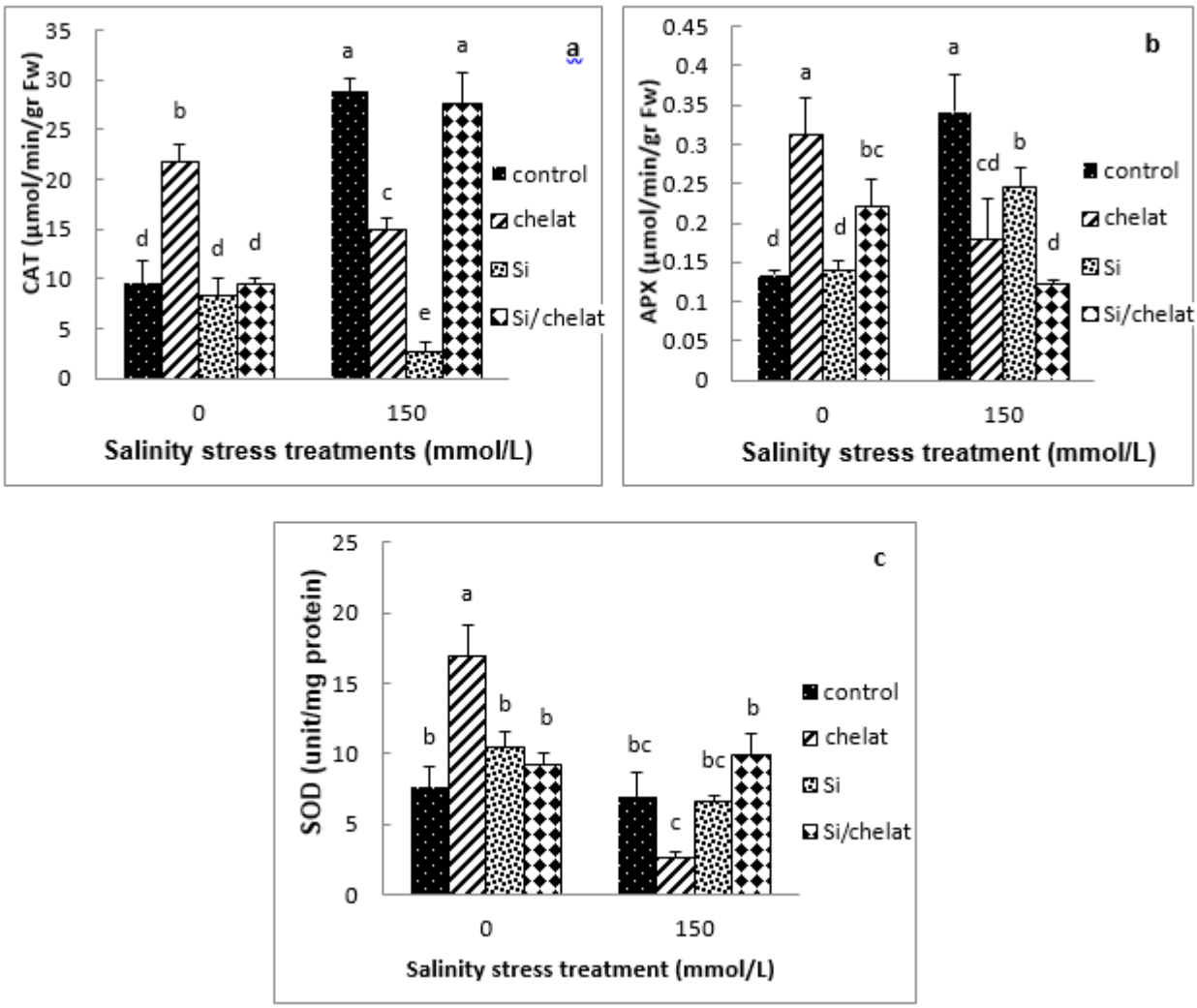

Figure 2. *Effect of Si (2 mmol/L), silicone nano-chelate (0.424 mg/L), salt (150 mmol/L) and their interaction on CAT (a), APX (b), and SOD (c). Columns with non-common letters indicate a significant difference between treatments based on Duncan's test (p <0.05).*

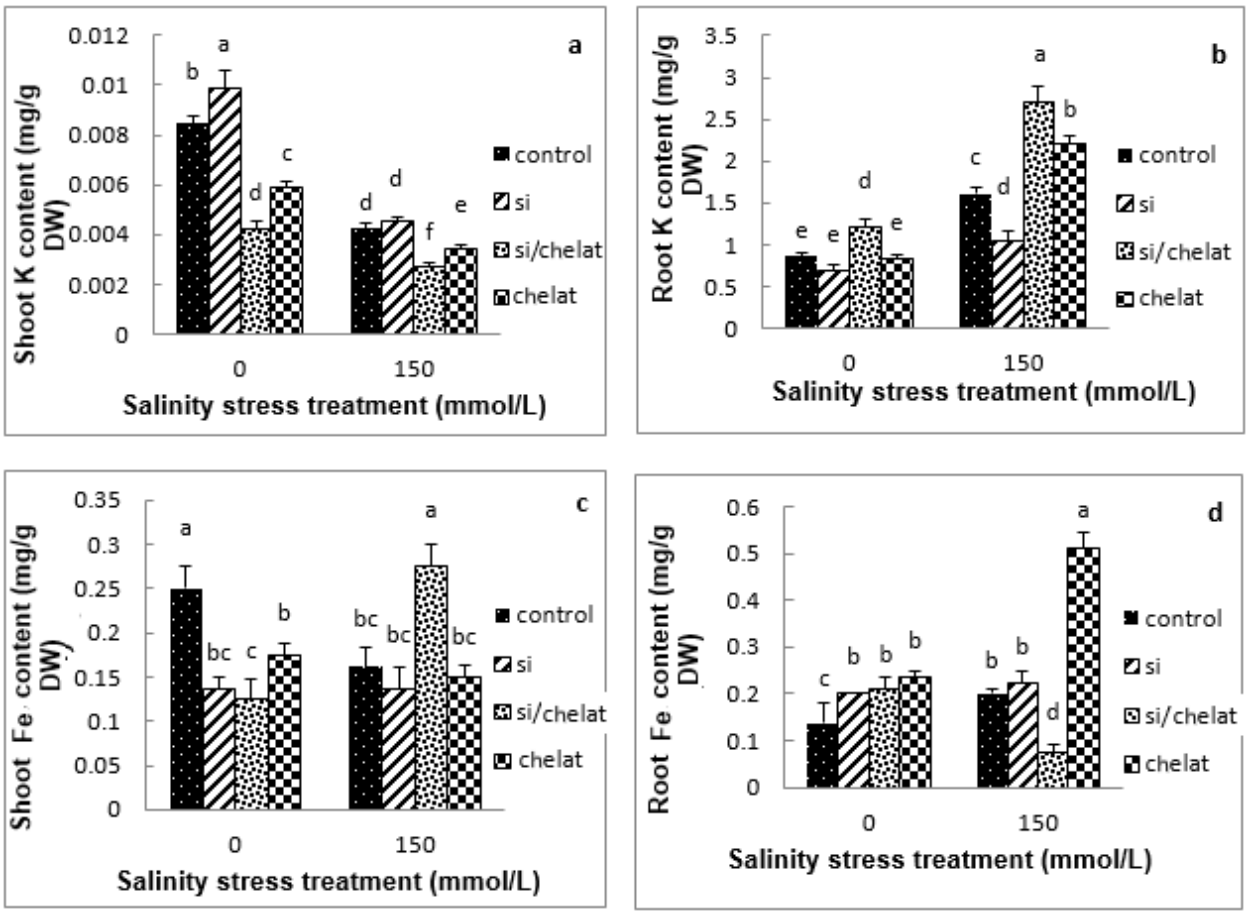

Figure 3. *Effect of Si (2 mmol/L), silicone nano-chelate (0.424 mg/L), salt (150 mmol/L) and their interaction on the content of K in shoots (a), the content of K in roots (b), the content of Fe in shoots (c) and content of Fe in roots (d). Columns with non-common letters indicate a significant difference between treatments based on Duncan's test ($p < 0.05$).*