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THE BOTANICAL EFFECTS OF SELENIUM AND ZINC ON BIOCHEMICAL ATTRIBUTES OF SPINACH UNDER VARYING SALT CONDITIONS

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Abstract
Spinach is rich in antioxidants as it is good provider of iron and folic acid, but these vegetables are spoiled by these chemicals and become toxic. The experiment was conducted at the research area of Botanic Garden, Government College University Faisalabad, Pakistan to examine the effect of foliar application of selenium and zinc on spinach (Spinacia oleracea L.) under salt stress. Seeds of spinach variety "Desi palak". Salinity 100 mM NaCl was applied after one week of germination. Sodium selenate was used as a source of selenium applied foliarly at 0.01%, 0.02 %, 0.03% Se with three replications under normal and saline conditions. Zinc sulphate was used as a source of zinc and was applied foliarly at 0.10%, 0.20%, 0.30% Zn with three replications under normal and saline conditions. Biochemical attributes were determined at seedling stage. The data collected was analyzed statistically by CoStat software. The data collected from the experiment shows that salinity reduces the physiological attributes like plant height, shoot and root length, shoot and root fresh & dry weight, number of leaves and leaf area.

INTRODUCTION

Human population is increasing day by day, scarcity of food is a problem for ever increasing population. There are many biotic and abiotic factors that effects plant growth and development, plant needs a suitable environment for their proper growth and development. Spinach (Spinacia oleraceae L.) is a leafy green vegetable and it exists in the Amaranthaceae family [1,2]. It is best known for its nutritional, anti-cancer and antioxidant composition. Spinach is rich in vitamin A, C, K, folate and minerals like iron, potassium and calcium. Spinach is also source of fibers and it contains less calories. Its green leaf all around the world are the most favourite ingredients for chefs. Spinach is very important for retaining alkaline reservoir of the body, vitamin, mineral and high carbohydrate contents [2,3].

Spinach (Spinacia oleraceae L.) is an annual leafy green plant which is basically native of central zone of Asia and Iran. The daily diet of most of the people is in shortage of essential nutrient elements, mainly micronutrient, so it is very important to increase the quality of agriculture products mainly vegetables which is used as food. Spinach is very important vegetable because it has many vitamins like A, B, C and antioxidants and different important minerals. Spinach is very beneficial, as medicine because it

consists of important nutrients of food like selenium and iron[4,5].

Spinach is at 10th position out of 45 vegetables and fruits due to its composition as it contains important nutrients like iron and selenium and vitamins like A, B, C. Spinach leaves are boiled to get benefits because oxalic acid that is present in the spinach leaves can react with Fe and Mg[4,6]. The boiled spinach leaves are used for the treatments of many diseases like fever, intestine stomach inflammations, and it also contains folic acid and antibacterial compounds that are beneficial for the cure of anemia.

Selenium is very important for the human beings, plants and animals, because it is needed for the proper growth and development of plants and animals and it can be provided through food crops and forages, and it is placed as a non-metal in the periodic table. Selenium is very important for human nutrition its normal value for human nutrition ranges from 0.01-2mg/kg in the soil[7,8].

This study aims to identify the role of selenium and zinc on various physiological, biochemical and ionic attributes of spinach under salt stress.

MATERIALS AND METHODS

The experiment was conducted to assess the physiological, morphological and biochemical attributes of spinach by foliar application of sodium selenate and zinc sulphate under salt stress. The experiment was conducted at botanical garden, Government College University Faisalabad, Pakistan.

Seed Material

The seeds of spinach variety (Desi palak) were obtained from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan.

The soil used for sowing was of river side and obtained from Ever Green nursery, Faisalabad. Soil analysis was performed at soil Fertility Department of Ayub Agricultural Research Institute Faisalabad, Pakistan. The seeds of spinach variety (Desi palak) were sown in pots and arranged in Completely Randomized Design (CRD) with three replicates of each treatment.

For the determination of soil texture and soil particles size was done through hydrometer method. For this purpose, 1 % soln. of sodium hexa metaphosphate, 30 g soil added to it and placed the reaction mixture for 24 h. Double distal water was used for dilution of reaction and with the help of stirrer soil mixed well. And soil texture and particles size were determined through the hydrometer, comparing with standard of international society for soil sciences.

For the determination of the soil pH the acidic or alkaline nature of the soil sample was determined. The pH meter was used for the determination of soil pH. Prepared buffers of standards (4.02, 7.02, 10.01) were used, after that take 20ml distilled water and add 5g soil sample in it and stir for one hour. After that solution was left down until soil settled to the bottom and pH meter dipped into the supernatant to find out the value, to get accurate results three times readings were noted.

Morphological Parameters

Plant height of spinach plants was recorded in centimeters with the help of simple scale. Whole spinach plant was taken and weighed with the help of micro weighing scale. Shoot fresh weight of spinach plants was measured in grams with the help of digital micro weighing scale. Plants were placed in the oven for 24-36 hours and after that weighed with the help of digital weighing balance. Spinach plant shoot were dried in incubator for 24-36 hours and then were subjected to digital balance for weighing. Root length of spinach plant was measured in centimeters using simple scale. Root fresh weight was taken in grams with the help of digital balance. Root were subjected to incubation for 24-36 hours and then weighed with the help of digital balance. Leaf area of each three replicates was measured in cm2 using scale in cm. Four to 6 plants from each pot were taken and 4-6 leaves were measured to take an average leaf area. The total numbers of leaves per plant were counted.

Statistical analysis:

The data collected was subjected to analysis of variance technique (ANOVA) by using computer software CoStat version 6.2, CoHort Software, 2003, Monterey, CA, USA. There were three replicates of each treatment, the mean values of each treatment was compared by using the Least Significant Difference (LSD) with (0.05) level of significance.

RESULTS AND DISCUSSION

Salinity significantly ($P<0.001$) reduced the shoot length of spinach plants when exposed to salt stress as compared to plants under control conditions (Fig

4.1). Spinach variety Desi palak under foliar application level 0.030% of NaSeO4 show maximum shoot length of 19.93 cm than other treatments of $ZnSO_4$ and Na_2SeO4. The lowest shoot length (13.26 cm) was observed in the plants that were treated with salt stress.

A non-significant difference of $ZnSO_4$ and Na_2SeO_4 was recorded in the spinach plants. The interaction between salinity and treatments was found non-significant. Overall results indicated that foliar application of $ZnSO_4$ and Na_2SeO_4 increased the shoot length of spinach desi genotype.

Biochemical paraments

Salinity significantly ($P<0.001$) reduced the root length of spinach plants when exposed to salt stress as compared to plant under control conditions (Fig 1). Spinach variety Desi palak under foliar application level 0.030% of Na_2SeO_4 and ZnSO4 level 0.30% exhibited maximum root length of 20.66 cm as compared to other treatments of $ZnSO_4$ and Na_2SeO_4. The lowest Root length (15.93 cm) was observed in the plants that were treated with salt stress.

A non-significant difference of $ZnSO_4$ and Na_2SeO_4 was recorded in the spinach plants. The interaction between salinity and treatments was found non-significant. Overall results indicated that foliar application of $ZnSO_4$ and Na_2SeO_4 increased the root length of spinach desi genotype.

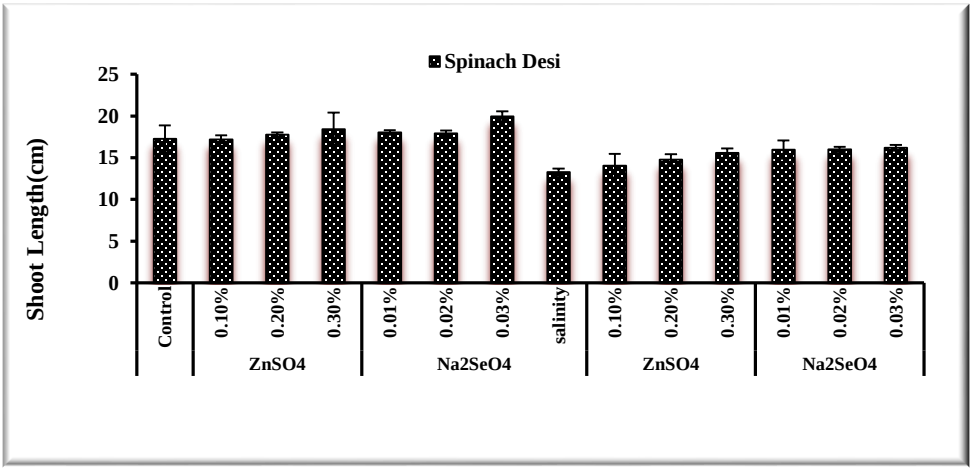

Fig 1. Effect of foliar application at different concentrations of $ZnSO_4$ and Na_2SeO_4 on spinach shoot length under control & salt stress conditions.

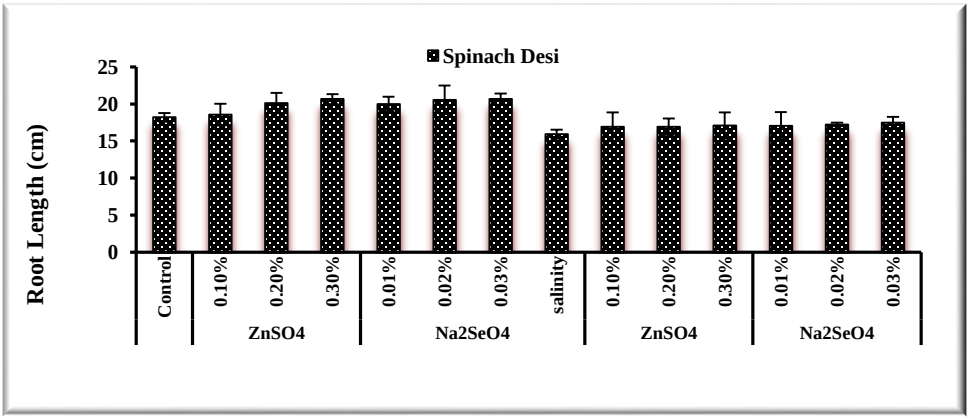

Fig 2. Effect of foliar application at different concentrations of $ZnSO_4$ and Na_2SeO_4 on spinach root length under control & salt stress conditions.

Salinity significantly (P<0.001) reduced the shoot fresh weight of spinach plants when exposed to salt stress as compared to plants under control conditions. Spinach variety Desi palak under foliar application level 0.030% of $NaSeO_4$ showed maximum shoot fresh weight of 17.24 (g) as compared to other treatments of $ZnSO_4$ and Na_2SeO_4. The lowest shoot fresh weight (6.17 g) was observed in the plants that were treated with salt stress.

Salinity significantly (P<0.01) reduced the shoot dry weight of spinach plants when exposed to salt stress as compared to plants under control conditions. Spinach variety Desi palak under foliar application level 0.030% of $NaSeO_4$ exhibited maximum shoot dry weight of 2.00 (g) as compared to other treatments of $ZnSO_4$ and Na_2SeO_4. The lowest shoot dry weight (0.72 g) was observed in the plants that were treated with salt stress.

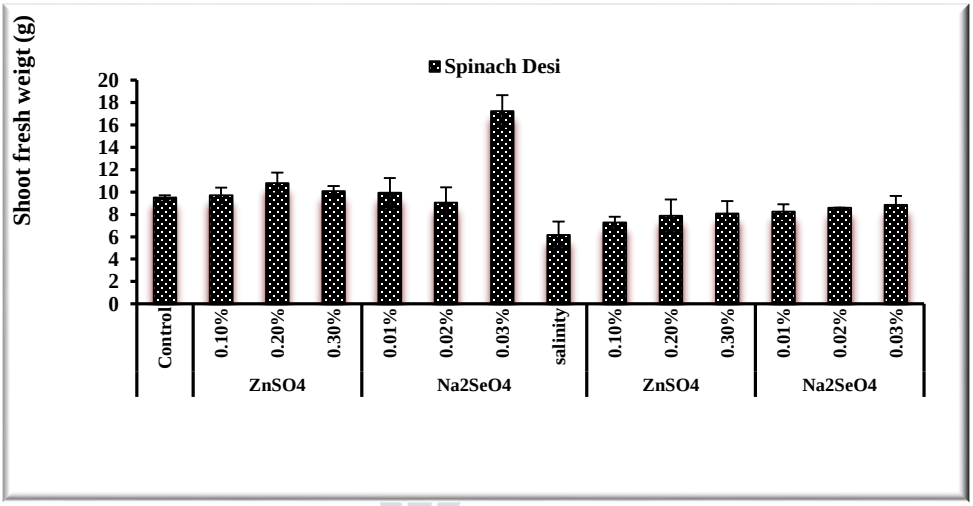

Fig 3. Effect of foliar application at different concentrations of $ZnSO_4$ and Na_2SeO_4 on spinach shoots fresh weight under control & salt stress conditions.

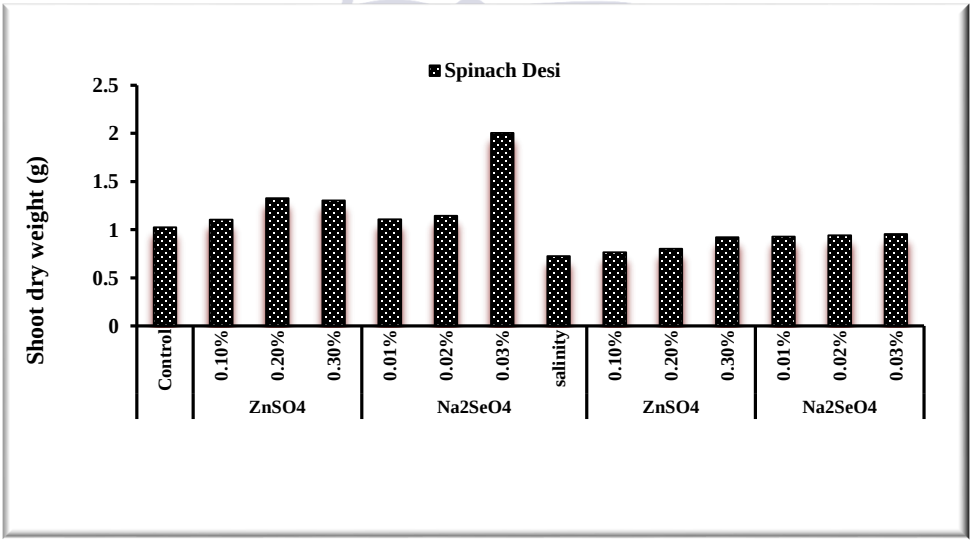

Fig 4. Effect of foliar application at different concentrations of $ZnSO_4$ and Na_2SeO_4 on spinach shoots dry weight under control & salt stress conditions.

Salinity significantly (P<0.001) reduced the root fresh weight of spinach plants when exposed to salt stress as compared to plants under control conditions. Spinach variety Desi palak under foliar application level 0.030% of Na_2SeO_4 exhibited maximum root fresh weight of 3.93 (g) as compared to other treatments of $ZnSO_4$ and Na_2SeO4. The lowest root fresh weight (1.40 g) was observed in the plants that were treated with salt stress.

Salinity significantly (P<0.01) reduced the root dry weight of spinach plants when exposed to salt stress as compared to plant under control conditions. Spinach variety Desi palak under foliar application level 0.030% of Na_2SeO_4 showed maximum root dry weight of 0.75 (g) as compared to other treatments of $ZnSO_4$ and Na_2SeO_4. The lowest root dry weight (0.29 g) was observed in the plants that were treated with salt.

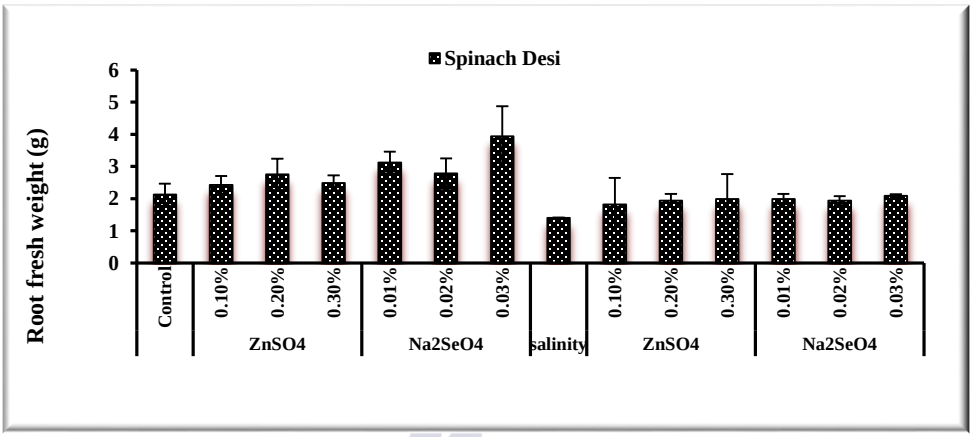

Fig 5. Effect of foliar application at different concentrations of $ZnSO_4$ and Na_2SeO_4 on spinach root fresh weight under control & salt stress conditions.

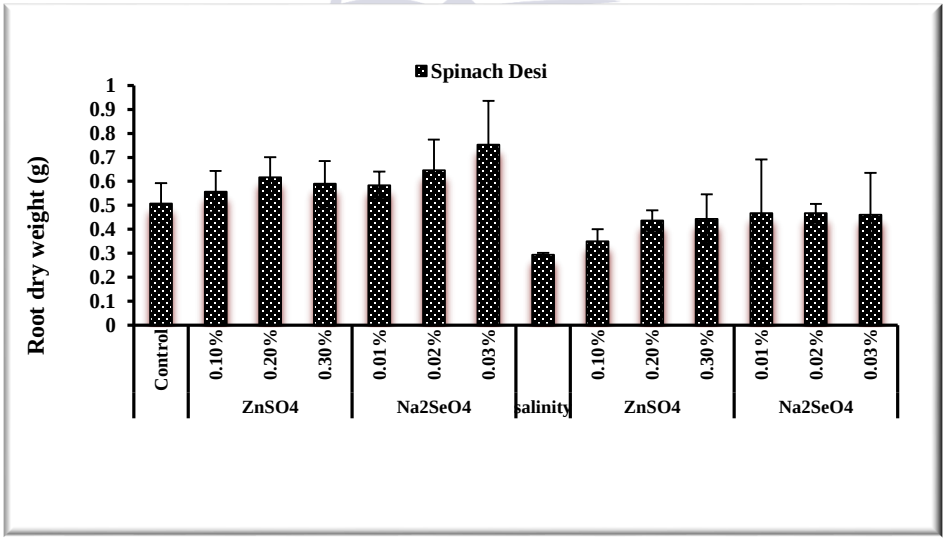

Fig 6. Effect of foliar application at different concentrations of $ZnSO_4$ and Na_2SeO_4 on spinach root dry weight under control & salt stress conditions.

Salinity significantly (P<0.001) reduced the no. of leaves/ plant of spinach plants when exposed to salt stress as compared to plants under control conditions. Spinach variety Desi palak plants that were treated with foliar application of level 0.03% of Na_2SeO_4 showed maximum 12.33 no. of leaves/ plant as compared to other treatments of $ZnSO_4$ and Na_2SeO_4. The lowest no. of leaves/plant 6.33 was observed in the plants that were treated with salt stress.

A significant difference (P< 0.05) of $ZnSO_4$ and Na_2SeO_4 was recorded in the spinach plants. The interaction between salinity and treatments was found non-significant. Overall results indicated that foliar application of $ZnSO_4$ and Na_2SeO_4 increased the no. of leaves/ plant of spinach desi genotype.

Salinity significantly (P<0.001) reduced the leaf area of spinach plants when exposed to salt stress as compared to plants under control conditions. Spinach variety Desi palak plants that were treated with foliar application of level 0.03% of Na2SeO4 exhibited maximum leaf area of 69.06 as compared to other treatments of $ZnSO_4$ and Na_2SeO_4. The lowest leaf area 24.33 was observed in the plants that were treated with salt stress.

A significant difference (P< 0.01) of $ZnSO_4$ and Na_2SeO_4 was recorded in the spinach plants. The interaction between salinity and treatments was found non-significant. Overall results indicated that foliar application of $ZnSO_4$ and Na_2SeO_4 increased the leaf area of spinach desi genotype.

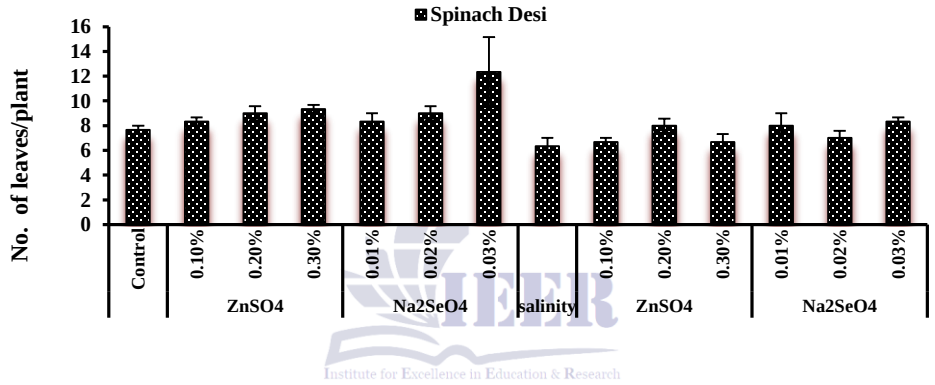

Fig 7. Effect of foliar application at different concentrations of $ZnSO_4$ and Na_2SeO_4 on leaves

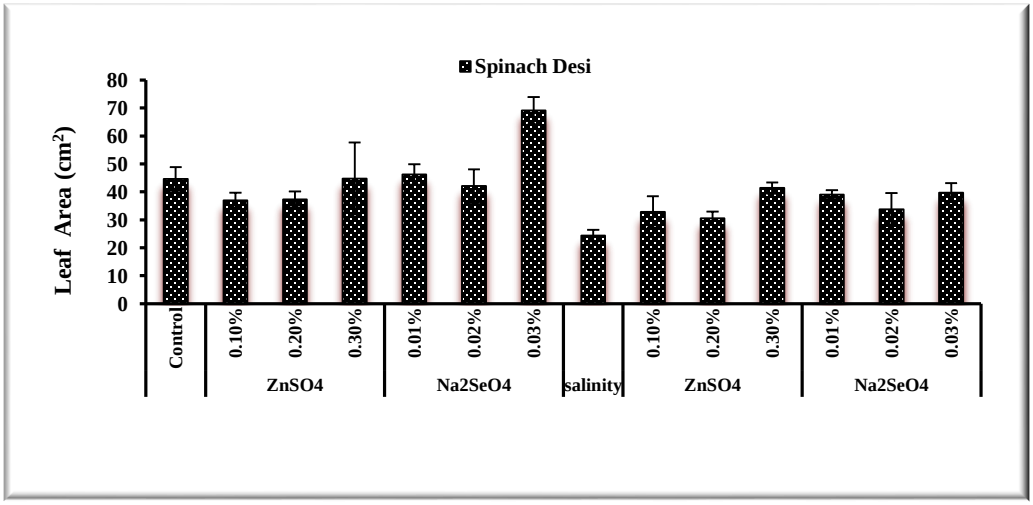

Fig 8. Effect of foliar application at different concentrations of $ZnSO_4$ and Na_2SeO_4 on spinach leaf area under control & salt stress conditions.

The abovementioned experiment was managed to assessed the effect of selenium and zinc on spinach (Spinacia oleraceae L.) physiological and biochemical attributes under salt stress.

Salt stress is the abiotic stress that harmfully affect plants, it is estimated that 800 million hectares' land is affected by salt stress through the whole world. High level of ions can affect the biochemical and physiological parameters that shows in the form of reduction in photosynthesis, ion toxicity and other metabolic processes this condition is present in the plants that grows under salt stress. Plants are damaged by the salt stress by reducing their ability to absorb water from the roots of plant and soil, as a result sodium chloride ion toxicity is occurred. Different factors that results salt stress are crop management control, plant sensitivity and salt levels[9,10].

Selenium is a micronutrient that is chemically resembled with sulfur which is necessary for human beings, plants, microorganisms and animals. Selenium has many physiological effects on the plant growth, although its high concentration is toxic for plants. Some plants can accumulate the selenium through specific and active way off accumulation, and some plants cannot accumulate selenium however its high concentration can cause toxicity of different elements, e.g selenomethionine and selenocysteine in place of methionine and cysteine into the proteins. The bonds that present between the selenium atoms are weaker and long and it can affect the catalytic activity of different enzymes and structures of different proteins[11,12]. Heavy metals can cause the shortage of these micronutrients and macronutrients that are necessary for daily life. Selenium can perform the same function as heavy metals like metabolism. Like these selenium level 0.03% gives maximum plant growth attributes than other levels of these chemicals[13,14].

Zinc sulphate can affect the plant growth and development, as it promotes the growth attributes of plants like plant height, shoot length, root length, root and shoot fresh and dry weights[15,16]. Zinc belongs to heavy metals that can affect the protein synthesis, enzymes activations lipids and carbohydrate metabolism. Zinc can also perform many functions like structural and regulatory function for example stabilization of catalase activity and superoxide dismutase. The results mentioned in the above tables and figures are similar to this information. Similar to these zinc level 0.30% gives better results than other levels of these chemicals[17,18].

CONCLUSION

The use of chemicals at higher concentrations is become toxic and it is harmful to the natural environment, so its affects human beings, plants and animals. Spinach is rich in antioxidants as it is good provider of iron and folic acid, but these vegetables are spoiled by these chemicals and become toxic. To investigate the effect of sodium selenate and zinc sulphate on the spinach (Spinacia oleraceae L.) physiological and biochemical attributes under salt stress. Sampling was done for physiological and biochemical attributes before flowering stage. The data collected from the experiment shows that salinity reduces the physiological attributes like plant height, shoot and root length, shoot and root fresh & dry weight, number of leaves and leaf area. Sodium selenate level 0.03% and and zinc sulphate level 0.30% give maximum values of these physiological parameters.

REFERENCES

Anwaar, S. A., Ali, S., Ali, S., Ishaque, W., Farid, M., Farooq, M. A., ... & Sharif, M. (2015). Silicon (Si) alleviates cotton (Gossypium hirsutum L.) from zinc (Zn) toxicity stress by limiting Zn uptake and oxidative damage. Environmental Science and Pollution Research, 22(5), 3441-3450.

Atti-Santos, A. C., Pansera, M. R., Paroul, N., Atti-Serafini, L., & Moyna, P. (2004). Seasonal variation of essential oil yield and composition of Thymus vulgaris L.(Lamiaceae) from South Brazil. Journal of Essential Oil Research, 16(4), 294-295.

Cakmak, I., & Horst, W.J. (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiologia Plantarum, 83(3), 463-468.

Gomez, J. M., Hernandez, J. A., Jimenez, A., Del Rio, L. A., & Sevilla, F. (1999). Differential response of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. Free Radical Research, 31(sup1), 11-18.

Gossett, D. R., Millhollon, E. P., & Lucas, M. C. (1994). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Science, 34(3), 706-714.

Gupta, U. C., & Gupta, S. C. (2000). Selenium in soils and crops, its deficiencies in livestock and humans: implications for management. Communications in soil science and plant analysis, 31(11-14), 1791-1807.

Henson, C. A., & Stone, J. M. (1989). Rapid high-performance liquid chromatographic separation of barley malt α-amylase on cyclobond columns. Journal of Chromatography A, 469, 361-367.

Jiang, C., Zu, C., Lu, D., Zheng, Q., Shen, J., Wang, H., & Li, D. (2017). Effect of exogenous selenium supply on photosynthesis, Na+ accumulation and antioxidative capacity of maize (Zea mays L.) under salinity stress. Scientific reports, 7, 42039.

Julkenen-Titto, R. 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Agric. Food Chem., 33: 213-217.

Lefsrud, M. G., Kopsell, D. A., & Kopsell, D. E. (2007). Nitrogen levels influence biomass, elemental accumulations, and pigment concentrations in spinach. Journal of plant nutrition, 30(2), 171-185.

Liu, S. L., Yang, R. J., Ma, M. D., Dan, F., Zhao, Y., Jiang, P., & Wang, M. H. (2015). Effects of exogenous NO on the growth, mineral nutrient content, antioxidant system, and ATPase activities of Trifoliumrepens L. plants under cadmium stress. Acta Physiologiae Plantarum, 37(1), 1721.

Maehly, A. C., & Chance, B. (1954). Methods of biochemical analysis. by Glick D., Interscience, New York, 454.

Parida, A. K., & Das, A. B. (2005). Salt tolerance and salinity effects on plants: a review. Ecotoxicology and environmental safety, 60(3), 324-349.

Sembratowicz, I., & Grela, E. (1997). Selenium in animal nutrition. Postepy Nauk Rolniczych (Poland).

Shafiq, S., Adeel, M., Raza, H., Iqbal, R., Ahmad, Z., Naeem, M., ... & Azmi, U. R. (2019). Effects of Foliar Application of Selenium in Maize (Zea Mays L.) under Cadmium Toxicity. In Biological Forum-An International Journal (Vol. 11, No. 2, pp. 27-37).

Vazin, F. (2012). Effect of zinc sulfate on quantitative and qualitative characteristics of corn (Zea mays) in drought stress. Cercetari agronomice in Moldova, 45(3), 15-24.

Xia, Q., Yang, Z., Shui, Y., Liu, X., Chen, J., Khan, S., ... & Gao, Z. (2020). Methods of Selenium Application Differentially Modulate Plant Growth, Selenium Accumulation and Speciation, Protein, Anthocyanins and Concentrations of Mineral Elements in Purple-Grained Wheat. Frontiers in Plant Science, 11, 1114.

Zahedi, S. M., Hosseini, M. S., Meybodi, N. D. H., & da Silva, J. A. T. (2019). Foliar application of selenium and nano-selenium affects pomegranate (Punica granatum cv. Malase Saveh) fruit yield and quality. South African Journal of Botany, 124, 350-358