

Full Length Research Paper

Effects of microelement fertilizers and phosphate biological fertilizer on some morphological traits of *Purple coneflower* in water stress condition

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In order to study the effects of microelement fertilizer and phosphate biological fertilizer “Phosphate Biofertilizer” on some morphological characteristics of *Echinacea purpurea* under water stress, an experiment was carried out using split-factorial method on the base of RCBD with three replicates in the agricultural research station of Islamic Azad University, Tabriz Branch, Iran. Experimental treatments administered included water stress as the main factor on three levels (irrigation after 70 mm evaporation from class A basin, irrigation after 120 mm evaporation from class A basin and irrigation after 170 mm evaporation from class A basin). The secondary factor includes: the application of micro elements in three levels (control, application of zinc sulphate and iron sulphate); and biological fertilizer in two levels (control and application of phosphate biofertilizer). The results obtained show that foliar application of iron and zinc sulphate had significant effect on shoot dry weight plant, plant height, flowering branch number and zinc and iron content of shoot of purple coneflower. Phosphate biofertilizer application also had significant effect on flowering branch number of each plant and plant-whole part, height and shoot dry yield. Application of water stress on purple coneflower resulted in 19.51% decrease in plant height, 6.91% in flowering branch number, 15.62% in shoot dry weight, 52.74% in zinc content of shoot and 38.83% in iron content of shoot. Microelement application of zinc and iron respectively caused a rise in 44.12 and 9.34% of shoot dry weight in stress condition. The highest effect on dry yield was equal to 69.44% and it was obtained in joint application of phosphate biofertilizer and zinc.

Key words: *Echinacea purpurea* (L.) Monch, zinc and iron content, biological fertilizer, water stress.

INTRODUCTION

Purple coneflower is the common name of *Echinaceae purpurea* (L.) Monch, a perennial grass plant. Its origin is reported to be from North America (Chevallier, 1996). One of the main benefits of this plant is that it increases the immunity power of the human body against illness. It is used as an important drug in treatment and prevention of illnesses such as cold, influenza and infections. Provision of suitable and fertile soil by the balanced application of chemical fertilizers and food elements on

this plant, is one aspect of the main cultivation technique to earn the most yield and acceptable quality from cultivated crops and to decrease the harmful effects of the environment on them (Chaudhry and Sarwar, 1999).

Records from previous decades, show that the application of chemical element on farm lands caused some environmental problems such as water pollution, low quality of agricultural productions and decrease in soil productivity rate (Sharma, 2002). Sustainable farming on the base of natural fertilizer application with the aim of omitting or decreasing chemical elements is a desirable approach to solve these difficulties.

Natural fertilizers are both economically desirable and stable soil sources, in maintaining long time production

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Table 1. The results of soil analysis on experimental field.

Zn (P.P.M)	Fe (P.P.M)	K (P.P.M)	P (P.P.M)	N	O.C (%)	EC (ds/m)	pH	Texture
0.96	2.28	490	15	0.097	0.92	1.91	7.93	S.L

and prevention of environmental pollution (Saleh, 2001). Zinc foliar application increases height, branch number per plant and dry weight of stem of grass pea (Thalooth et al., 2006). Also zinc, magnesium and iron foliar application increases growth parameters, yield and plant parts significantly (Thalooth et al., 2006). Glyn (2002) reports that different levels of microelements influenced dry weight of tarragon. Anna Mallay et al. (2004) confirmed that by the application of phosphate solvent bacteria, a significant yield improvement was obtained in (*Phyllanthus amarus*). Wasule et al. (2002) mentioned that phosphate solvent bacteria and *Bradyrhizobium japonicum* application on soybean increased significantly improved some characteristics such as nodulation, dry weight of nodules and plant dry weight. Drought is one of the main restrictive factor which causes production yield in agriculture (Mitra, 2001). Repcak et al. (2001) observed that water stress increased useful medicinal material of *Chamomilla recutita* L. Results from researches done by Sreevallia et al. (2001), it was observed that water stress increased root yield and decreased medicinal plant leaf yield of Periwinkle. Shubhra et al. (2004) during researches on *Calendula officinalis* found that the height and flower number per plant under water stress decreased significantly. According to reports, medicinal plants cultivated under conditions such as dry, salty and stresses are limited. It should be noted that most parts of Iran have salty lands or parts with restricted water sources, so the importance of this research in this field is clear.

MATERIALS AND METHODS

Purple coneflower seeds were cultivated in the lines with 10cm apart and 2 up 3 cm depth in research station of Islamic Azad University Tabriz branch in 2010. By regular and timely irrigation and control of weeds in a greenhouse, the seeds germinated after 15 days. Because of slow growth in the early stages, the weeds were uprooted by hand during growing period. Then Seedlings were transplanted to the main land at the 4-5 leaf stage and placed 45 cm and 25 cm apart on 22nd June. As a split-factorial experiment based on RCBD in 3 replications in which stress was the main factor and microelement and phosphate biofertilizer were the subplot factors. Water stress was in 3 levels as: A₁: Irrigate after 70 mm evaporation from class A basin, A₂: Irrigate after 120 mm evaporation from class A basin, A₃: Irrigate after 170 mm evaporation from class A basin, Microelements in 3 levels: B₁: Not application (control), B₂: Zinc sulphate and B₃: Iron sulphate, and biological fertilizer in 2 levels: C₁: Not application of phosphate biofertilizer and C₂: Application of phosphate biofertilizer. Distribution of the base nitrogen and potassium fertilizers were done according to soil analysis (Table 1). 200kg Urea fertilizer was applied per hectare in 3 stages of cultivation: beginning, stemming

and flowering. Then Microelement foliar application was used in 8 leaf stages of plant with 0.005 density. Thereafter, Phosphate biofertilizer was also used twice along with farrow in 2 replication after one month distance. Phosphate biofertilizer contained *Pantoea agglomerans* and *Pseudomonas putida* bacteria which have phosphate enzymic activities and agglomeration bacteria has H⁺ activity, so the first one has efficiency in solving organic unsoluble phosphate and the second in solving unsolvable phosphates. They efficiently raise the phosphate use of plants.

The Irrigation process was carried out once a week up to flowering stage then flowering water stress was used. Measuring traits were performed when plants were fully flowered. thereafter to omit the margin effects, two margin lines were drawn, and two plants from the beginning and the end of the line were removed. The average plant height, flowering branch number and dry matter of aerial organs were calculated. To measure the iron and zinc ion content the atomic absorption method was used. For data variance analysis calculation using Mstat-c, and mean comparisons was done by Duncan's multi test and excel sheets were used to draw the figures.

RESULTS AND DISCUSSION

The results obtained from data variance analysis showed that water stress significantly affected plant height, flowering branch number, dry aerial organ yield and iron rate of aerial organ. Microelement application also was significant on all studied characteristics. The use of natural phosphate biofertilizer was significant on height, dry yield, the flowering branch number and zinc rate of above ground. Studied factors effects were significant on the most *Purple coneflower* characteristics (Table 2).

Plant height

Variance analysis showed that effect of water stress and foliar application of microelements and also effect of foliar application of microelements and application and control of phosphate biofertilizer was significant, interaction of water stress and application and control of phosphate biofertilizer was significant. Comparisons of the means of AB (water stress and microelements) showed the highest plant related to zinc sulphate foliar application under full irrigation conditions with 82 cm length. The smallest height related to control under irrigation conditions after evaporation of 170 mm was 56 cm (Figure 1). The decrease in plant height under control condition by increasing stress from 120 to 170 mm was 6.32% and 19.51% respectively for the controls. Zinc and iron application under full irrigation conditions of 120 mm resulted in significant plant height. Increase in application

Table 2. Analysis of variance of some characteristics.

S.O.V.	df	Mean square				
		Plant height	Flowering branch number	Above ground dry yield	Above ground zinc content	Above ground Iron content
Replication	2	20.952**	0.506	1.108	0.256 ^{ns}	0.517 ^{ns}
Water stress (A)	2	670.707**	16.007**	43.853**	6.281 ^{ns}	5.614**
Error	4	0.822	0.272	1.349	0.121	0.089
Microelements (B)	2	551.576**	15.159**	847.588**	13.787**	13.968**
Stress*microelements(AB)	4	56.984**	7.514**	7.418*	0.310**	0.021 ^{ns}
Phosphate biofertilizer(C)	1	278.347**	160.856**	94.512**	4.1**	3.910 ^{ns}
A*C	2	9.031*	17.641**	3.219 ^{ns}	0.041 ^{ns}	0.014 ^{ns}
B*C	2	15.339**	0.551 ^{ns}	92.557**	0.202 ^{ns}	0.010 ^{ns}
A*B*C	4	3.914 ^{ns}	1.086*	0.886	0.034 ^{ns}	0.049 ^{ns}
Error	30	1.959	0.308	2.579	0.077	0.128
cv%		1.96	5.90	4.70	17.31	12.58

*, ** and ns: Significant at 5 and 1% levels and non-significant, respectively.

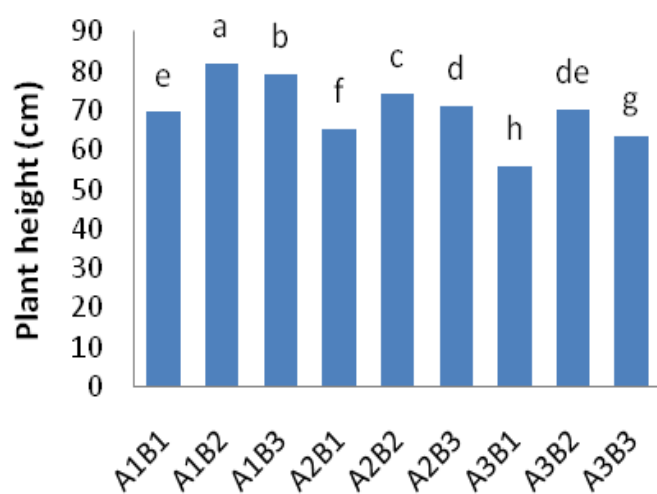


Figure 1. Effect of microelements on plant height under water stress A₁, Full irrigation; A₂, irrigation after 120 mm evaporation of basin; A₃, irrigation after 170 mm evaporation of basin; B₁, no application of microelement; B₂, zinc sulphate foliar application; B₃, iron sulphate foliar application.

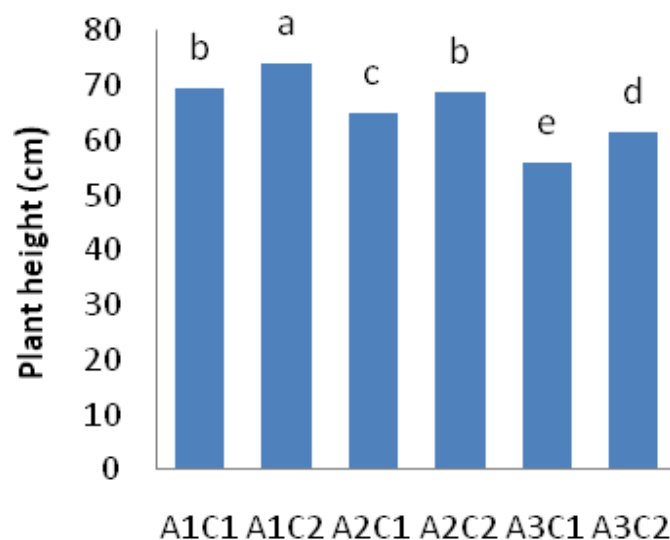


Figure 2. Effect of phosphate biofertilizer on plant height under water stress. A₁, Full irrigation; A₂, irrigation after 120 mm evaporation of basin; A₃, irrigation after 170 mm evaporation of basin; C₁, no application of phosphate biofertilizer; C₂, application of phosphate biofertilizer.

of zinc and iron was 6.69 and 2.29% respectively. The increase in stress to 170 mm significantly destroyed the effect of zinc so the iron application could not prevent height decline of bush during the control period. The study on corn under stress, showed that zinc presence is necessary for Indol Acetic Acid synthesis, also zinc shortage influences growth and development of grana, inside the chloroplast (Mengel and Kirkby, 1987). Thalooh et al. (2006) showed that zinc foliar application increase height, branch number per plant and stem dry weight. Mean comparison interaction effects of AC (water stress and phosphate biofertilizer) show that the most height of plant related to A1C2 treatment by 74 cm height

(application of phosphate biofertilizer under full irrigate condition) and the smallest height related to A3C1 treatment (no application of phosphate biofertilizer under 170 mm water stress) with 56cm height (Figure 2). Phosphate biofertilizer application under different irrigation conditions increased plant height. The increase was 6.37% for full irrigation, 5.87% in 120 mm and 10.18% in 170 mm. According to this result phosphate biofertilizer had the most effect under stress condition. Applying water stress resulted in decrease plant growth. Perhaps the reason for the decrease in the grow rate is

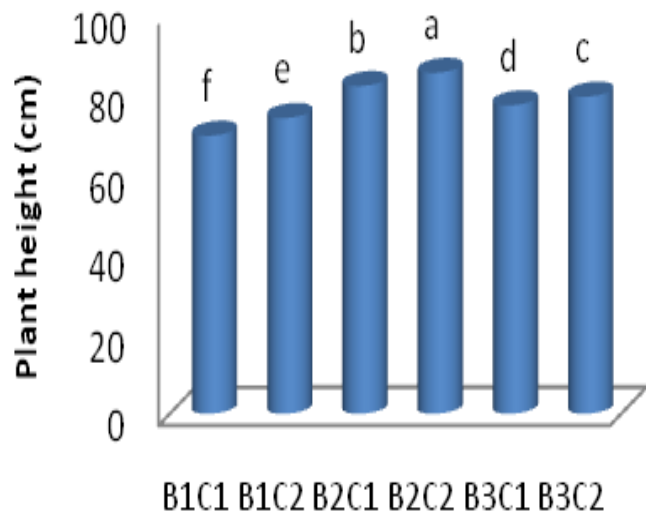


Figure 3. Effect of microelements and phosphate biofertilizer on plant height under water stress. B₁, No application of microelement; B₂, zinc sulphate foliar application; B₃, iron sulphate foliar application; C₁, no application of phosphate biofertilizer; C₂, application of phosphate biofertilizer.

as a result of the decrease in cell divisions and cell growth. The role of hormones is also important on the treatments under stress and the hormone rate of ABA increased. This hormone prevents IAA and CA activities which control cell division and cause growth rate (Dandria et al., 1995). Comparison of BC shows that the highest plant related to B2C2 treatment (zinc sulphate foliar application with phosphate biofertilizer application) was 85.3 cm and the smallest increase related to B1C1 treatment (control treatment) was 69.57 cm (Figure 3). Phosphate biofertilizer application when compared in controlled application produced significant height rise but it was more than it when the microelements did not apply. However, the highest effect on plant height increased related to phosphate and zinc application at the same time, was equal to 22.61%. One of the benefits of plant growth motiven bacteria is production of hormones and motive plant growth, root system development and improvement of water and nutrition absorption (Kravchenko et al., 1994). Plant biological fertilizers application in *Thymus vulgaris* increase plant growth significantly (Youssef et al., 2004).

Flowering branch number

Comparisons of the means of microelement foliar application effect and the use of phosphate biofertilizer under water stress condition on studied cases showed that the branches with more flower numbers were found with zinc sulphate foliar application treatment and phosphate biofertilizer use under full irrigation condition with 13.4 flowering branches which had no significant differences between iron sulphate foliar application and

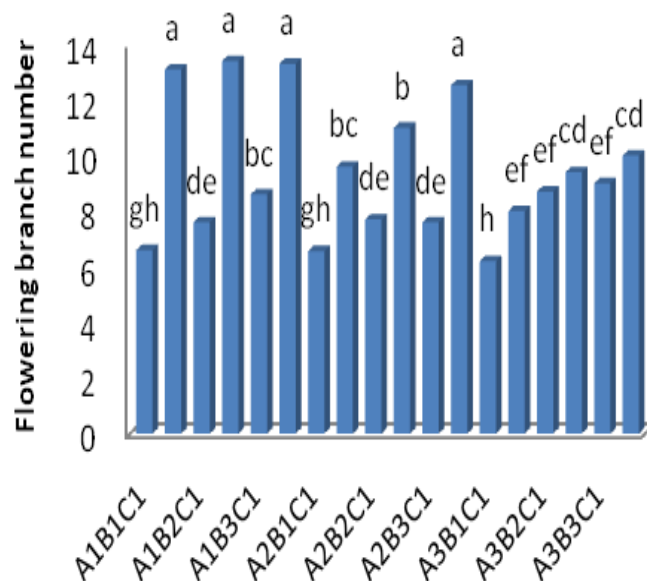


Figure 4. Effect of microelement fertilizers and phosphate biofertilizer on flowering branch number under water stress. A₁, Full irrigation; A₂, irrigation after 120 mm evaporation of basin; A₃, irrigation after 170 mm evaporation of basin; B₁, no application of microelement; B₂, zinc sulphate foliar application; B₃, iron sulphate foliar application; C₁, no application of phosphate biofertilizer; C₂, application of phosphate biofertilizer.

phosphate biofertilizer under full irrigate condition treatments. Phosphate biofertilizer under full irrigation treatment conditions, zinc sulphate foliar application and the use of phosphate biofertilizer in irrigation after 120 mm evaporation of evaporating basin by 13.3, 13.1, 12.53 produced flowering branches, respectively (Figure 4). The less flowering branch numbers related to A3B1C1 treatment (Non application of microelement fertilizer and biological fertilizer under water stress condition) produced 6.2 flowering branches. Simultaneous application of phosphate biofertilizer, zinc, iron and phosphate biofertilizer in all stress levels increased flowering branch number, but the highest increase in results : 50.3%, 49.93% was obtained by full irrigation. By increasing stress level up to 120 mm, the flowering branch numbers decreased but it was not significant in the application of zinc and phosphate biofertilizer at the same time. Stress increase up to 170 mm decreased flowering branch numbers, while zinc and phosphate biofertilizer application at the same time with iron and phosphate biofertilizer increased flowering branch number to 35.2% and 34.46%, respectively. The result was more than when controlled with the same level of stress. Stress occurrence causes photosynthesis material production and plant production development decrease, The signal of less growth development is the decrease in sub stem number of plants. The results obtained showed that biological fertilizer causes flowering branch number increase and yield improvement. Fungus and bacteria and their bilateral effects could be effective in root and

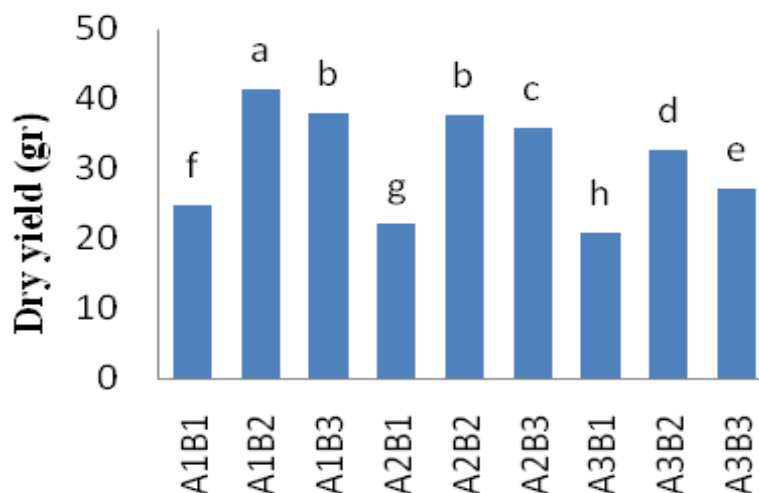


Figure 5. Effect of microelements under water stress on dry yield. A₁, Full irrigation; A₂, irrigation after 120 mm evaporation of basin; A₃, irrigation after 170 mm evaporation of basin; B₁, no application of microelement; B₂, zinc sulphate foliar application; B₃, iron sulphate foliar application.

above ground growth (Medina et al., 2003). Zinc increases photosynthesis and hydrocarbon production, as a result, plant growth improves. Zinc, Mn, iron foliar application increase S growth parameters, yield and its components significantly (Thalooth et al., 2006).

Above ground dry yield

Mean comparisons showed that zinc sulphate foliar application under full irrigation with 41.41 g had the highest dry weight of aerial organ between different treatments (Figure 5). The less above ground dry weight related to A3B1 treatment (not application of microelement fertilizer under water stress) with 20.96 g. The results show that dry yield decrease under non application of microelements by increasing stress up to 120 and 170 mm rather than when controlled with 90.2 and 15.62%, respectively. Zinc and iron application under full irrigation with 120 and 170 mm stress, resulted in significant rise in dry yield rather than non application of them which gave results of 44.12 and 9.34%, respectively. Results obtained from mean comparisons showed that from dry aerial organ yield point of view bilateral effect of microelement foliar application, application and not application of phosphate biofertilizer is significant. Therefore, the highest dry weight occurred in zinc sulphate foliar treatment and phosphate biofertilizer application with 42.9 g. The less dry weight occurring in controlled treatment was 24.84 g (non application of phosphate biofertilizer and microelements) (Figure 6). Phosphate biofertilizer application and non application of it in all levels and increased microelements application resulted in dry yield. But this increase under control of microelements was more than its application.

However, the highest effect on dry yield obtained by phosphate biofertilizer and zinc simultaneously was equal to 69.44%. This research showed that leaf application of iron, zinc, Mn microelements to stem height causes dry material yield in Corn (Whitty and Chambliss, 2005). Dry material yield increase could occur as a result of auxin biosynthesis increase due to the presence of zinc element (Sharafi et al., 2002). During some researches it became clear that different levels of microelements influenced taragon dry weight (Glyn, 2002) which corresponds with results above. Ratti et al. (2001) declared that phosphate solvent bacteria when applied to some plant growth hormones have positive effect on increasing dry weight of lemon grass medicinal plant.

Zinc content of above ground

Average comparisons showed that the highest zinc rate in above ground of Purple coneflower occurred the A1B2 treatment (foliar application of zinc under full irrigate condition) with 3.81 mg per kg of dry material, and there were significant difference with other treatments (Figure 7). The less zinc occurred in A3B1 treatment (no application of microelements under water stress) with 0.69 mg per kg of dry mater. The results showed that foliar application of zinc caused increase in the content of aerial organs of purple coneflower. Also, zinc foliar application resulted in iron rate improvement in aerial organ. Above ground zinc rate decreased under control conditions by increasing stress to levels 120 and 170 mm and when controlled, it was 25.34 and 52.74%, respectively. Zinc application and full irrigation condition with stress application of 120 and 170 mm increased

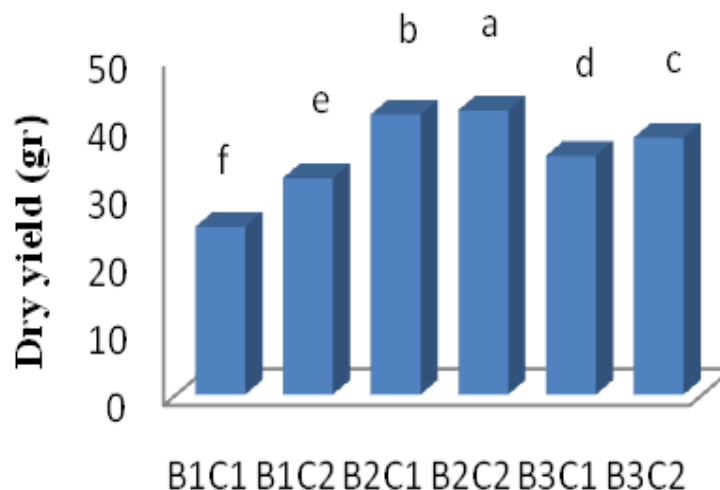


Figure 6. Effect of microelements and phosphate biofertilizer under water stress on dry yield. B₁, No application of microelement; B₂, zinc sulphate foliar application; B₃, iron sulphate foliar application; C₁, no application of phosphate biofertilizer; C₂, application of phosphate biofertilizer.

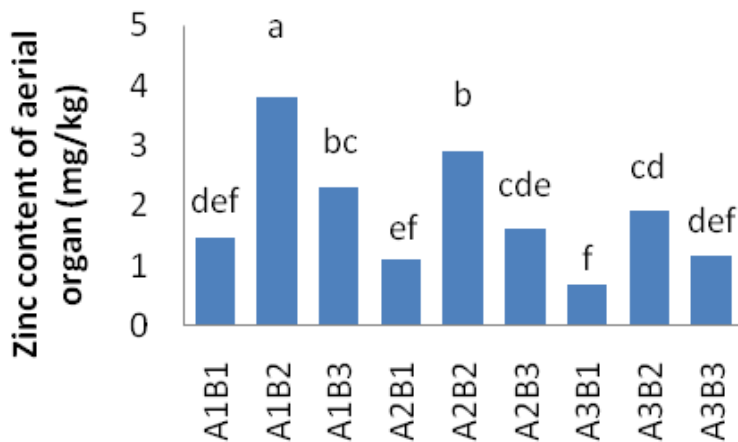


Figure 7. Effect of microelements on zinc content of aerial organ under water stress. A₁, Full irrigation; A₂, irrigation after 120 mm evaporation of basin; A₃, irrigation after 170 mm evaporation of basin; B₁, no application of microelement; B₂, zinc sulphate foliar application; B₃, iron sulphate foliar application; C₁, no application of phosphate biofertilizer; C₂, application of phosphate biofertilizer.

significantly the zinc rate of above ground than when controlled. The increased in zinc application was 30.82%. Iron application under full irrigation with stress of 120 and 170 mm increased the zinc in aerial organ. Brown et al (1993) showed that with zinc and potassium application, the absorption of some nutritious elements such as Iron and Mn increases. It seems that water stress application, decreases zinc absorption of soil. Phosphate biofertilizer application decreases zinc density of aerial organ so that zinc density in aerial organ related to the non application of biological fertilizers with 1.46 mg per kg of dry

material treatment, which had significant differences with biological fertilizer application with 0.92 mg per kg of dry material treatment (Figure 8). In control of phosphate biofertilizer, zinc rate in aerial organ was 36.99% and more. High application phosphorous decreases the absorption of less used elements especially zinc in farms (Malakouti, 2003).

Iron content of above ground

Mean comparisons show that highest iron rate in

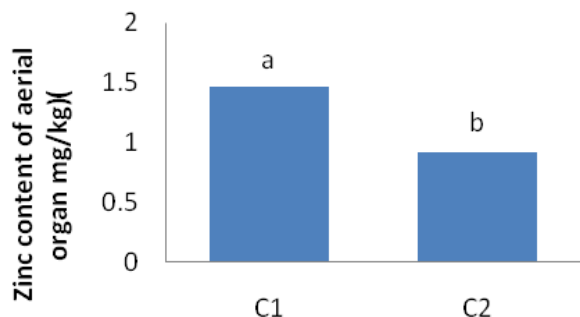


Figure 8. Effect of phosphate biofertilizer on zinc content of aerial organ. C₁, no application of phosphate biofertilizer; C₂, application of phosphate biofertilizer.

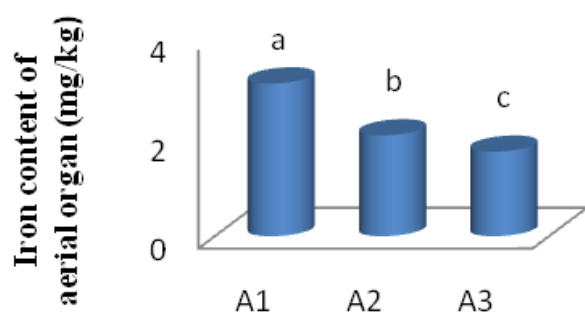


Figure 9. Water stress condition on Iron content of aerial organ. A₁, Full irrigation; A₂, irrigation after 120 mm evaporation of basin; A₃, irrigation after 170 mm evaporation of basin.

aerial organ was found in A1 treatment (full irrigation condition) with 3.09 mg per kg of dry material. The lowest iron rate was found in the A3 (water stress) with 1.89 mg per kg of dry material (Figure 9). Iron rate decreased in aerial organ under stress increases up to 120 mm, when not stressed 16.83% and when stressed increases up to 170 mm was 38.83%. Results showed that iron foliar application increases its rate in aerial plant organ. Also iron foliar application had positive effect on zinc absorption. Foliar application of microelements also had significant effect on iron rate of aerial organ so the most iron rate, related to B3 treatment (Iron sulphate foliar application) with 4.72 mg per kg dry matter and the less rate was in B1 treatment (not iron foliar application) with 3.09 mg per kg of dry material (Figure 10). Iron sulphate foliar application increased iron rate of aerial organ up to 52.75% rather than when controlled. Zinc application had no significant effect on aerial organ iron rate. However zinc sulphate foliar application increased 5.5% Iron rate of aerial organ rather than when controlled.

Conclusion

Totally, it can be concluded that application of

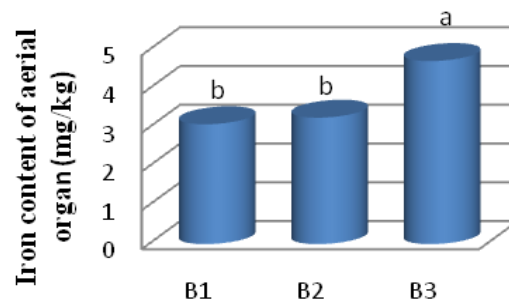


Figure 10. Effect of microelement fertilizers on Iron content of aerial organ. B₁, No application of microelement; B₂, zinc sulphate foliar application; B₃, iron sulphate foliar application.

microelement fertilizers increases plant height, flower branch number, plant dry yield and also high density of this element in above ground. According to the results obtained, foliar application of zinc and iron sulphate in 8 leaf stage of plant with 0.005 under full irrigation condition can be recommended. The use of phosphate biological fertilizer increases dry plant material and Purple coneflower flower branch number, which increases plant yield. Water stress had negative effect on morphological characteristics of purple coneflower. Iron and zinc microelement application can increase dry yield of *Purple coneflower* up to 44.12 and 9.34% under stress condition than when controlled. The absence of phosphate biofertilizer application in all levels of microelements application caused significant dry yield rise. The highest effect on dry yield obtained from the use of phosphate biofertilizer and zinc at the same time was 69.44%.

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REFERENCES

- Anna MA, Lakshmi PTV, Lalithakumari D, Murugesan K (2004). Optimization of biofertilizers on growth, biomass and seed yield of *Phyllanthus amarus* (Bhumyamalaki) in sandy loam soil. *J. Med. Aromat. Plants Sci.*, 26: 21-28.
- Brown PH, Cakmak I, Zhang Q (1993). Form and function of zinc in plants. In: A.D. Robson (ed). *Zinc in soils and plants*. Kluwer Academic publishers. Dordrecht, The Netherlands, pp. 93-106.
- Chaudhry AU, Sarwar M (1999). Optimization of nitrogen fertilizer in cotton (*Gossypium hirsutum* L.). *Pak. J. Biol. Sci.*, 2: 242-243.
- Chevallier A (1996). *The encyclopedia of medicinal plants*. Dorling Kindersley Ltd. Pub1. London. pp. 46-63.
- Dandria R, Chiaranda FQ, Magliulo V, Mori M (1995). Yield and soil water uptake of sunflower sown in spring and summer. *Agron. J.*, 87: 1122-1128.
- Glyn MF (2002). Mineral nutrition, production and artemisinin content in *Artemisia annua* L. *Acta Hort.*, 426: 721-728.

- Kravchenko LV, Leonova EI, Tikhonovich IA (1994). Effect of root exudates of non-legume plants on the response of auxin production by associated diazotrophs. *Microb. Releases*, 2: 267-271.
- Malakouti MJ (2003). The role of zinc in plant growth and enhancing animal and human health. Regional expert consultation in plant, animal and human. Interaction and Impact, Damascus, Syria, pp. 38-45.
- Medina A, Probanza A, Gutierrez Mañero FJ, Azcón R (2003). Interactions of arbuscular-mycorrhizal fungi and *Bacillus* strains and their effects on plant growth, microbial rhizosphere activity (thymidine and leucine incorporation) and fungal biomass (ergosterol and chitin). *Appl. Soil Ecol.*, 22: 15–28.
- Mengel K, Kirkby EA (1987). Principles of plant Nutrition, 4th Edition. International Potash Institute, Bern, Switzerland.
- Mitra J (2001). Genetics and genetic improvement of drought resistance in crop plants. *Curr. Sci.*, 80: 758- 763.
- Ratti N, Kumar S, Verma HN, Gautam SP (2001). Improvement in bioavailability of tricalcium phosphate to *Cymbopogon martini* var. motia by rhizobacteria, AMF and azospirillum inoculation. *Microbiol. Res.*, 156: 145- 149.
- Repčak M, Imrich J, Franekova M (2001). Umbelliferone, a stress metabolite of *Chamomilla recutita* L. Rauschert. *J. Plant Physiol.*, 158: 1085-1087.
- Saleh RN (2001). Biofertilizers and their role in order to reach to sustainable agriculture. A compilation of papers of necessity for the production of biofertilizers in Iran, pp. 1- 54.
- Sharafi S, Tajbakhsh M, Majidi M, Pourmirza A (2002). Effect of iron and zinc fertilizer on yield and yield components of two forage corn cultivars in Urmia. *Soil Water*, 12: 85-94.
- Sharma AK (2002). A handbook of organic farming. Agrobios, India, pp. 627-639.
- Shubhra K, Dayal J, Goswami CL, Munjal R (2004). Effects of water-deficit on oil of calendula aerial parts. *Biol. Plant.*, 48(3): 445-448.
- Sreevalli Y, Baskaran K, Chandrashekara R, Kuikkarni R, Sushil Hasan S, Samresh D, Kukre j, Ashok A, Sharma Singh K, Srikant S, Rakesh T (2001). Preliminary observations on the effect of irrigation frequency and genotypes on yield and alkaloid concentration in Petriwinkle. *J. Med. Aromat. Plant Sci.*, 22: 356-358.
- Thalooth AT, Tawfik MM, Magda MH (2006). A comparative study on the effect of foliar application of zinc, potassium and magnesium on growth. yield and some chemical constituents of mungbean plants grown under water Stress Conditions. *World J. Agric. Sci.*, 2(1): 37-46.
- Wasule DL, Wadalkar SR, Buldo AN (2002). Effect of phosphate solubilizing Bacteria on role of Rhizobium on Nodulation by soybean. Proceeding of the 15 th Meeting on Microbial Phosphate Solubilization. Spain: Salamanca University Publication, pp. 38-49.
- Whitty EN, Chambliss CG (2005). Fertilization of field and forage crops. Nevada State University Publication, p. 21.
- Youssef AA, Edris AE, Gomaa AM (2004). A comparative study between some plant growth regulators and certain growth hormones producing microorganisms on growth and essential oil composition of *Salvia officinalis* L. *Plant Ann. Agric. Sci.*, 49: 299-311.