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EFFECTS OF MICRONUTRIENTS ON THE VEGETATIVE GROWTH AND SHOOT NUTRIENT STATUS OF CHICKPEA (*CICER ARIETINUM* L.) GROWN UNDER DROUGHT STRESS CONDITION

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Abstract

This study evaluated the effects of micronutrients (B, Fe and Zn) on the vegetative growth and shoot nutrients (K, Ca, Fe and Zn) status of chickpea (*Cicer arietinum* L.) grown under different moisture regimes (50%, Well-Watered; 25%, Mild-Stressed and 12.5%, Severe-Stressed) in greenhouse condition. Micronutrients were supplied alone and in combination as foliar spray to plants with severe-stressed condition. Results showed a significant decline in shoot height ($p < 0.0001$), fresh weight ($p < 0.0001$), dry weight ($p < 0.0001$) and relative water content ($p = 0.0001$) from well-watered to severe-stressed condition, although no remarkable change was observed after the application of micronutrients. Significant reduction in root fresh weight ($p = <0.0001$) and dry weight ($p = 0.0071$) was also seen in severe-stressed condition compared to that in the well-watered condition. Micronutrients were found to stimulate growth of root dry weight and root length under severe-drought condition. Significant increase of leaf area ($p = <0.0001$) and decrease of leaf chlorophyll content ($p = <0.0001$) were noted under severe-drought condition compared to that of well-watered condition. Concentrations of K, Ca, Zn and Fe increased in plant shoot tissue after the application of micronutrients although depending on the combination of treatments. Results thus indicated a promising role of micronutrient use in the amelioration of drought stress for the cultivation of chickpea.

Introduction

Legumes are important crop plants since they provide protein rich diet for humans and livestocks[(1)]. Legume foods supply plenty of health benefits as they are rich in vitamins, minerals, folic acids and secondary metabolites[(2,3)]. These plants are also used as soil quality improver because their tissue contains elevated N contents[(4)]. Legumes, therefore, have the ample potentials to help achieve sustainable agriculture, livelihoods as well as food security and safety for the rural people.

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Drought is one of the most important limiting factors for the yield of crop plants. A number of factors including global warming, lowering underground water level and reduced precipitation cause onset of drought condition[5]. It has become a serious threat to food security and livelihoods in many developing countries including Bangladesh that are vulnerable to impacts of global climate change. Growth and yield of legumes are sensitive to drought stress not only in the arid and semi-arid regions but also in areas where water shortage is becoming a constraint. Therefore, alleviating drought stress in agriculture has become an essential requirement to meet the increasing food demand across the globe. Nevertheless, it is important to find rapid and affordable approaches to mitigate drought stress for crop plants.

Amelioration of drought stress in crop plants through application of micronutrients offers multiple benefits including enhanced nutritional food value for humans and livestocks. Although macronutrients are well recognized for boosting yield of crop plants relatively less attention is paid on the use of micronutrient despite their immense potentials in providing biofortified foods and stress mitigation in agriculture[6,7]. Furthermore, although B, Fe and Zn play important role in growth, development and drought tolerance of plants[8,9], their role in alleviating this stress condition has not yet been well studied for legumes. The present study, therefore, aimed to investigate the effects of B, Fe and Zn on the vegetative growth and shoot nutrient status of chickpea (*Cicer arietinum* L.) grown under water-stressed condition. The findings of this study will thus provide insights into the functional role of micronutrients in conferring drought tolerance and hence pave the way to achieve better management of drought stress for the cultivation of chickpea.

Materials and Methods

Preparing seeds and pots for the experiment: Seeds of chickpea (*C. arietinum* L.) variety BARI Chola-7 were collected from Bangladesh Agricultural Research Institute, Joydebpur, Gazipur to use in this study. Before germination, seeds were surface sterilized by soaking into 3% sodium hypochlorite (NaOCl). Seeds were found more than 95% viable on germination test. Plastic pots of 1.5 liter in volume were used to grow plants. Soils collected from the Botanical Garden, University of Dhaka were sieved through a 2-mm mesh size to remove litters, roots and rocks. Then, 500 g of this soil was kept into a pot. Soil used was silt-loam in texture. The other properties of soil included pH 6.4, electrical conductivity 173 μS/cm, organic C 0.41%, total N 0.076%, total P 0.063%, Ca 12,900 ppm, Mg 11,705 ppm, K 32,103 ppm. Among the micronutrients, concentrations of Fe and Zn were 35,215 and 101.4 ppm, respectively while that of B was below detection level.

Growing plants in the greenhouse condition: Seeds were germinated in the Petri dish under controlled laboratory condition. Then, 3-days old seedlings were transferred to the pots kept in the greenhouse located at the University of Dhaka campus. Light intensity used was 1,629 lux measured by Illumination meter (Topcon IM-2D, Japan) and temperature was maintained 17^{o}C at 12 h day/night. Two weeks after the emergence of cotyledons, plants were subjected to treatments of soil moisture and micronutrients. Plants were grown in three replicated pots for each treatment.

Preparation of treatment conditions: In order to grow plants, pots were filled with soil with the moisture content of 50% (Well-Watered, WW), 25% (Mild-Stressed, MS) and 12.5% (Severe-Stressed, SS) conditions. The three micronutrients opted in the study included Fe, Zn and B. In this experiment, 0.5% Fe, 0.5% Zn and 0.2% B solutions as well as their combinations were prepared (Table 1). Sources of Zn, Fe and B were Zinc sulphate heptahydrate (Z_nSO_4. $7H_2O$), Iron (II) sulfate heptahydrate ($FeSO_4$. $7H_2O$) and Sodium tetraborate decahydrate ($Na_2B_4O_7$. $10H_2O$), respectively. Nutrients were applied at one week interval by spraying solutions uniformly on the leaves of plants grown for three weeks. In order to maintain the moisture treatments constant during the period of experiment, the pots were weighed every alternate day, and watered if the weight was lost by evapotranspiration as per the approach described elsewhere(10).

Table 1. Different soil moisture regimes and foliar micronutrient spray treatments applied to the chick pea plants in the experiment

Sl.	Treatments	Abbreviation
1	Well-watered condition, 50% moisture	WW
2	Mild Stress condition, 25% moisture	MS
3	Severe Stress condition, 12.5% moisture	SS
4	SS + Foliar spray: 0.5% Zn ($ZnSO_4$. $7H_2O$)	SS + Zn@0.5%
5	SS + Foliar spray: 0.5% Fe ($FeSO_4$. $7H_2O$)	SS + Fe@0.5%
6	SS + Foliar spray: 0.2% ($Na_2B_4O_7$. $10H_2O$)	SS + B@0.2%
7	SS + Foliar spray: 0.5% Zn + 0.2% B	SS + Zn + B
8	SS + Foliar spray: 0.5% Zn + 0.5% Fe	SS + Zn + Fe
9	SS + Foliar spray: 0.2% B + 0.5% Fe	SS + B + Fe
10	SS + Foliar spray: 0.5% Zn + 0.5% Fe + 0.2% B	SS + Zn + B + Fe

Measurement of shoot growth parameters: Shoot height was measured with the help of a ruler scale at the time just before harvesting of plants. Shoot parts were cut and weighed for the fresh weight. Then, shoots were oven dried at 80^{o}C for 24 hours for the dry weight. Temperature for drying leaves in the oven was set on the basis of the results obtained from previous study(11). Relative water content of plant shoot was determined by soaking plants into water for 24 h and taking the weights with the help of an electric balance before and after soaking.

Determination of leaf growth parameters: After spreading on a plain glass sheet, leaf length and width were determined by using a ruler scale. Fully expanded youngest leaf was taken to determine leaf area. Leaf area (LA) was determined by using the formula, LA (cm^2) = 0.73 ∗ length ∗ width, where, 0.73 is the correction factor for determination of leaf area in Fabaceae family(12). Leaf length and width were also determined by following standard protocol(13). Leaf chlorophyll content was determined with the help of a chlorophyll meter

(SPAD-502 Plus, Konica, Minolta, Japan). Three fully expanded youngest leaves per plant and three plants per pot were selected to determine chlorophyll content.

Measurement of root growth parameters: Root systems were spread on a tray with water and length of the longest root was measured with the help of a ruler scale. Root systems were cleaned carefully by gentle flow of tap water. Excess soil and debris were removed from the root with the help of blotting paper. Then, fresh weight of root was taken. Roots were kept into separate envelops and then oven dried at 80^{o}C for 24 h and weighed for the dry weight.

Determination of nutrient concentration in shoot tissues: Leaves dried in oven (Model-Json-100) at 80^{o}C for 24 h were taken into mortar pestle for grinding. Finely powdered plant materials of 0.1g were measured in a digital weight balance and taken to make pellet into a pellet maker (Specac, UK). Then, 5-ton pressure was applied to make a pellet. After preparing a pellet, it was placed on the cadmium (Cd-109) source section of Energy Dispersive X-ray Fluorescence (EDXRF) machine. Finally, analysis was performed from the EDXRF reading using OLCAL21J calibration. As the analysis was based on direct comparison, the standards of similar matrices were used to construct the calibration curve in order to avoid any matrix effect. Concentrations of two macronutrients, potassium (K) and calcium (Ca) and two micronutrients, zinc (Zn) and iron (Fe) were measured by EDXRF technique available in the Chemistry Division, Atomic Energy Centre, Dhaka.

Statistical analysis: One-way Analysis of Variance was performed by using JMP 4.0 software (SAS Institute, Carry, NC, USA) to examine the effects of treatments on the studied parameters. Tukey-Kramer HSD test was done to compare the level of significance among the means values.

Results and Discussion

Effects of moisture and micronutrient treatments on shoot parameters: A significant decline in shoot height ($p < 0.0001$), fresh weight ($p < 0.0001$), dry weight ($p < 0.0001$) and relative water content ($p = 0.0001$) was observed from well-watered to severe-stressed condition, although no growth stimulation of these parameters was found after application of micronutrients (Fig. 1). Detrimental effects of water deficits on plant growth parameters were reported by previous study[14-16]. These results suggest that water deficiency retarded the shoot growth causing the stunted growth of plants.

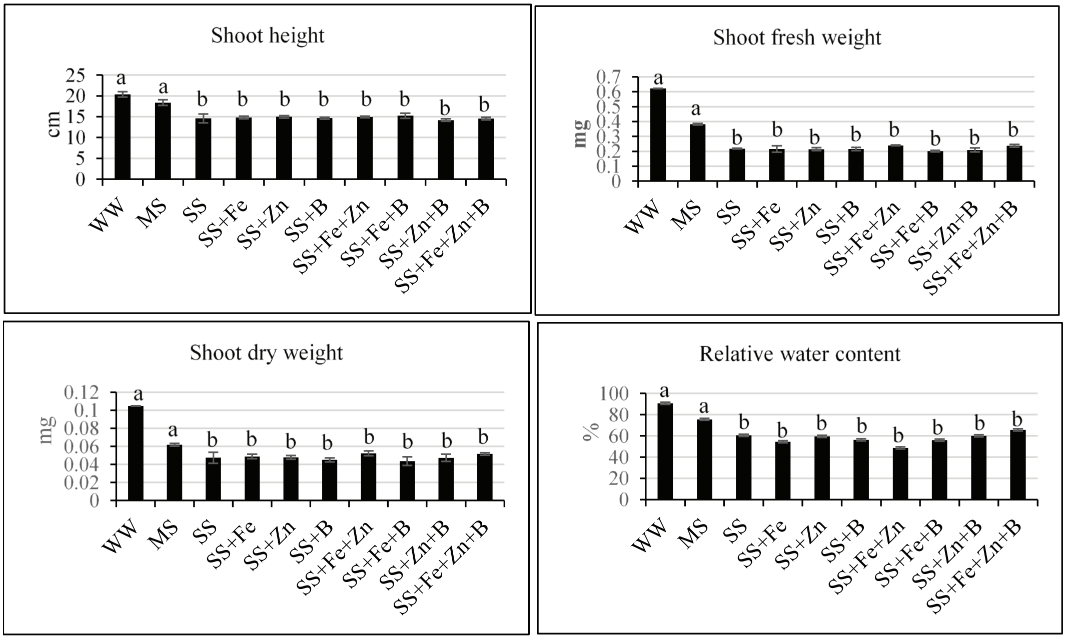

Fig. 1. Shoot height, fresh weight, dry weight and relative water content of chickpea plant under different soil moisture (50%, Well-Watered, WW; 25%, Mild-Stressed, MS; 12.5%, Severe-Stressed, SS) and micronutrient (Fe, Zn and B) treatments. Different letters indicate significant difference among the treatments. Tukey-Kramer HSD test was done to compare the level of significance among the means values.

Effects of moisture and micronutrient treatments on root growth parameters: Significant effects of treatments were found on the studied root parameters including fresh weight ($p = <0.0001$), dry weight ($p = 0.0071$) and root:shoot length ($p = 0.0008$) except root length that was not statistically significant ($p = 0.2485$: Fig. 2). Data showed that mean values of root length and root:shoot ration were relatively higher in severe-stressed condition than well-watered condition. On the other hand, root fresh and dry weights drastically decreased under severe-stressed condition compared to well-watered condition. Root dry weight was notably higher under the treatments of B, Fe and Zn alone or in combination than that of the severe-stressed condition. Roots played an important role in adaptation of plants under drought condition perhaps by increasing its length in search of water. Results of the present study, thus, corroborated with the findings of other studies that reported increased root length and root:shoot length of a number of crop plants grown under abiotic stresses such as drought[14-18] and saline[19,20] conditions. Drought resistance is characterized by an extensive root growth and small reduction of shoot growth in dry conditions[21].

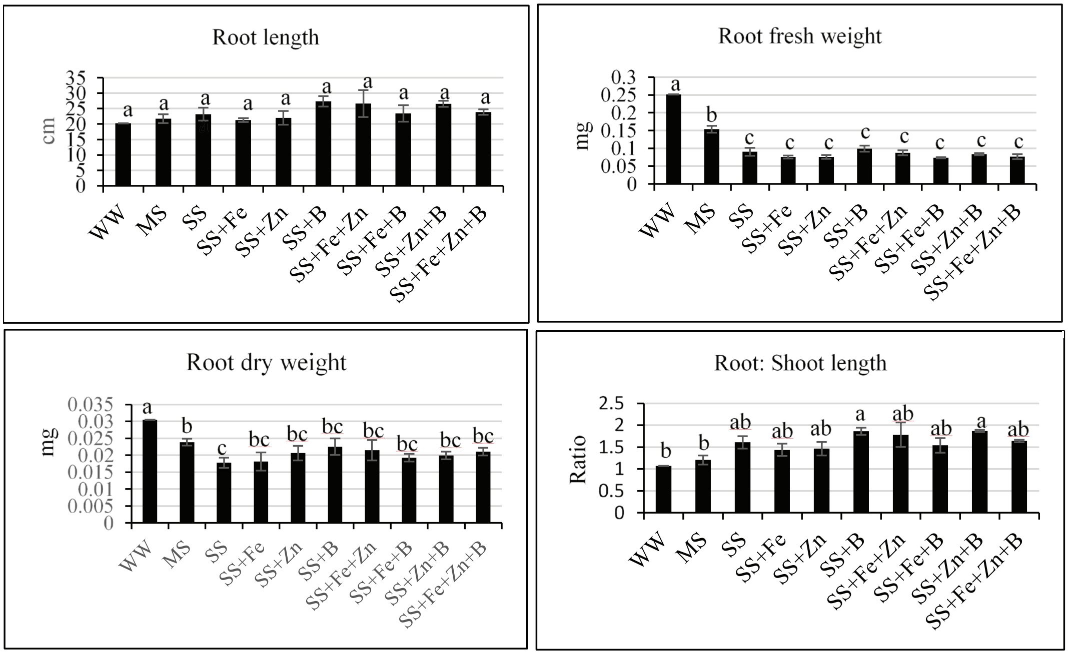

Fig. 2. Root length, fresh weight, dry weight and root:shoot length of chickpea plant under soil moisture (50%, Well-watered, WW; 25%, Mild-stressed, MS; 12.5%, Severe-stressed, SS) and micronutrient (Fe, Zn and B) treatments. Different letters indicate significant difference among the treatments. Tukey-Kramer HSD test was done to compare the level of significance among the means values.

Effects of moisture and micronutrient treatments on leaf parameters: Effects of soil moisture and micronutrient treatments on leaf parameters are shown in Fig. 3. Although leaf length did not differ significantly ($p = 0.4357$) among the treatments, it was higher in micronutrient treatments than in well-watered to severe-stressed conditions. Significantly higher values of leaf width ($p = <0.0001$) and area ($p = <0.0001$) were found in severe-stressed condition than the well-water condition. Similar results were found by other studies where application of micronutrients (e.g. B and Zn) stimulated leaf area[22]. Increased leaf area could help plants increase the leaf size resulting expansion of photosynthetic area to capture maximum sunlight for photosynthesis during drought condition. Leaf chlorophyll content, on the other hand, decreased significantly ($p = 0.0081$) and gradually from well-watered to sever-stressed conditions and it did not increase remarkably with the application of micronutrients. Drought stress led to lower chlorophyll content[15,23].

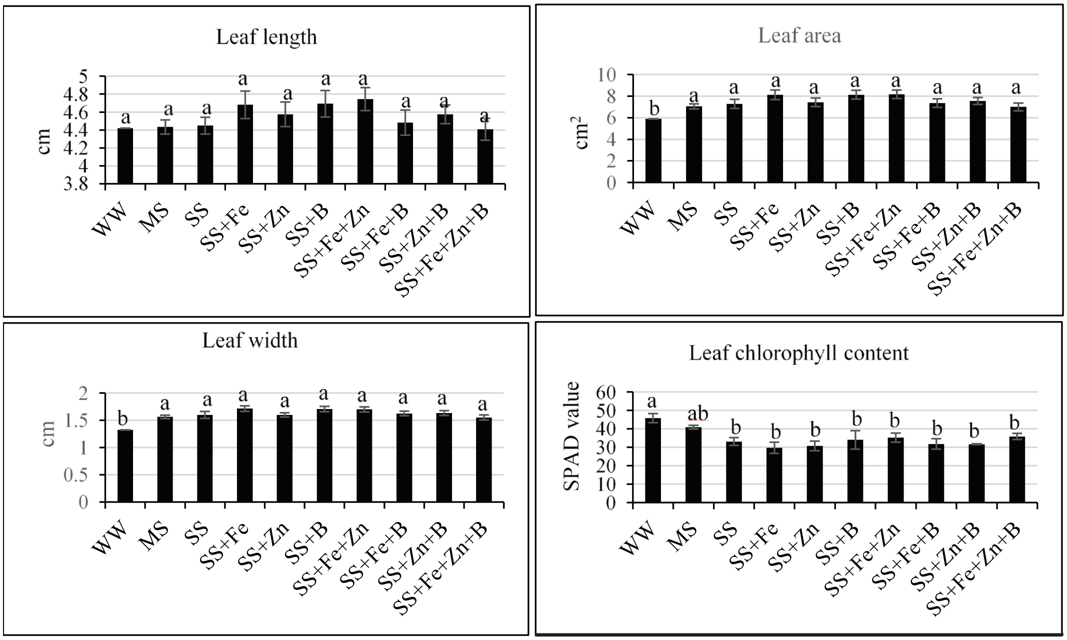

Fig. 3. Leaf length, width, area and chlorophyll content of chickpea plant under different soil moisture (50%, Well-watered, WW; 25%, Mild-stressed, MS and 12.5%, Severe-stressed, SS) and micronutrient (Fe, Zn and B) treatments. Different letters indicate significant difference among the treatments. Tukey-Kramer HSD test was done to compare the level of significance among the means values.

Effects of moisture and micronutrient treatments on shoot nutrient status: Significant effects of soil moisture and micronutrient treatments were found on the concentrations of K ($p < 0.0001$), Ca ($p < .0001$), Fe ($p < 0.0001$) and Zn ($p < 0.0001$) of the shoot tissue (Fig. 4). Concentration of K decreased gradually with the increase of soil moisture deficit. The maximum K concentrations were found under the combined application of B and Fe followed by that of B, Fe and Zn compared to even that in the well-watered condition. Although Ca concentration in the shoot tissue did not differ significantly among the moisture treatments it was significantly higher in the combined treatments of Fe and Zn followed by Fe and B as well as B alone where the values were higher than that in the well-watered condition. The concentration of Ca in the shoot under these two treatments was much higher than that in the soil. Fe concentration was higher in mild- and severe-stressed conditions than the well-watered condition indicating an accumulation of this element in plant shoot under drought condition. Concentration of Fe was higher in the shoots under the treatments where Fe was applied either alone or in combination with B and Zn. However, Fe concentrations in shoot tissue under all treatments was much lower than that in the soil. Optimum amount of K maintains cellular membrane stability, osmotic adjustments and detoxify the reactive oxygen species (ROS), thereby, contributes towards the sustainable crop growth in stress condition[24]. Previous studies reported that K enabled plants adapt with drought stress via regulation of morphology, physiology including root exudation and signal pathways as well as rhizosphere community[25,26]. Role of Ca on drought tolerance of maize by influencing growth, tissue water, proline content and H2O2 activity was reported in other study[27]. Data demonstrated that although single or combined application of Fe increased the shoot Fe concentration in plants, the values were much lower than that in the soil.

Increased Zn concentration was noted in shoot tissue when B, Zn and Fe were applied to the drought-stressed plants alone or in combination of them except single application of Fe. Concentrations of Zn in the shoot tissues under these treatments were higher than that in soil under the same treatments. Zn helped plants counteracted drought by improving membrane stability, hormone synthesis, the photosynthetic process and the scavenging of ROS[7,28]. Other studies also reported an enhanced concentration of micronutrients by the application of B, Zn and Fe[15,29]. Micronutrients (B, Zn and Fe) play pivotal role by functioning as the co-factors of the enzymes in stimulating the activities of antioxidant enzymes, reducing ROS as well as maintaining osmotic adjustment and tolerance under drought stress[30,31]. Overall, the results of the present study indicated a promising role of the studied micronutrients (B, Zn and Fe) on the growth and mineral nutrient status of the shoot tissue of chickpea. However, further studies are suggested to ascertain the dose effects as well as biochemical and molecular characteristics of the effects of micronutrients for enhanced understanding of drought tolerance in chickpea.

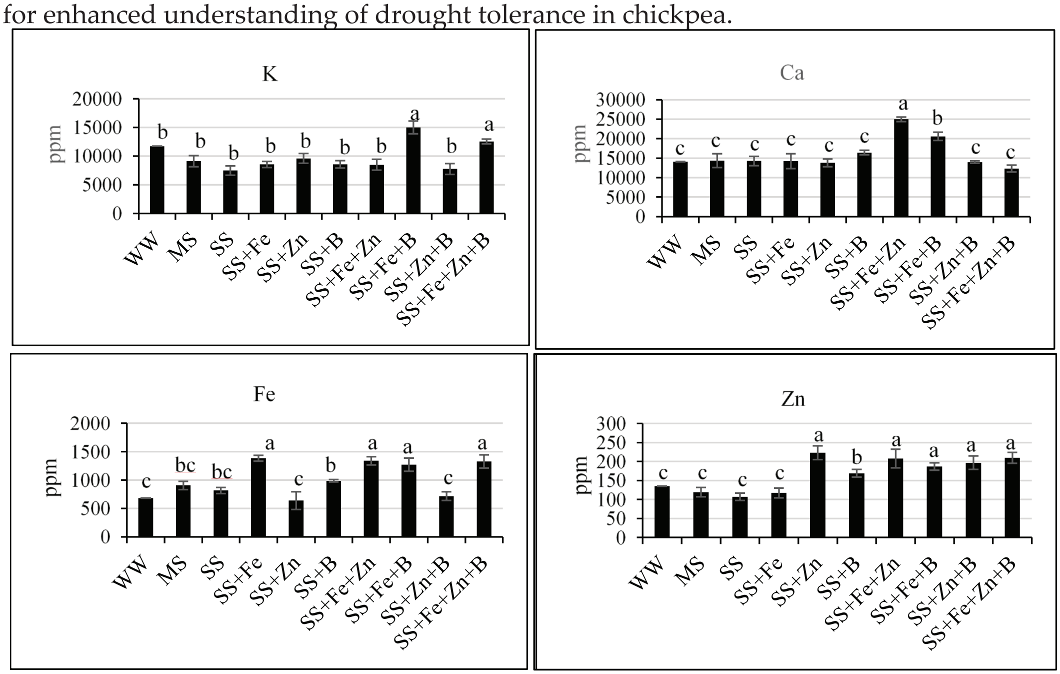

Fig. 4. Concentrations of K, Ca, Fe and Zn in the shoot tissue of chickpea grown under soil moisture (50%, Well-watered, WW; 25%, Mild-stressed, MS and 12.5%, Severe-stressed, SS) and micronutrient (Fe, Zn and B) treatments. Different letters indicate significant difference among the treatments. Tukey-Kramer HSD test was done to compare the level of significance among the mean values.

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