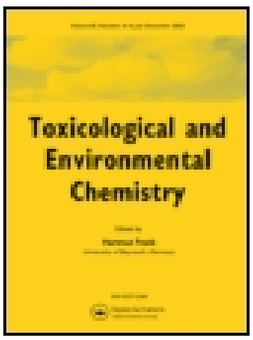

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Selenium improves physiological responses and nutrient absorption in wheat (*Triticum aestivum* L.) grown under salinity

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ABSTRACT

Salinity is a serious limiting factor for crop growth and production. The present study was conducted to investigate the response of wheat grown at salinities of 0.12, 0.30 and 0.60 S m^{-1} on soil supplemented with 0, 0.5, 1, and 4 mg kg^{-1} Se as selenite. Chlorophyll *a* and *b*, carotenoid contents, Fe, Zn and Se in shoots as well as shoot dry weight were negatively affected by increased salinity. Se had a dual effect: at 0.5 mg kg^{-1}, chlorophyll *b*, proline, and shoot Fe content were increased, catalase activity was stimulated; there was no effect on Zn content and shoot dry weight. At the two higher concentrations, Se led to decreases in chlorophyll content, nutrient concentration, and shoot dry weight. Thus, moderate addition of Se to soil could be a strategy to improve physiological responses and micronutrient status in wheat under salinity stress.

KEYWORDS
Selenite; micronutrients; salinity; chlorophyll; catalase

Introduction

Salinity of soil or water is an environmental stress factor reducing the area of cultivable land as well as crop productivity and quality. Saline soils are widespread throughout the world and cover about 400 million ha. According to Lin et al. (2015), about 7 million ha soil in Europe and 195 million ha in Asian suffer from salinity.

Abiotic stress including salinity leads to overproduction of reactive oxygen species (ROS) causing progressive oxidative damage and, ultimately, cell death. In order to alleviate adverse effects of salinity on plants, strategies to enhance salt-tolerance have been developed, e.g. some methods based on differentiated mineral nutrition. Supplementation with Zn and Fe have been found to diminish adverse effects of salinity (Keshavarz and Saadat 2016). Besides these elements, Se has gained attention due to its potential in mitigating numerous abiotic stress factors. In several publications is reported that Se might be beneficial in protecting plants against metal toxicity (Huang et al. 2017), drought (Hajiboland et al. 2015) and high temperatures (Hawrylak-Nowak et al. 2018). The special attention devoted to Se might be related to its role for animal and human health (White et al. 2017). In some countries, supplementation of fertilizers with Se has been adopted to enhance the Se content in plants, especially in wheat as a staple food (Alfthan et al. 2015). Several studies have been conducted regarding the effects of Se on non-food and food crops such as wheat and the appropriateness and effectiveness of Se biofortification (Wang et al. 2017).

Wheat (*Triticum aestivum* L.) is an important staple food, with Zn and Fe determining its high nutritional value. It is believed that dietary deficiency of these micronutrients affects more than two billion people worldwide (Meena et al. 2017). Thus, it is important to investigate the effect of Se on the uptake of these two micronutrients in wheat.

Se enhances the tolerance of plants for abiotic stress by alleviation of oxidative damage (Jiang et al. 2017). A protective role of Se in plants exposed to salinity in hydroponic systems have been reported by Hasanuzzaman, Hossain, and Fujita (2011) and Mozafariyan, Kamelmanesh, and Hawrylak-Nowak (2016). Given the limited number of reports, the present study aims to evaluate some physiological and growth responses of salt-stressed wheat to Se. For this purpose, shoot dry weight (DW), some physiological parameters, and selected micronutrient contents are determined. The results provide information for strategies for

alleviating some negative effects of salinity on wheat by Se, at the same time explaining the impact of Se on the levels of Fe and Zn.

Materials and Methods

Reagents

All chemicals, analytical grade, were purchased from Merck (Darmstadt, Germany), sulphosalicylic acid was obtained from Sigma-Aldrich (Steinheim, Germany).

Experimental setup

Silt loam soil of low salinity and Se (Table 1) was taken from Khorasan Razavi Agricultural and Natural Research Station in Mashhad, Iran. Soil pH and electrical conductivity (EC) were measured with a digital pH meter (Metrohm 620, Herisau, Switzerland) and an EC-meter (Jenway 4010, Dunmow, Essex, England). Soil texture, organic carbon (OC), total nitrogen, and available phosphorus and potassium were measured as described by Khoshgoftar et al. (2004). Available Se (Soltanpour and Workman 1980) and Fe, Mn and Zn (Lindsay and Norvell 1978) were extracted with an aqueous solution of 1 mol L^{-1} $NH_4HCO_3^-$ and 0.005 mol L^{-1} diethylenetriaminepentaacetic acid (DTPA) and determined by atomic absorption spectrometry (AAS) (Spectr AA, 55B, Varian, Palo Alto, CA, USA) at the Department of Ecology, Environment and Plant Sciences, Stockholm University, Sweden. For Se measurements, the AAS instrument was equipped with a continuous hydride generator.

A bulk soil of 300 kg was dried at room temperature (20-25 °C) for 10 days, thoroughly mixed and sieved with a 5-mm mesh-diameter sieve. Six kg dried soil was allocated to each pot, supplemented with 150 mg kg^{-1} N, 22 mg kg^{-1} P, 83 mg kg^{-1} K, 10 mg kg^{-1} Zn, 5 mg kg^{-1} Mn and 7 mg kg^{-1} Fe by dissolving 1.43 g urea, 0.5 g $NH_4H_2PO_4$, 1.32 g KNO_3, 0.26 g $ZnSO_4.7H_2O$, 0.1 g $MnSO_4.H_2O$ and 0.7 g Fe-ethylenediamine-di(o-hydroxyphenyl) acetate in 200 mL distilled water, pouring and mixing this solution onto an aliquot of 300 g of soil, and then adding and mixing the latter to the whole soil sample of 6 kg. Urea was applied in three equal splits, once before sowing and two times during the growing period, providing a total of 150 mg N kg^{-1} soil. Se was added in the same way, i.e. by taking 4 aliquots of 300 g each from the dried soil and adding to either one a solution of 0 mg, 9.98 mg, 19.98 mg, and

79.90 mg $Na_2SeO_3.5H_2O$ in 200 mL distilled water, mixing the 4 aliquots separately, and adding them to 4 soil samples of 6 kg each, resulting in 4 soils supplemented with Se at 0, 0.5, 1, and 4 mg kg^{-1}. They were in factorial combination with three levels of irrigation water salinity at 0.12, 0.30, and 0.60 S m^{-1} (adopted as the lowest, moderate and highest salinity) with three replications, giving a total of 36 pots. The electrical conductivity of the tap water used was 0.12 S m^{-1}, so no salt was added to the tap water for low salinity. For an EC value of 0.30 S m^{-1}, 0.67 g $CaCl_2$ and 0.53 g NaCl were added to one liter of tap water, and 1.8 g and 1.4 g for an EC value of 0.60 S m^{-1}.

The fertilized soil not supplemented with Se, as control, and the three Se-supplemented soils were placed into polyethylene pots (37 cm height, 30 cm diameter), irrigated to field capacity every five days by weighing the pots and adding amounts of water equal to their weight loss, and left for 30 days to attain equilibrium. Then, eight seeds of wheat (*Triticum aestivum* L., cv. Falat), obtained from Khorasan Razavi Agricultural and Natural Research (Mashhad, Iran), were sown per pot and thinned to four uniform plants 10 days after sowing. The vegetation was performed under greenhouse conditions with a relative humidity of 70-80 %, a temperature of 26-29 °C/14-16 °C (day/night), and a photoperiod of 16/8 h at a photon flux density of 300 μmol m^{-2} s^{-1} provided by natural light supplemented with fluorescent lamps (Osram Vialox, Nove Zamky, Slovakia). During the growing period, the pots were weighed, irrigated and maintained at field capacity conditions every day. Since plants were small, irrigation was applied once a week, but increased to 4-5 times weekly at the end of the growing period, so a total volume of 6 L of the corresponding saline water was added per pot over the 60 days experimental period. Then, about 4 g of newly expanded leaf samples were taken from each pot for determination of the concentration of photosynthetic pigments, free proline, and catalase activity. Half of the leaves (2 g) were cut into smaller pieces, frozen in liquid nitrogen and stored at -80 °C for later analysis. Shoots were harvested 1 cm above the soil level and rinsed twice with distilled water, once with 20 mmol L^{-1} EDTA, and again once with distilled water. They were wiped dry with paper and oven-dried at 50 °C to constant weight. The DW of the shoots was recorded, and they were bagged separately and maintained in a cold room.

Determination of chlorophyll and carotenoid contents

Fresh leaf samples (0.2 g each) were homogenized using a mortar and pestle in 10 mL 96 % methanol in the dark at 4 °C. The extract was centrifuged at 2500 × g for 10 min and the supernatant was diluted to the four fold volume with methanol. Absorbance of the methanol-diluted supernatant was measured at 666 nm for chlorophyll *a*, 653 nm for chlorophyll *b*, and 470 nm for carotenoid using a spectrophotometer (CECIL, Cambridge, UK). The content of chlorophyll *a* and *b* and the carotenoid was calculated using the equations given by Dere, Gunes, and Sivaci (1998).

Determination of free proline concentration

Free proline was determined according to Bates, Waldren, and Teare (1973). Accordingly, 0.5 g fresh leaf sample was homogenized in 10 mL of an aqueous solution of 3 % (w/v) sulphosalicylic acid. The solution was filtered and 2 mL of the filtrate was reacted with 2 mL acidic ninhydrin and 2 mL glacial acetic acid for 1 h in a water bath at 100 °C. After cooling in an ice bath, the reaction mixture was extracted with 4 mL toluene and vortexed for 20 s. The absorbance at 520 nm was measured and the content of free proline was calculated using a standard curve for proline.

Catalase extraction and assay

Fresh leaf sample of 0.5 g was ground using mortar and pestle in 5 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7) in an ice bath and centrifuged at 10000 × g for 15 min at 4 °C. The supernatant was used for determination of catalase (CAT; EC 1.11.1.6) activity, based on the decomposition of H_2O_2 by monitoring the decrease in absorbance at 240 nm (an extinction coefficient of 39.4 L mol^{-1} cm^{-1}) at a final volume of 1 mL, consisting of 25 mmol L^{-1} phosphate buffer (pH 7.0), 10 mmol L^{-1} H_2O_2, and the crude enzyme extract (Hasanuzzaman, Hossain, and Fujita 2011).

Determination of the concentrations of Fe, Zn, and Se

The dried shoots were ground in a stainless steel Wiley mill (Fisher Scientific, Pittsburgh, PA, USA) to pass through an 18-mesh (1 mm) screen. Aliquots of 1 g of each sample and 1 g aliquot of *Lagarosiphon* reference material (*Lagarosiphon major*, CRM 60; Community Bureau of Reference - Commission of the European Communities) were placed into 70-mL Pyrex tubes (DWK Life Sciences, Wertheim, Germany), 15 mL of a mixture of concentrated nitric-perchloric acids (7/3, v/v) was added to each tube. The tubes were placed in a heating block (Tecator, Hoganas, Sweden) for wet digestion using a temperature program (Autostep; 50 °C, ramp 15 min, hold 1 h; 70 °C, ramp 15 min, hold 1.45 h; 100 °C, ramp 15 min, hold 1.45 h; 120 °C, ramp 15 min, hold 1.15 h; 150 °C, ramp 1.3 h, hold 1.3 h; 180 °C, ramp 15 min, hold 45 min; 225 °C, ramp 30 min, hold 3 h). The tubes were cooled and filled up to 10 mL using distilled water. Then, they were analyzed for Se, Fe and Zn by AAS (Banuelos and Meek 1990; Djanaguiraman, Prasad, and Seppanen 2010).

Statistical analysis

Data were analyzed by the Proc GLM procedure of SAS PC version 9.4 and the means were compared by Tukey's test (HSD). Differences at $P<0.05$ were considered significant.

Results

The increasing salinity reduced the concentrations of both forms of chlorophyll (Figures 1A and 1B). The chlorophyll *a* concentration at the highest salinity was 23 % lower than that at lowest salinity (Figure 1A). For chlorophyll *b*, 39 % reduction was noted. Se at 4 mg kg^{-1} reduced both chlorophyll *a* and chlorophyll *b* content, which was more pronounced at higher salinity. At lowest salinity, plants supplemented with 0.5 and 1 mg kg^{-1} Se had higher chlorophyll *b* concentrations than those without Se, but no beneficial effect of Se was found at the two higher salinities (Figure 1B).

As the salinity increased, the carotenoid concentrations decreased and compared to 0.12 S m^{-1} they were lower by about 19 % at 0.30 S m^{-1} and 24 % at 0.60 S m^{-1} (Figure 1C). At all three salinities, the carotenoid content was mostly unchanged with 0.5 or 1 mg kg^{-1} Se and was reduced by 4 mg kg^{-1} Se.

A substantial increase in the free proline content was observed in plants grown at the two higher salinities (17 % at moderate, 37 % at highest salinity), compared to the lowest salinity (Figure 2A). Regardless of the salinity level, the mean concentration of this amino acid was 71 µg g^{-1} for plants grown on non-Se supplemented soils and 84 µg g^{-1} for those grown on soil supplemented with 4 mg kg^{-1} Se. In contrast, catalase was adversely affected as at the highest salinity its activity was nearly 23 % lower than at lowest salinity (Figure 2B). Se, depending on its concentration, exerted a dual effect on catalase activity. At all three salinities, the activity of catalase increased when Se was added at 0.5 mg kg^{-1}, and decreased by about 25 % at a Se supplementation of 4 mg kg^{-1} (Figure 2B).

The Se content in shoots was positively influenced by Se supplementation and negatively affected by salinity (Figure 3A). The Se concentration in shoots rose from about 0.17 mg kg^{-1} DW in plants grown on soil not supplemented with Se to 14 mg kg^{-1} DW (approximately 80-fold) in those grown on soil supplemented with 4 mg kg^{-1} Se. Salinity had an antagonistic effect on Se absorption: at lowest salinity, the Se concentration in shoots increased by about 4.5 mg kg^{-1} DW for each mg kg^{-1} Se applied to soil, a value of 3.7 mg kg^{-1} DW was noted at moderate salinity, and at highest salinity the value was only 2.3 mg kg^{-1} DW (Figure 3A).

Fe and Zn in the shoots decreased with increasing salinity, viz lower by 23 % for Fe and by 30 % for Zn at 0.60 S m^{-1} relative to 0.12 S m^{-1} (Figure 3B and 3C), while the reduction in Zn concentration at salinity of 0.30 S m^{-1} relative to 0.12 S m^{-1} was negligible, decreasing to 73.2 mg kg^{-1} DW from about 76 mg kg^{-1} DW at 0.12 S m^{-1}. The corresponding Fe values were 92 mg kg^{-1} DW and 104 mg kg^{-1} DW.

The initial increase in absorption of Fe with Se at 0.5 mg kg^{-1} was followed by a decrease when the soil was supplemented with Se at 1 or 4 mg kg^{-1} (Figure 3B). In contrast, the uptake of Zn was not affected by Se at 0.5 mg kg^{-1} and decreased only for Se supplementation at 4 mg kg^{-1} (Figure 3C).

Increased salinity caused a remarkable reduction in the shoot DW (Figure 4), as at the highest salinity it was about 36 % lower than that at lowest salinity. Se had no beneficial effect on the shoot DW at none of salinities. At the lowest salinity, plants grown on soil supplemented with Se at 0.5 mg kg^{-1} tended to have better growth, but this increase was marginal (Figure 4) and at moderate and high salinities no increase was observed. For Se at 4 mg kg^{-1} soil, a considerable decrease in the shoot yield was observed, dependent on the

salinity level. At lowest salinity, Se (at any concentration) had no negative effect on the shoot DW. At moderate salinity, the shoot DW of plants supplied with Se at 4 mg kg^{-1} decreased by 14 %, at the highest salinity by 23 % relative to those without Se (Figure 4).

Discussion

Decrease in photosynthetic pigment concentrations under salinity might have different reasons. According to Ouzounidou et al. (2016), reduced chlorophyll content in plants subjected to stress is attributed to the destruction of lipids of the chloroplast membranes, to the inhibition of protochlorophyllide reductase, and to the prevention of chlorophyll biosynthesis caused by deficiency or imbalance of nutrients.

In plants grown at lowest salinity, Se at 0.5 mg kg^{-1} increased the chlorophyll *b* content, which is in agreement with the findings of Hajiboland et al. (2015) that Se caused an increase in chlorophyll *b* content in drought-stressed wheat. It has been demonstrated that nuclear and plasma membranes became blurred, swollen, or disintegrated under salinity stress, while Se sustained the integrity of chloroplasts and mitochondria and thus improved chlorophyll content. The positive effect of Se on the chlorophyll content is also attributed to its involvement in the protection of the chloroplast structure and to the role of this element in reducing oxidative damage induced by ROS (Kong, Wang, and Bi 2005; Jiang et al. 2017).

At high level, Se affected all photosynthetic pigments negatively. This is in agreement with the results recently obtained by Jiang et al. (2017). Se at high concentrations causes Fe deficiency in plants because there are strong interactions between both elements. This can result in a reduced chlorophyll content, since Fe is an essential component of the photosynthetic apparatus and plays a crucial role in the biosynthesis of chlorophyll and its precursors (Molnárová and Fargašová 2009; Guerrero et al. 2014).

Se also had a stimulating effect on the accumulation of free proline, a result confirmed by others including Mozafariyan, Kamelmanesh, and Hawrylak-Nowak (2016). By stimulating the activities of enzymes involved in proline metabolism, Se led to increased proline accumulation followed by enhancement of defense mechanisms (Ahmad et al. 2016).

Increased salinity caused a substantial reduction in Se concentrations in the wheat shoots. This is in agreement with the findings of Mikkelsen, Page, and Haghnia (1988) and Renkema et al. (2012) who showed that wheat subjected to salinity imposed by SO_4^{2-} and Cl^- ions and

simultaneously to SeO_4^{2-} accumulated less Se than those exposed to SeO_4^{2-} alone. Some researchers have pointed out that the inhibition of Se uptake at higher salinities may be caused by the toxicity of Cl^- to the cell membranes, whereas others argue that it arises from the antagonistic, competetive effect in the uptake of SO_4^{2-} on SeO_4^{2-}. Transport of both anions in plant roots is mediated by a common carrier in the root plasma membrane, and thus they compete for the same binding sites. A smaller antagonistic effect on Se accumulation has also been reported in the simultaneous presence of SeO_4^{2-} and Cl^- anions. Therefore, the uptake of Se is inhibited largely by SO_4^{2-} and, to a lesser extent, by Cl^- (Mikkelsen, Page, and Haghnia 1988; El Mehdawi et al. 2018).

Since Se increases the growth of plant roots, it appears that it improves access to Fe present in the soil by increasing the volume of plant roots enhancing the absorption of Fe (Simojoki, Xue, and Lukkari 2003). The data also indicated that Se increased wheat shoot yield only slightly, an expected result since Se is not considered essential for plant growth. Similarly, Hajiboland, Sadeghzadeh, and Sadeghzadeh (2014) showed that, although Se improved physiological and antioxidant parameters of wheat, it did not improve yield.

A considerable reduction in dry matter yield was noted at high concentrations of Se. It has been found that Se is toxic by two mechanisms, i.e. by misincorporation of selenocysteine and selenomethionine in place of cysteine and methionine in the protein structure, and by induction of oxidative stress. Se acts as a pro-oxidant at high concentrations and causes over-production of ROS, resulting in intensified oxidative stress and decreased yield (Gupta and Gupta 2017).

Conclusion

Salinity is a soil condition that occurs mainly in arid and semiarid regions as a serious limiting factor for crop growth and production. In order to alleviate some adverse effects of salinity on plants, different strategies have been developed. In the present investigation, some physiological and growth responses of salt-stressed wheat to Se were evaluated. It can be concluded that Se at 0.5 mg kg^{-1} improves Fe uptake and increases catalase activity although without growth-stimulating effect. The results obtained can lead to better understanding of the mechanisms of damage by salinity and may indicate a possibility how to use selenite for improving physiological responses and micronutrient status in wheat under salinity.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 1 Properties of soil used for the experiment

pH		8.1
OC		0.63 %
Saturation		37 %
N (Total)	mg kg^{-1}	600
EC_e	S m^{-1}	0.09
P (Available)	mg kg^{-1}	8.4
K (Available)	mg kg^{-1}	220
Zn (Available)	mg kg^{-1}	1.6
Mn (Available)	mg kg^{-1}	10.3
Fe (Available)	mg kg^{-1}	4.4
Se (Available)	µg kg^{-1}	2.6

Figure captions

Figure 1 Effects of Se added to soil on chlorophyll *a* (A), *b* (B), and carotenoid (C) content in wheat grown at different salinities. Means (± SD, N = 3) with different letters on top of the bars are significantly different (P<0.05) according to Tukey's LSD test.

Figure 2 Effect of Se added to soil on free proline (A) and the activity of catalase (B) in wheat grown in different salinities. Means (± SD, N = 3) with different letters on top of the bars are significantly different (P<0.05) according to Tukey's LSD test.

Figure 3 Effect of Se concentration on Se (A), Fe (B), and Zn (C) content in the shoots of wheat grown in different salinities. Means (± SD, N = 3) with different letters on top of the bars are significantly different (P<0.05) according to Tukey's LSD test.

Figure 4 Effect of Se concentration on the DW of wheat shoots in different salinities. Means (± SD, N = 3) with different letters on top of the bars are significantly different (P<0.05) according to Tukey's LSD test.

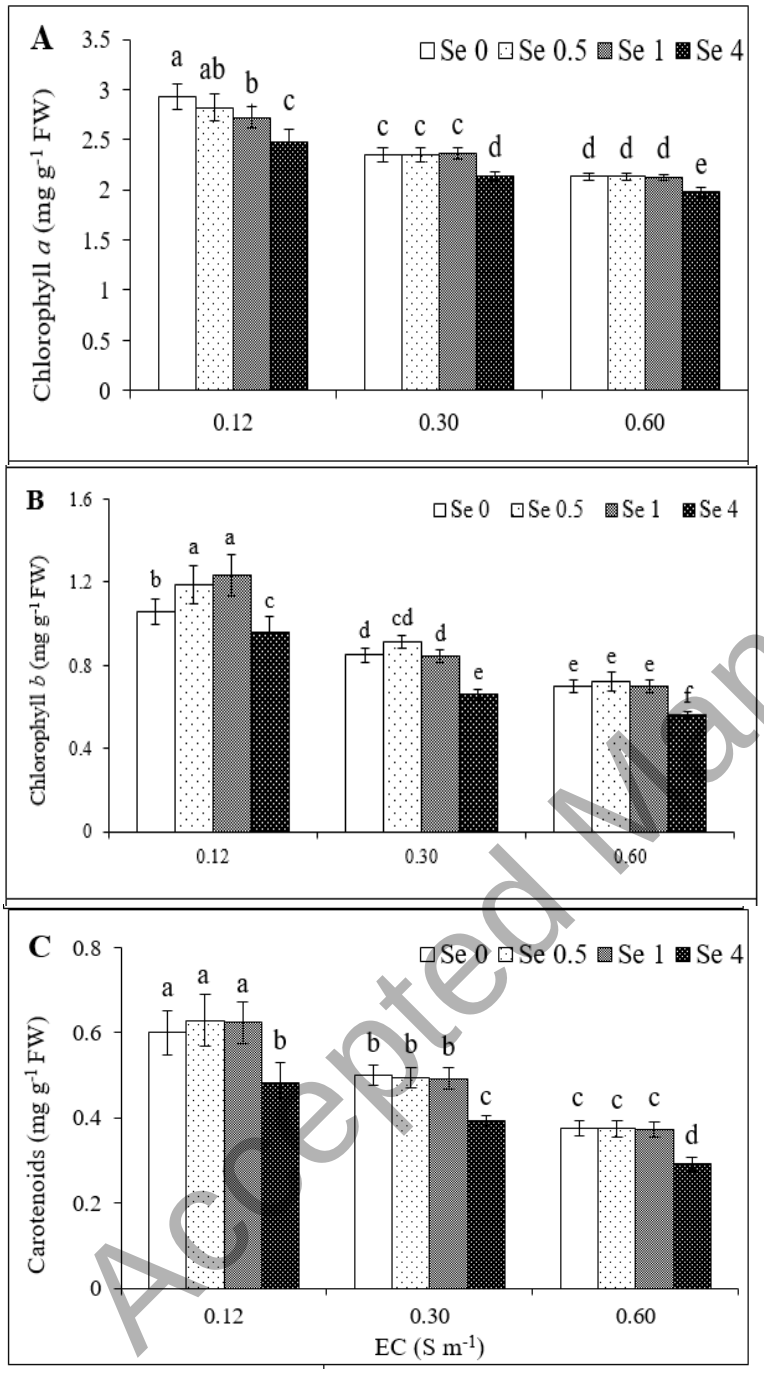

Figure 1

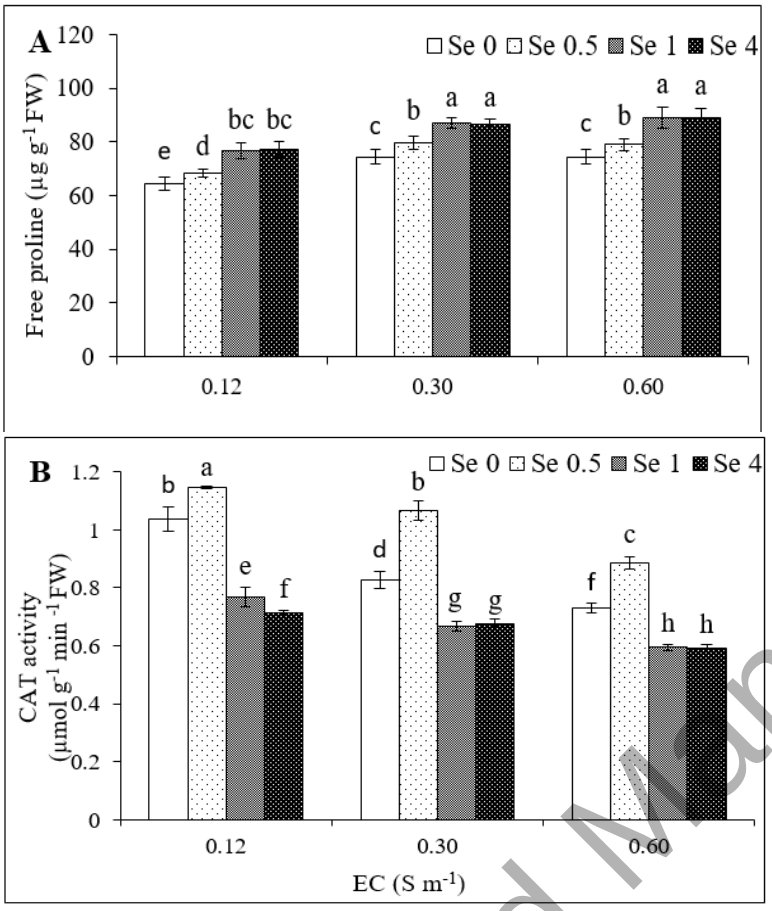

Figure 2

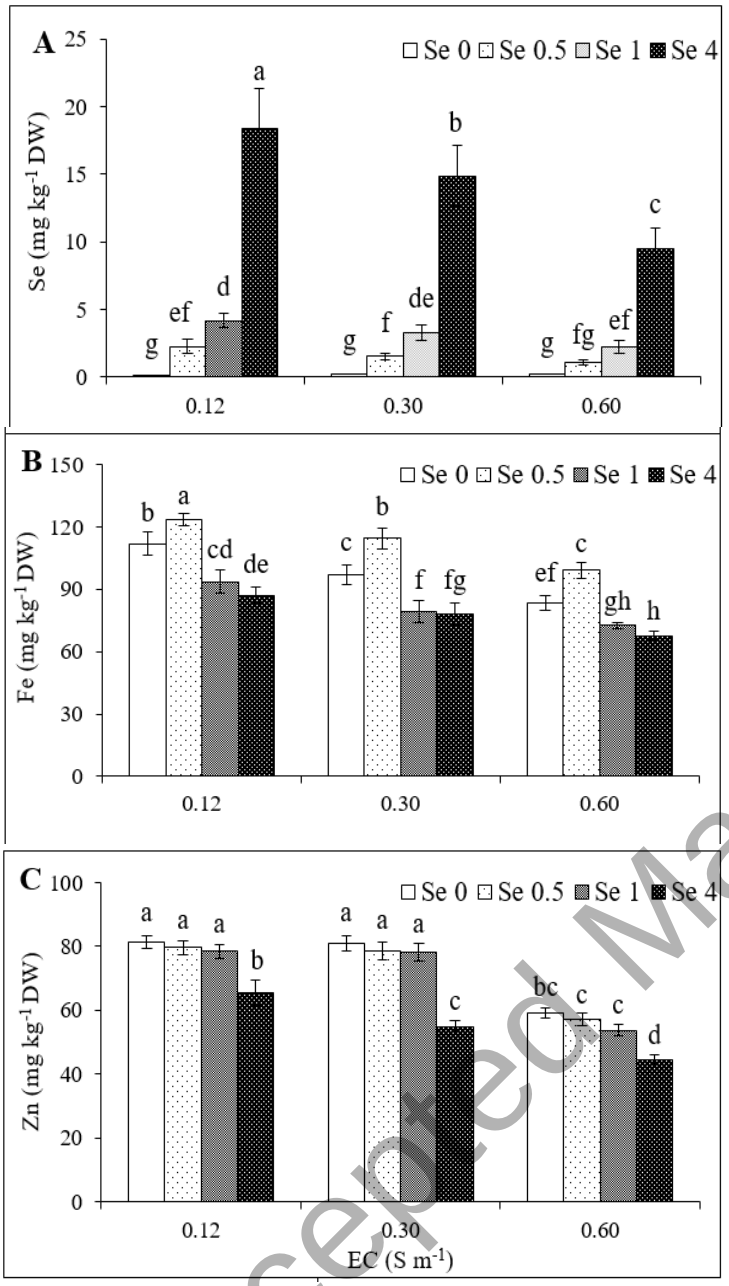

Figure 3

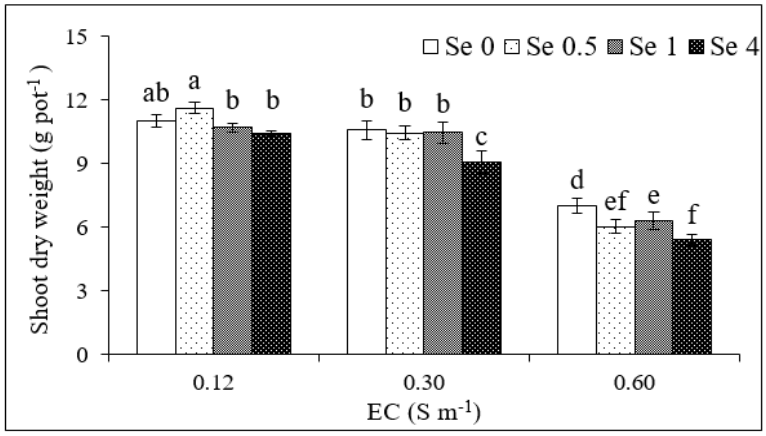

Figure 4