

Exogenous Application of Zinc and Manganese for Improve Chemical Constituents in *Brassica Juncea* under Drought Stress

Seema Ali¹, Shabeena^{1,2}, Husna¹, Laila Aziz¹, Zubia Rahim², Sidra Sahar²,
Nousheen Pervez¹ and Humaira Gul^{1*}

¹Department of Botany, Abdul Wali Khan University, Mardan, Khyber Pukhtunkhuwa, Pakistan.

²Department of Botany, Women University Swabi, Khyber Pukhtunkhuwa, Pakistan.

Received: February 22, 2017

Accepted: May 3, 2017

ABSTRACT

During germination, plants are exposed to various biotic and abiotic stress. Drought stress is a biotic stress which effect plant growth efficiency. To determine the influence of exogenous application of Manganese (as manganese sulfate) and zinc (as zinc sulphate) on chemical constituents (Reducing and non-reducing sugars, total carbohydrates, total proteins, total flavonoids, total phenols and total antioxidant status) of *Brassica juncea* grown under drought stress, an experiment was conducted in the botanical garden of Abdul Wali Khan University Mardan. Plants were subjected to drought stress as 7, 14 and 21 days. As foliar spray zinc sulphate (Zn) and manganese sulfate were applied alone and in combination at concentrations of 3000mg/L and 4000 mg/L. Results showed that total proteins, total carbohydrates, reducing and non-reducing sugars and total antioxidant status showed significant increase while phenols and total flavonoids showed significant reduction under drought stress. Plants that treated (foliar spray) with different concentrations of ZnSO₄ and MnSO₄(3000 and 4000 mg/l) showed significant improvement in above mentioned parameters in control as well as in drought stress plants.

KEYWORDS: Drought, antioxidant status, total carbohydrates, total proteins, phenols, flavonoids.

INTRODUCTION

Abiotic stress means any negative impact of non-living factors of the environment on the most advantageous performance of an organism. In both natural and agricultural systems plants are commonly encountered by abiotic stress like high and low temperature, drought, alkalinity, salinity, UV stress, flooding, high wind or oxidizing agents and toxicity [1]. Two conditions either surplus of water or water deficiency may cause water stress. Plant growth and its efficiency is affected by drought stress[2, 3] The sternness of water stress is random as it rely on various factors like rainfall distribution, nature of climates and nature of soils [4].

To improve the plant growth the term drought managing implies that human interruption can decrease susceptibility and shocks. Roots and foliage are the organs through which plants absorb nutrients. The nutrients which are needed in great quantities are mostly supplied to plants through roots than through foliage. Foliar application of the nutrient are preferred when the soil conditions are unfavorable. For foliar application, fertilizer materials are needed in smaller quantities than when supplying to the soil. Since long in literature, the important mineral elements are known having role in development and growth of plants [5].

One of the most important genus within the family Cruciferae is *Brassica*, having 37 different species. In the tribe Brassiceae, *Brassica juncea* is one of 51 genera and economically the most fundamental genus of the family [6]. It has high medicinal values and used to cure different diseases. Its leaves used as antiscorbutic, diuretic, stimulant and stomachic, relieve headache, Muscular and skeletal pains, diaphoretic. It is also used as liniment for rheumatic pain Anti helmintic, anti dysentric, diaphoretic, Fever and cold, for bladder inflammation or hemorrhage in china [7].

One of the critical element for the growth in animals and plants is the zinc (Zn). It is used as a plant nutrient as well as essential for various plant processes like oxidation reduction reactions, enzymatic reactions and metabolic activities. The Zn acts a key role by effecting plant metabolic activities like cytochrome synthesis, stabilization of ribosomal fractions and hydrogenase and carbonic anhydrase activities [8, 9]. Poor growth, undersized petioles and internodes and small leaves are the common symptoms of Zn deficiency which results in the “rosette” symptom in the primary growth stages of dicots and “fan shaped” stems in monocots [10].

Manganese (Mn) is an important micronutrient in most organisms. Certainly, it is common to see a peppering of brighter, cleared spots across the leaf blade and necrotic pits develop. Initially, the deficiency of Mn effect the rapidly increasing leaves, a few nodes back from the tip. But as the symptom intensifies, both older and younger

leaves are affected [11]. Present study was under taken in order to determine the results of drought and foliarly applied micronutrients (Manganese and zinc) on some biochemical aspects of *brassica juncea*.

MATERIAL AND METHODS

The present experiment was performed to examine the effect of zinc sulfate and manganese sulfate on relative water content, leaf water loss, electrolyte leakage and pollen viability, total phenols, total flavonoids and total antioxidants of *Brassica juncea* grown under drought stress. Seeds of *Brassica juncea* were obtained from Agricultural Research Institute, Tamab Peshawar, Khyber Pukhtunkhwa.

Growth condition and treatments:

For this experiment 84 pots were used. Seven sets were made from these 84 pots. Detail of these 7 sets is follows:

1st Set: With no zinc and manganese, the set consists of control and three treatments of drought (7, 14 and 21 days).

2nd Set: Zinc applied as zinc sulfate @ 3000 mg/L, the set consists of control and three drought treatments (7, 14 and 21 days).

3rd Set: Zinc provided a zinc sulfate @ 4000 mg/L, the set comprises of control and three drought treatments (7, 14 and 21 days).

4th Set: Manganese applied as manganese sulfate @ 3000 mg/L, the set consists of control and three treatments of drought (7, 14 and 21 days).

5th Set: Manganese provided as manganese sulfate @ 4000 mg/L, the set comprises of control and three drought treatments (7, 14 and 21 days).

6th Set: Zinc and manganese provided as zinc sulfate and manganese sulfate in combination @ 3000 mg/L, the set comprises of control and three drought treatments (7, 14 and 21 days).

7th Set: Zinc and manganese provided as zinc sulfate and manganese sulfate in combination @ 4000 mg/L, the set comprises of control and three drought treatments (7, 14 and 21 days).

These plastic pots of 17.5 cm in diameter and 6.5 cm deep have outlet at the base for drainage. Out of 7 sets, each set comprises of 12 pots with 4 treatments i) control (non-saline), ii) 7 days drought, iii) 14 days drought and iv) 21 days drought. For each treatment 3 replicates were kept. 1 Kg sandy loam soil was washed carefully and filled in each pot. Strong Hoagland's solution was used for saturation of soil of every pot. 0.1% mercuric chloride was used for surface sterilization of seeds for one minute. After this with distilled water these seeds were washed. Seeds were used of approximately equal number and of uniform size. In every pot 5 seeds were sown. Pots were then irrigated daily, each with 50 ml of tap water i.e., with an equal amount. When plants were fully germinated, they were thinned out (one seedling per pot). All these 84 pots were then placed in the Botanical Garden of Department of Botany, Abdul Wali Khan University, Mardan. Drought treatment was given and then tap water of 1.5L was used for irrigation of every pot as twice per week. As drought treatment was completed, zinc sulfate and manganese sulfate were given exogenously through foliar spray to pots of different sets in different concentrations.

Reducing Sugars Estimation:

The reducing sugars were estimated by applying arsenomolybdate reagent introduced by Nelson, 1944 [12] for colorimetric determination of the cuprous oxide formed in the oxidation of the sugars by alkaline cooper tartarate reagent.

Total Carbohydrate Content:

According to modified anthrone reagent total carbohydrate content was determined following Fales, 1951 protocol [13].

Analysis of Total Protein:

After extraction, total proteins were analyzed by Bradford method [14].

Estimation of total Phenol:

total phenolic contents of leaves were estimated by following Singh and Malik protocol [15].

Total Flavonoids Estimation:

Total flavonoid content of the sample extracts was concluded by using the method of aluminium chloride [16]

Total Antioxidants:

Modified method was used for determination of the ferric ion reducing power capability of samples [17].

Experimental design and statistical analysis: The experimental design was completely randomized Design (CRD) with three drought levels and three replicates. Collected DATA was examined statistically by SPSS software for analysis of variance (ANOVA), and the Duncan's multiple range test ($P < 0.05$) was used to compare the means.

RESULTS AND DISCUSSION

Reducing Sugar and Non-Reducing Sugar:

In a large series of plants grown at low level of humidity, the amount of sugars have been long known to increased [18]. In our observation plants treated with drought stress pronounced significant ($P < 0.05$) increase in total soluble sugar (reducing and non-reducing sugar) level Fig. 1 & 2. Both in germinated embryos and endosperm a significant increase in DW from 0 h to 14 h of stress infliction corresponded with an enhancement in total soluble sugar content and reducing content [19]. These results can be compared with the previous annotations where infliction of water stress was related to a high level of sugars in soybean seedling [20], corn [21], in the seeds and leaves of wheat [22], chickpea [23] and cotton [24]. Effective osmoregulation under water stress conditions is also helped by this enhancement in the sugar levels of stressed tissues [25]. Our observation also stated that plants treated with ZnSO_4 and MnSO_4 alone or in combine form showed significant ($P < 0.05$) increase in the level of total sugar, which showed resemblance with the consequence of Prakash *et al.* [26] who illustrated that the significant improvement in reducing sugar was noticed by applying micro nutrients and the fruits of treated trees showed more reducing sugar as compared to control. Among the three micro nutrients zinc proved more beneficial expressing. This result is in agreement with those obtained by Abdel-Ghany [27] and Ahmed [28]. They also stated the same pattern in their reports.

Total Carbohydrates:

In our observation drought treated plants showed significant ($P < 0.001$) increase in total carbohydrate level in plant Fig. 3. Our findings are in concurrence with the results of Mafakheri [29]. He observed that during both the flowering and vegetative phases in drought resistant chickpea cultivars water soluble carbohydrate is increased significantly by severe drought stress. Praxedes [30] and Asish [31] also observed similar results in their experiments. We also reported through our observations that plants treated with different doses of ZnSO_4 and MnSO_4 (3000mg/l, 4000mg/l) showed significant ($P < 0.05$) increase in total carbohydrate content. Observation of Aline [32] also reported the same pattern that higher contents of total carbohydrate and soluble carbohydrates by foliar application of Zn were enhanced as compare to control plants. In our experiment plants treated with different doses of MnSO_4 (3000mg/l, 4000mg/l) showed significant ($P < 0.05$) increase in carbohydrates level. Mousavi in 2011 [33] also reported that foliar spray of Mn showed increase in carbohydrate metabolism.

Total Proteins:

In our experiment drought treated plants showed significant ($P < 0.001$) increase in total protein level Fig. 4. Our results showed little resemblance with result of Asish [31], who reported that although very small decrease upon drought treatment, protein contents showed a statistically significant. As compared to stressed plants, the protein contents increased significantly in Zn and Mn treated recovered stressed plants likely to be equal to their corresponding controls. Plants treated with doses of ZnSO_4 and MnSO_4 (3000mg/l, 4000mg/l) showed non-significant increase in protein content. Our findings are in correspondence with the outcomes of Raholla *et al* in 2007 [34], who examined a rise in protein contents by application of Zn and Mn. Aline *et al* in 2013 [32] also described that higher protein content in grains is promoted by foliar application of Zn. In our observation plants treated with ZnSO_4 (3000mg/l, 4000mg/l) showed significant ($P < 0.05$) increase in this parameter as compared to control set. Mousavi *et al* in 2007 [34] also reported that Zn Sulphate promotes increased protein content in potato crop when applied exogenously in higher doses.

Total Phenols:

Total phenols exhibited reduction in different levels of drought as compared to control plants (Fig. 5). Plants can accumulate phenolic compounds under various stress conditions such as low temperature, light, hydric deficit [35]. Factors resulting from the applied drought stress here suggest the offset of stimulating effect of aeration in Sphagnum-dominated peat caused the significant ($P < .0001$) decrease in phenol activities. Acidification induced by drought is the major evident change of chemical properties [36]. They also indicated that acidification may caused poorer phenol activity in peat lands, and suggests that one of the main factors hindering phenol activity is the

acidification incited by drought stress. Our data showed that total phenolics are relatively stable among the experimental categories. The water deficit did not alter total phenolics in Opal and Cayuga cultured in shade and water deficit conditions but it induced their decrease in Ruvi. Under water deficit conditions, the accumulation of total phenolics was slightly stimulated in Thorn free, mainly in plants cultured in full sunlight conditions. Contrary, total phenolics decreased in Lochness, mainly in shaded plants [37]. ZnSO₄ and MnSO₄ illustrated non-significant enhancement in this parameter as compared to control. Zn significantly reduced the H₂O₂ content in drought stress. Increasing the amount of Zn application at higher drought levels significantly reduced the H₂O₂ content. As compared to drought treatment used alone, Zn treatment resulted a conspicuous enhancement in phenolic content [38]. The evidence illustrate that phenolic content is increased in drought stressed pistachio seedlings by zinc treatment, thus alleviating drought effects and ameliorating the growth of seedlings [39].

Total Flavonoids:

Flavonoids showed significant ($P < 0.001$) decline in different levels of drought as compared to control plants (Fig. 6). Flavonoids have multiple protective functions such as protective against heavy metal stress [40-42] and have anti oxidative activity [43-45]. While application of flavonoids through foliar spray might not be enough for shielding against drought stress because of probable problems with uptake and proper delivery of flavonoids to the appropriate tissues, e.g. in the mutant lines, over expression of genes coding for components of flavonoid biosynthesis or transcription factors could be a fruitful approach for clarifying their role in drought tolerance [46]. In this study, total flavonoids exhibited reduction in different levels of drought as compared to control plants. In the biosynthesis approach of the flavonoids which was directly correlated with the flavonoid content and was greatly affected by deficit water stress. In addition, anthocyanins, which are synthesized by the same method as flavonoids, are tolerant to stressors such as salt stress and drought stress [47]. ZnSO₄ and MnSO₄ exhibited significant ($P < 0.05$) decline as compared to control. Above results illustrated that the micronutrients greatly affect the total flavonoids and it mainly decreased the flavonoid content. The reduction of flavonoid content was consistent with the increase of micronutrients activity [48].

Total Antioxidant:

Antioxidant exhibited significant ($P < 0.05$) promotion in different levels of drought but that were no significant difference between control and other plants (Fig. 7). Our results were same as of Blokhina *et al.* 2003 [49]. He studied that a lot of antioxidant enzymes illustrated maximum enzymatic activity in stress treatment but there were significant differences between recovered and control plants. As compared with Borujerd, Birjand had more enzyme activity especially under stress treatments. Thus this experiment illustrated better performance of *Kochia* in Birjand area than that of Borujerd and could settle in unfavourable situation. Fazeli *et al.* [50] examined the response of antioxidant enzymes under drought stress. The changes in antioxidant metabolism correlated with water dearth in this experiment are in concurrence with the results of Sgherri *et al.* [51], who described that by exposing to a phase of water scarcity and re watering, activity of some antioxidants is enhanced such as ascorbate peroxidase activity in the leaves of wheat. Similarly their findings showed that different antioxidant elements can react differently to water shortage which was indicated by diminished activity of superoxide dismutase. As compared to control, significant ($P < 0.05$) increase was showed for ZnSO₄ and MnSO₄. Our results were in concurrence with the outcomes described by [52-55]. Decreased harmful effects of H₂O₂ in drought stress conditions is contributed by the concurrent enhancement in the activity of these enzymes. Rahmati *et al.* found that excess of Mn increase antioxidant activity compared with control treatment [56]. Moreover, analysis of variance showed that there were significant differences ($P < 0.05$) between activity levels of these drought stress condition were increased, with the increase of micronutrients especially Zn and Mn treatment [57].

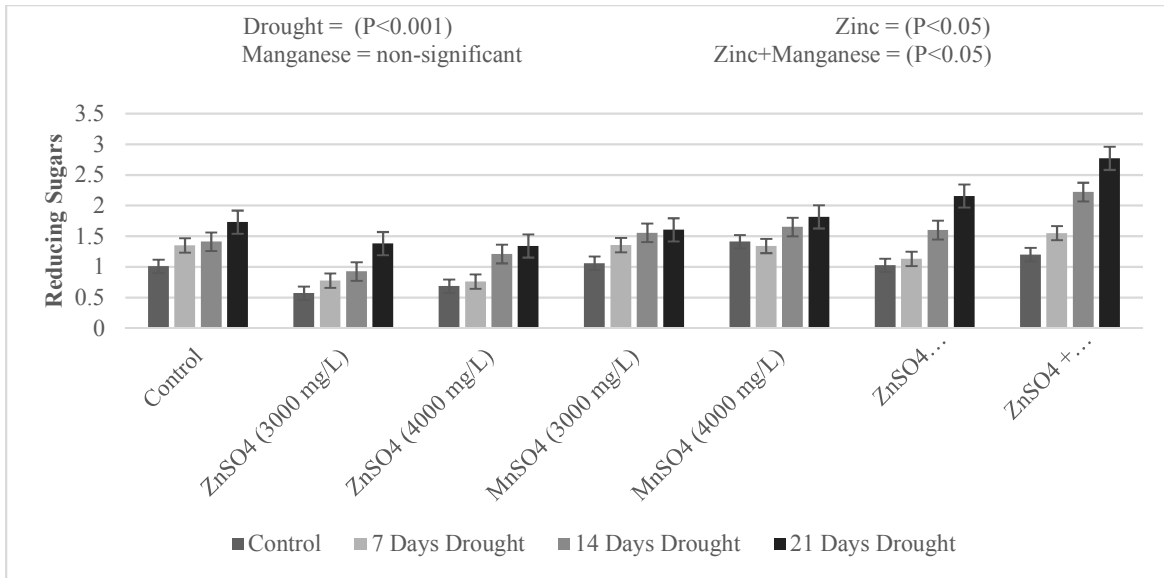


Figure 1. Influence of micronutrient (Zinc and Manganese) on reducing sugars (mg/gmfr.wt) of *Brassica juncea* grown under drought stress.

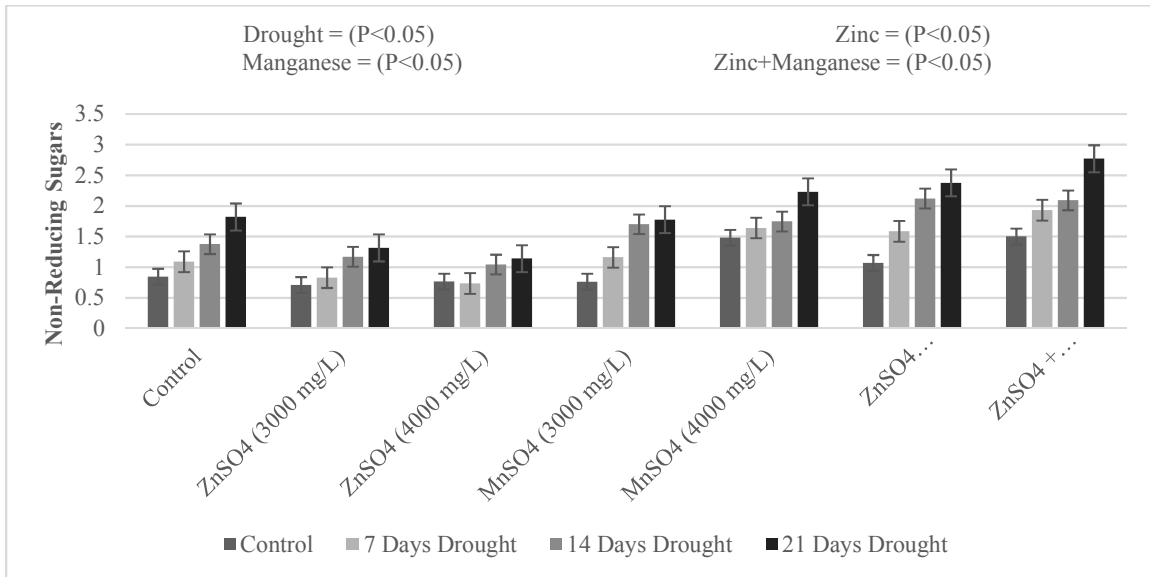


Figure 2. Influence of micronutrient (Zinc and Manganese) on non-reducing sugars (mg/gmfr.wt) of *Brassica juncea* grown under drought stress.

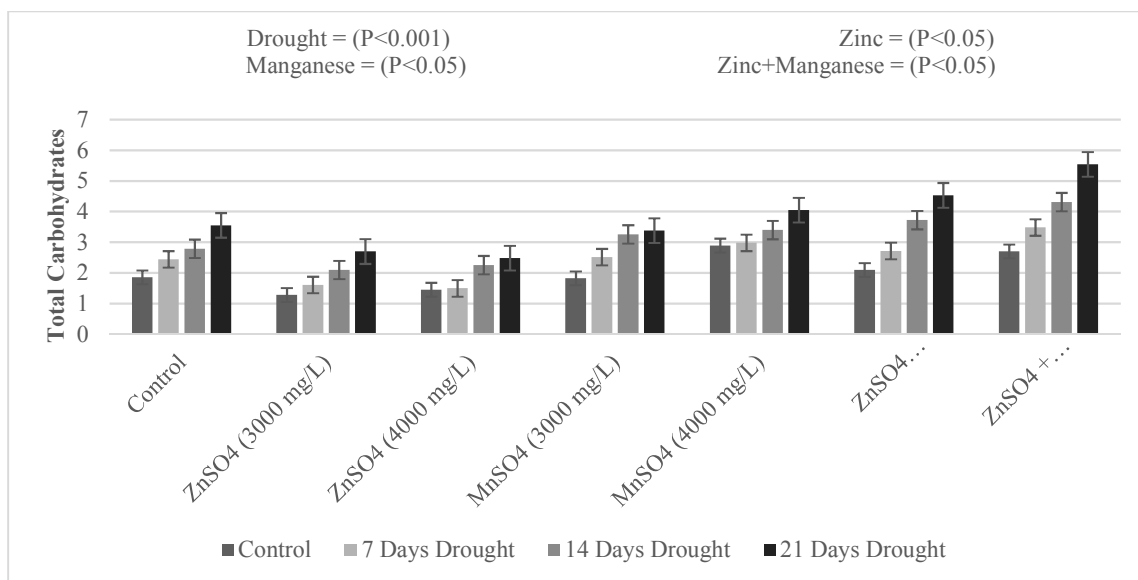


Figure 3. Influence of micronutrient (Zinc and Manganese) on total carbohydrates (mg/gm fr. wt) of *Brassica juncea* grown under drought stress.

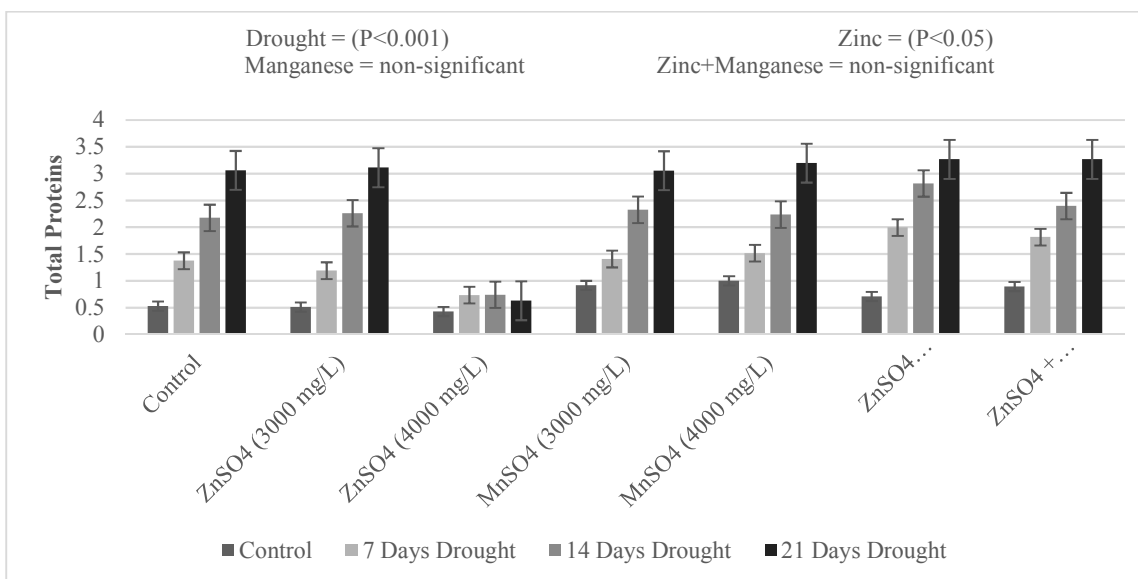


Figure 4. Influence of micronutrient (Zinc and Manganese) on total proteins (mg/gm fr. wt) of *Brassica juncea* grown under drought stress.

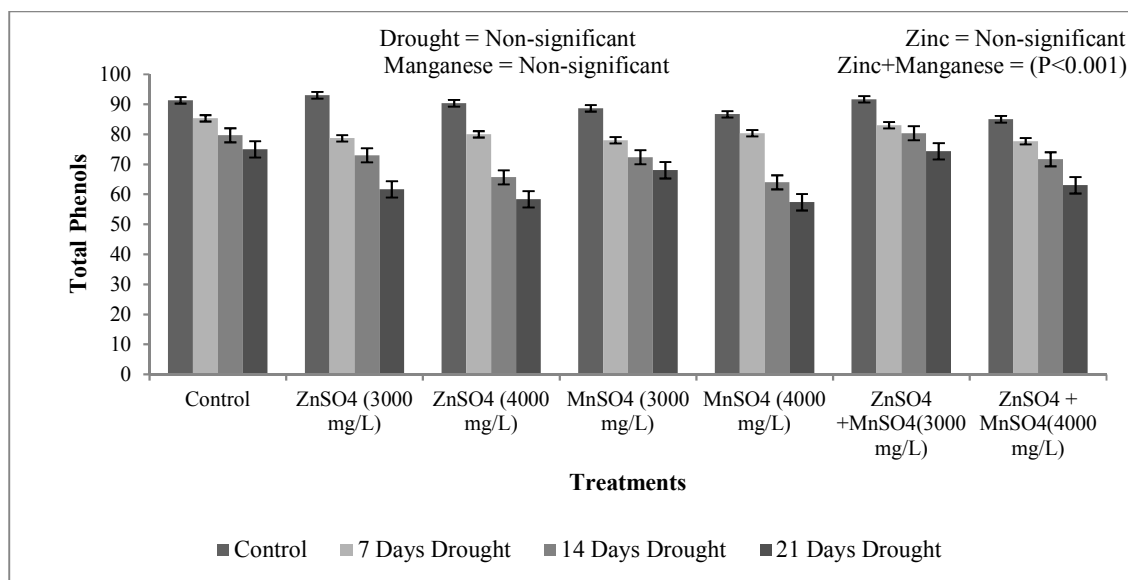


Figure 5. Interactive effects of drought and micronutrients (Zinc and Manganese) on total phenols (mg/gmfr.Wt) of *Brassica juncea*.

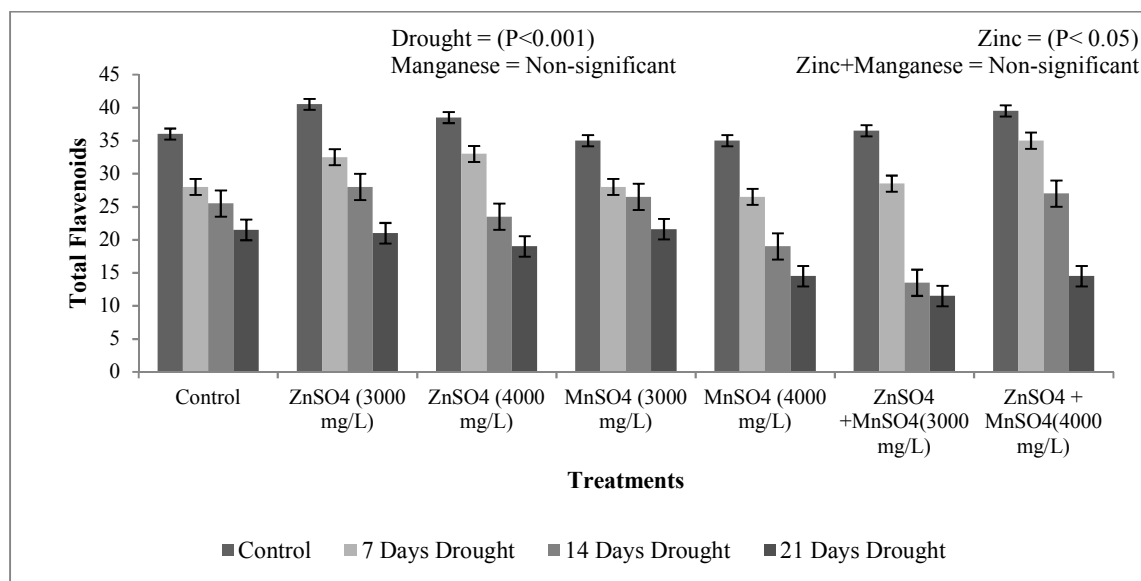


Figure 6. Interactive effects of drought and micronutrients (Zinc and Manganese) on total flavonoids (mg/gmfr.Wt) of *Brassica juncea*.

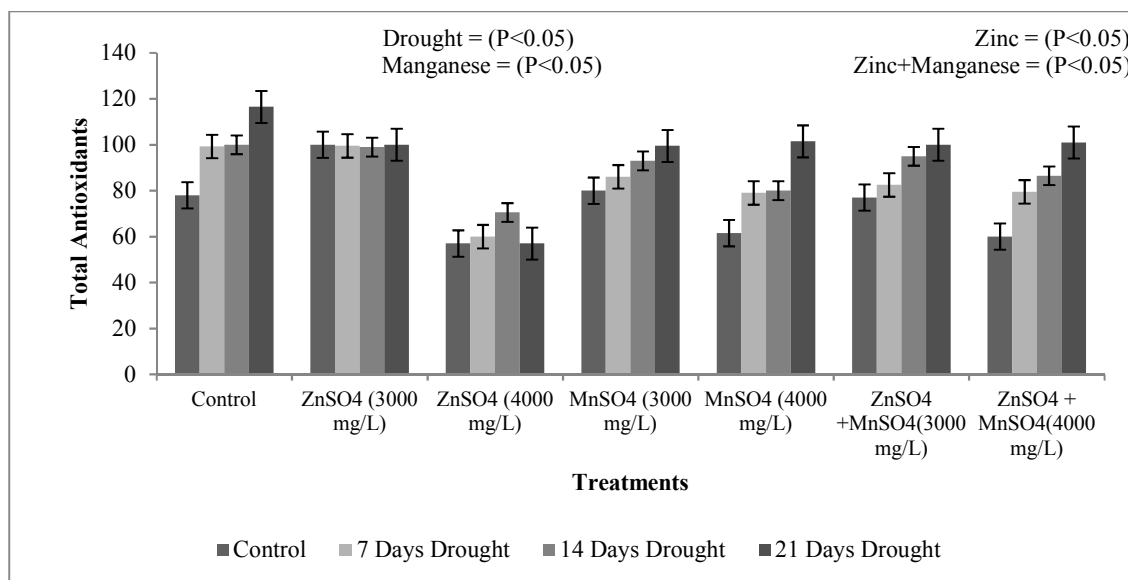


Figure 7. Interactive effects of drought and micronutrients (Zinc and Manganese) on total antioxidant status(mg/gmfr.Wt) of *Brassica juncea*.

REFERENCES

1. Atkinson, N.J. and P.E. Urwin, *The interaction of plant biotic and abiotic stresses: from genes to the field*. Journal of experimental botany, 2012. **63**(10): p. 3523-3543.
2. Yuan, J.S., et al., *Plant systems biology comes of age*. Trends in plant science, 2008. **13**(4): p. 165-171.
3. Riaz, A., et al., *Morphological and biochemical responses of turf grasses to water deficit conditions*. Pak. J. Bot, 2010. **42**(5): p. 3441-3448.
4. Wery, J., et al., *Screening techniques and sources of tolerance to extremes of moisture and air temperature in cool season food legumes*. Euphytica, 1993. **73**(1-2): p. 73-83.
5. Hewitt, E.J., *Sand and water culture methods used in the study of plant nutrition*. Sand and water culture methods used in the study of plant nutrition., 1952.
6. Tardieu, F. and R. Tuberosa, *Dissection and modelling of abiotic stress tolerance in plants*. Current opinion in plant biology, 2010. **13**(2): p. 206-212.
7. Burkill, I.H., *A dictionary of the economic products of the Malay Peninsula*. A Dictionary of the Economic Products of the Malay Peninsula., 1966. **2**(2nd edition).
8. Brown, P.H., I. Cakmak, and Q. Zhang, *Form and function of zinc plants*, in *Zinc in soils and plants*. 1993, Springer. p. 93-106.
9. Hänsch, R. and R.R. Mendel, *Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl)*. Current opinion in plant biology, 2009. **12**(3): p. 259-266.
10. Snowball, K. and A.D. Robson, *Symptoms of nutrient deficiencies: lupins*. 1986: Department of Soil Science and Plant Nutrition, Institute of Agriculture, University of Western Australia.
11. O'Sullivan, J.N., C.J. Asher, and F.P.C. Blamey, *Nutrient disorders of sweet potato*. Vol. 48. 1997: Australian Centre for International Agricultural Research Canberra.
12. Nelson, N., *A photometric adaptation of the Somogyi method for the determination of glucose*. J. biol. Chem, 1944. **153**(2): p. 375-380.
13. Fales, F.W., *The assimilation and degradation of carbohydrates by yeast cells*. Journal of Biological Chemistry, 1951. **193**(1): p. 113-124.
14. Bradford, M.M., *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding*. Analytical biochemistry, 1976. **72**(1-2): p. 248-254.
15. Malik, C.P. and M. Singh, *Plant enzymology and histo-enzymology*. 1980.

16. El Far, M.M. and H.A. Taie, *Antioxidant activities, total anthocyanins, phenolics and flavonoids contents of some sweetpotato genotypes under stress of different concentrations of sucrose and sorbitol*. Australian Journal of Basic and Applied Sciences, 2009. **3**(4): p. 3609-3616.
17. Yen, G.-C. and H.-Y. Chen, *Antioxidant activity of various tea extracts in relation to their antimutagenicity*. Journal of Agricultural and Food Chemistry, 1995. **43**(1): p. 27-32.
18. Rascio, A., et al., *The accumulation of solutes and water binding strength in durum wheat*. Physiologia Plantarum, 1994. **90**(4): p. 715-721.
19. Gill, P.K., et al., *Osmotic stress-induced changes in germination, growth and soluble sugar content of Sorghum bicolor (L.) Moench seeds*. Bulg J Plant Physiol, 2002. **28**(3-4): p. 12-25.
20. Meyer, R. and J. Boyer, *Osmoregulation, solute distribution, and growth in soybean seedlings having low water potentials*. Planta, 1981. **151**(5): p. 482-489.
21. Barlow, E., L. Boersma, and J. Young, *Root temperature and soil water potential effects on growth and soluble carbohydrate concentration of corn seedlings*. Crop Science, 1976. **16**(1): p. 59-62.
22. Kameli, A. and D. Lösel, *Growth and sugar accumulation in durum wheat plants under water stress*. New Phytologist, 1996. **132**(1): p. 57-62.
23. Gupta, A., et al., *Effect of polyethylene glycol-induced water stress on germination and reserve carbohydrate metabolism in chickpea cultivars differing in tolerance to water deficit*. Plant physiology and biochemistry, 1993. **31**(3): p. 369-378.
24. Timpa, J.D., et al., *Effects of water stress on the organic acid and carbohydrate compositions of cotton plants*. Plant physiology, 1986. **82**(3): p. 724-728.
25. SPYROPOULOS, C.G., *Control of sucrose metabolism in polyethylene glycol-stressed carob (Ceratonia siliqua L.) young seedlings. The role of sucrose*. Journal of Experimental Botany, 1982. **33**(6): p. 1210-1219.
26. Singh, P., R. Gangwar, and V. Singh, *Response of boron, zinc and copper on quality of aonla fruits cv. Banarasi*. HortFlora Research Spectrum, 2012. **1**(1): p. 89-91.
27. Abdelghany, A.E. and M.H. Ahmad, *Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds*. Aquaculture Research, 2002. **33**(6): p. 415-423.
28. Ahmad, M.H., M. Abdel-Tawwab, and Y.A. Khattab, *Effect of dietary protein levels on growth performance and protein utilization in Nile tilapia (Oreochromis niloticus L.) with different initial body weights*. in *The sixth international symposium on tilapia in aquaculture, Manila, Philippine*. 2004. Citeseer.
29. Mafakheri, A., et al., *Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (Cicer arietinum) cultivars*. Australian Journal of Crop Science, 2011. **5**(10): p. 1255.
30. Praxedes, S.C., et al., *Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (Coffea canephora Pierre var. kouillou) leaves*. Environmental and Experimental Botany, 2006. **56**(3): p. 263-273.
31. Parida, A.K., et al., *Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery*. Plant Biotechnology Reports, 2007. **1**(1): p. 37-48.
32. Puga, A.P., et al., *Efecto de diferentes métodos de aplicación y dosis de zinc sobre la composición química de los granos de maíz y sorgo, cultivados en un suelo Oxisol*. Ciencia e investigación agraria, 2013. **40**(1): p. 97-108.
33. Mousavi, S.R., M. Shahsavari, and M. Rezaei, *A general overview on manganese (Mn) importance for crops production*. Australian Journal of Basic and Applied Sciences, 2011. **5**(9): p. 1799-1803.
34. Mousavi, S.R., M. Galavi, and G. Ahmadvand, *Effect of zinc and manganese foliar application on yield, quality and enrichment on potato (Solanum tuberosum L.)*. Asian Journal of Plant Sciences, 2007.
35. Sakihama, Y., et al., *Reduction of phenoxyl radicals mediated by monodehydroascorbate reductase*. Biochemical and biophysical research communications, 2000. **279**(3): p. 949-954.
36. Pind, A., C. Freeman, and M. Lock, *Enzymic degradation of phenolic materials in peatlands—measurement of phenol oxidase activity*. Plant and Soil, 1994. **159**(2): p. 227-231.
37. Hatier, J.-H.B. and K.S. Gould, *Foliar anthocyanins as modulators of stress signals*. Journal of Theoretical Biology, 2008. **253**(3): p. 625-627.
38. Lacan, D. and J.-C. Baccou, *High levels of antioxidant enzymes correlate with delayed senescence in nonnetted muskmelon fruits*. Planta, 1998. **204**(3): p. 377-382.
39. Dionisio-Sese, M.L. and S. Tobita, *Antioxidant responses of rice seedlings to salinity stress*. Plant science, 1998. **135**(1): p. 1-9.
40. Tumova, L. and M. Backovska, *Effect of Chitosan on the Production of Flavonoids by the Culture of Ononis arvensis L. in vitro*. CESKA A SLOVENSKA FARMACIE, 1998. **47**: p. 110-114.

41. Kim, M.S., et al., *Effect of fungal elicitor and heavy metals on the production of flavonol glycosides in cell cultures of Ginkgo biloba*. Journal of microbiology and biotechnology, 1999. **9**(5): p. 661-667.
42. Michalak, A., *Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress*. Polish Journal of Environmental Studies, 2006. **15**(4): p. 523.
43. Aherne, S.A. and N.M. O'Brien, *Mechanism of protection by the flavonoids, quercetin and rutin, against tert-butylhydroperoxide-and menadione-induced DNA single strand breaks in Caco-2 cells*. Free Radical Biology and Medicine, 2000. **29**(6): p. 507-514.
44. Havsteen, B.H., *The biochemistry and medical significance of the flavonoids*. Pharmacology & therapeutics, 2002. **96**(2): p. 67-202.
45. Rusak, G., H.O. Gutzeit, and J.L. Müller, *Structurally related flavonoids with antioxidative properties differentially affect cell cycle progression and apoptosis of human acute leukemia cells*. Nutrition Research, 2005. **25**(2): p. 143-155.
46. Stracke, R., et al., *Differential regulation of closely related R2R3-MYB transcription factors controls flavonol accumulation in different parts of the Arabidopsis thaliana seedling*. The Plant Journal, 2007. **50**(4): p. 660-677.
47. Gould, K.S., *Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves*. BioMed Research International, 2004. **2004**(5): p. 314-320.
48. Schützendübel, A. and A. Polle, *Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization*. Journal of experimental botany, 2002. **53**(372): p. 1351-1365.
49. Blokhina, O., E. Virolainen, and K.V. Fagerstedt, *Antioxidants, oxidative damage and oxygen deprivation stress: a review*. Annals of botany, 2003. **91**(2): p. 179-194.
50. Fazeli, F., M. Ghorbanli, and V. Niknam, *Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars*. Biologia Plantarum, 2007. **51**(1): p. 98-103.
51. Sgherri, C.L.M., M. Maffei, and F. Navari-Izzo, *Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering*. Journal of Plant Physiology, 2000. **157**(3): p. 273-279.
52. Habibi, D., et al. *Antioxidative enzymes in sunflower subjected to drought stress*. in *4th International Crop Science Congress, Australia*. 2004.
53. Jiang, Y. and B. Huang, *Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation*. Crop Science, 2001. **41**(2): p. 436-442.
54. Bailly, C., et al., *Antioxidant systems in sunflower (Helianthus annuus L.) seeds as affected by priming*. Seed Science Research, 2000. **10**(01): p. 35-42.
55. Malan, C., M.M. Greyling, and J. Gressel, *Correlation between CuZn superoxide dismutase and glutathione reductase, and environmental and xenobiotic stress tolerance in maize inbreds*. Plant Science, 1990. **69**(2): p. 157-166.
56. Rahmati, M., M. Yazdani, and F. Ghanati. *Effect of excess amount of Mn on activation of certain enzymes of antioxidant system in suspension-cultured tea cells*. in *The 2nd Congress on Applied Biology (International Approach)*. 2004.
57. Wang, S.-H., et al., *Copper-induced stress and antioxidative responses in roots of Brassica juncea L.* Botanical Bulletin of Academia Sinica, 2004. **45**.