Nutritional responses of Thymus exposed leaf spraying under soil nitrogen deficiency

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Abstract

Thyme is a valuable plant used in medicine, perfumery and food industry. Mineral deficiencies often limit the growth of plants. Nitrogen deficiency in agricultural soils is a problem throughout the world. The present study evaluated effects of applying Fe and Zn in absorption of some important nutrients of Thymus vulgaris L. under nitrogen deficiency. Potted experiments were designed to study effect of spraying some micronutrients on nutrient contents of thyme. Nitrogen deficiency applied whereas application of N decreased to half, 1/4 and zero. At the same time, foliar spraying of Fe2+ and Zn2+ in concentration of 1% and 2% was replicated each 15 days. Results showed that application of Zn 2% cause increase in NO3- content of thymus root and in shoots of deficient plants. Also foliar spraying of Zn and Fe cause increase in P and NO3- content of thymus plants under nitrogen deficiency conditions. There was significant correlation ($P<0.05$, $r>0.9$) among micronutrients, P and NO3- content under N deficiency. Analysis of variance showed that the difference in all nutrients content between nitrogen treatments, among spray of different micronutrients and nitrogen treatments × spray was significant in roots and shoots of plants and soil. Foliar application of iron and zinc significantly enhanced the absorption of other nutrients in thymus plants under deficiency conditions, but influence of zinc was higher. It seems that foliar fertilizer improves nutrition of plant in nitrogen deficiency conditions and decrease environmental hazard by decreasing the applying of chemical fertilizers in soils.

Keywords: Tolerance; leaf nutrition; lamiaceae; micronutrients; deficiency

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Introduction

Thyme (*Thymus vulgaris* L.) is a flowering plant of Lamiaceae. The essential oil of thyme is an antioxidative agent. Also constituents of thyme are used as preserving products (Van den Hoven et al., 2003). Thyme contains more essential oil than other species, therefor it is usually applied in pharmaceutical dosage type (Verpoorte, 2000).

Plant nutrition is the most necessary factor in plant production. Harmony of macro and micronutrients fertilization in plant nutrition is essential for high yield (Sawan et al., 2001).

Nitrogen (N) is very important, because its presence in plant protein structure. N have an important role in synthesis of different enzymes.

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N supply was essential of physiological process and production of wheat (Marino et al., 2016). Primary and secondary metabolism affected by nitrogen deficiency. Deficiency of N in Arabidopsis, led to coordinate repression assigned to photosynthesis and induction of secondary metabolism (Scheible et al., 2004). N availability limitation reduced root growth in order to intensify uptake from deficient rhizosphere (Nguyen et al., 2003).

Fertilizers can be supplied to plants as foliar sprays. Foliar spray is a technique of plant nutrition by using liquid fertilizer to leaves directly (Bernal et al., 2007). Application of nutrients with foliar type is more efficiently than root application when nutrients availability in soil is low (Erdal et al., 2004). Between micronutrients, Cu, Fe, Zn and Mn are using foliar fertilizers form, micronutrients such as iron (Fe) and zinc (Zn) are universally used as foliar fertilizers in order to compensate deficiency (Kaya et al., 2005). Foliar spraying of fertilizers including micronutrients such as Zn and Fe have been shown to be suitable for field use, have a good effectiveness and rapid plant response (Fernández et al., 2013). Also, foliar fertilizers help to avoid toxicity that may occur after application of same micronutrients in soil (Obreza et al., 2010).

The solubility and availability Zn and Fe of soils were dependent on pH, texture, organic carbon content, soil moisture, calcium carbonate content, redox potential, interactions with other nutrients, plant parameter and climate properties. Application of micronutrients cause improved plant growth and performance to absorb nutrients and photosynthesis, because these have important role in different biochemical processes (Kalidasu et al., 2008). The solubility of Zn and Fe decrease in calcareous soils with high pH. Adsorption of Fe and Zn on the lime and clays surfaces could reduce the availability of them. Therefore, the effectiveness of Zn and Fe fertilizers is decrease when using to soil (Marschner, 1995).

Iron has essential functions in metabolism, for example in catalase enzymes activating and in the pathway of glycolate and chlorophyll structure. Zinc is important micronutrient for cell division. Therefore, Zinc deficiency reduces plant growth, fruits quality, flowering stage and seed germination (Marschner, 1995). Fe and Zn on plant effects as a metal component of many enzymes. Moreover, they have important roles in metabolism (Baloch et al., 2008).

The aim of present study was evaluation comparative effects of applying Fe and Zn in Thymus vulgaris L. under nitrogen deficiency conditions.

Materials and Methods

Plant material

Potted experiments were designed to study effect of foliar spraying some of micronutrients on mineral nutrient concentrations in Thymus plant under nitrogen deficiency. Soil preparation was done in November 2017. We used sandy loam as texture of the soil (77.5 % sand, 7.5 % silt and 15 % clay). Seedlings of Thymus Vulgaris L. were obtained from Zarrin ghiah (Agricultural Research Center, Urmia). The seedlings of thymus, were planted in pots (20 cm depth and 18 cm diameter) and irrigated with 1/4 strength Hoagland solution (Table 1). Irrigation continued until harvesting time with 1/2 and full strength Hoagland solution. Nitrogen deficiency applied on three months' plants. To apply nitrogen deficiency, the KNO3 compounds gradually decreased half way, then a quarter and finally dropped to zero. Instead of combining $Ca(NO_3)_2$, $4H_2O$ and $NH_4H_2PO_4$, $Ca(SO_4)_2$ and KH_2PO_4 were added to Hoagland solution. In this way nitrogen is removed from the solution and other nutrients are applied. The application of N decreased to half, 1/4 and zero each for 15 days. At the same time, foliar spraying of Fe^{2+} and Zn^{2+} in concentration of 1 % and 2 % (w/v) was replicated each 15 days. Plants harvested after 45 days, when they showed deficiency and spraying symptoms. Plants were harvested and leaves and roots of plants were weighed separately and dried at 70 °C for 48 hours. Dried plants were ground to increase homogeneity.

Plant measurements

Grounded tissues were put into centrifuge tubes and 1 mM HNO_3 was added. The tubes were

Table 1
Composition of modified Hoagland nutritional solution for growing plants

Normal			Deficiency		
Composition	Storage Concentration	Solution volume of storage per litter of final solution	Composition	Storage Concentration	Solution volume of storage per litter of final solution
	gL^{-1}	mL		gL^{-1}	mL
Main element			Main element		
KNO_3	101.10	6.0	-	-	-
$Ca(NO_3)_2$, $4H_2O$	236.16	4.0	$Ca(SO_4)_2$	145.15	4.0
$NH_4H_2Po_4$	115.08	2.0	KH_2PO_4	136.09	2.0
$MgSO_4$, $7H_2O$	246.48	1.0	$MgSO_4$, $7H_2O$	246.48	1.0
Micronutrients			Micronutrients		
KCl	1.864		KCl	1.864	
H_3BO_3	0.773		H_3BO_3	0.773	
$MnSO_4TH_2O$	0.169		$MnSO_4TH_2O$	0.169	
$ZnSO_4$, $7H_2O$	0.288	2.0	$ZnSO_4$, $7H_2O$	0.288	2.0
$CuSO_4$, $5H_2O$	0.062		$CuSO_4$, $5H_2O$	0.062	
H_2MoO_4(%85 MoO_3)	0.040		H_2MoO_4(%85 MoO_3)	0.040	
NaFeDTPA(%10Fe)	30.0	0.3-1.0	NaFeDTPA(%10Fe)	30.0	0.3-1.0
$NiSO_4.6H_2O$	0.066	2.0	$NiSO_4.6H_2O$	0.066	2.0
$Na_2SiO_3.9H_2O$	284.20	1.0	$Na_2SiO_3.9H_2O$	284.20	1.0

left overnight for extraction. The next morning, tubes were placed in boiling water bath for 15 min. Then deionized water was added to each tube and returned to the water bath, again. Samples were centrifuged at 5,000 rpm and the supernatant poured into plastic tubes. A further deionized water was added and tubes returned to the water bath, again. Sample tubes were centrifuged and the supernatant poured into tubes and the volume made up to 10 ml by addition of deionized water (Walker et al., 2004). Fe^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} concentrations were measured by atomic absorption. P concentration was determined by Murphy and Riley (1962). 0.25 g of dry plant material weighted and transferred into tube. a few pumice boiling granules, and about 3 g catalyst mixtures added using a calibrated spoon. then 10 ml concentrated H_2SO_4 added using a dispenser, and stirred with vortex tube stirrer until mixed. tubes Placed in a block-digester set at 100 °C for 20 minutes, and the tubes removed to wash down any material adhering to the neck of the tube with the same concentrated H_2SO_4. Thoroughly agitated the tube contents, and then placed the tubes back on the block-digester set at 380 °C for 2 hours after clearing. After digestion completed,

Table 2
The texture and amount of primary soil elements are listed in the table below

pH	EC (ds/m)	OM (%)	CCE (%)	sand	silt	clay	Texture
7.44	3.21	2.38	17	77.5	7.5	15	Sandy loam

removed tubes, cooled, and bring to 100 mL volume with deionized water. Each batch of samples for digestion should contain at least one reagent blank (no plant), and one chemical standard (weigh 0.1 g EDTA standard digest), and one standard plant sample (internal reference).
NO_3– concentration was measured by nitration of salicylic acid (Cataldo et al., 1975). Briefly, 0.5 ml aliquots were mixed with 0.8 ml of 5% (w/v) salicylic acid in concentrated H_2SO_4. After 20 min at room temperature, 19 ml of 2N NaOH were added slowly to raise the pH above 12. The samples were cooled to room temperature and absorbance at 410 nm was determined by using a spectrophotometer (UV-visible, WPA S2100).

Soil analysis

Soil texture was assayed according to hydrometer method (Gee and Bauder, 1986). The hydrometer method of silt and clay measurement relies in the effect of particle size on the differential settling velocities within a water column. By this method after 40 second all sand-sized particles (0.02 mm and larger) settle out of the suspension and after 4 h, particles larger than clay (0.002 mm) settle out of the suspension.

Soil pH and carbonate calcium equivalent (CCE) was assayed according to McLean (1982) method. For pH measurement, 50 ml deionized water added to 50 g air-dry soil. Then mixed and allowed to stand for 30 minutes. The suspension stirred every 10 minutes during this period. After 1 hour, the suspension stirred. The combined electrode put in suspension and reading tacked after 30 seconds.

For CCE assay, carbonate is dissolved in the excess of hydrochloric acid (HCl). The remainder of the acid is titrated against sodium hydroxide (NaOH). In this reaction, CO_2 gas is released and the acid not used in the dissolution of carbonates is back-titrated with NaOH solution. In the titration method, two equivalents of acid are assumed to react with one mole of $CaCO_3$.

Electrical conductivity (EC) was determined by Roade (1982). Weigh of 50 g air-dry soil and 50 ml deionized water added, mix well and allowed stand for 30 minutes. After 1 hour, the suspension stirred. First, a round Whatman No. 42 filter paper putted in the Buchner funnel. Second, the filter paper moistens with deionized water and the vacuum pump start. The filtration continued until the soil on the Buchner funnel starts cracking. the clear filtrate transferred into a 50-ml bottle and take the reading. Also Organic matter (OM) was measured by Walkly and Black (1934) method with modification. So that, 10 ml 1 N potassium dichromate solution added to 1 g air-dry soil (0.15 mm), 20 ml concentrated H_2SO_4 added and the suspension mixed. After 30 minutes, 200 ml deionized water added and then 10 ml concentrated H_3PO_4 added, and allowed the mixture to cool. 10–15 drops diphenylamine indicator added and then a Teflon-coated magnetic stirring bar added and the beaker placed on a magnetic stirrer. Titration done with 0.5 M ferrous ammonium sulphate solution, until the colour changes from violet-blue to green.

The properties of primary soil were presented in Table 2. Available ion concentrations were assayed, too. Fe^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} were extracted by DTPA and TEA method and their contents were measured by atomic absorption (Lindsay and Norwel, 1979). N content was assayed by phenol disulphonic acid method (Bremner, 1965). 1 g air-dry soil (0.15 mm) weighted into tube. about 5.0-5.5 g catalyst mixture, a few pumice boiling granules and 15 ml concentrated H_2SO_4 added. A glass funnel placed in the neck of the tube, and then tubes placed in the rack, and leaved overnight. The tubes rack placed in the block-digester and temperature slowly increased to 370 °C. The H_2SO_4 should

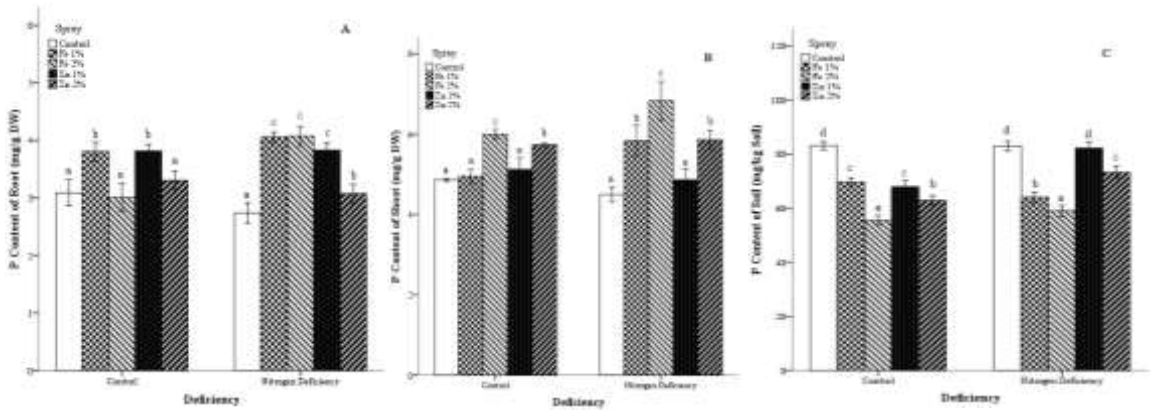

Fig. I. P content of roots (A) and shoots (B) of Thymus plant and soil (C) one month after nitrogen deficiency and spray of micronutrients. Different letters above the columns indicate significant difference (P<0.05) between the treatments according to Duncan's test.

condense about half-way up the tube neck; and when solution clears, heating continued for about 3 hours. the tubes rack lifted out of the block-digester, carefully placed on a rack holder, and let tubes cool to room temperature. about 15 ml deionized water slowly added to the tubes, cooled, and bring to volume. Each batch of samples for digestion should contain at least one reagent blank (no soil), and one chemical standard.

Also P content was determined by Olsen et al. (1954). 5 g air-dry soil (2-mm) weighted into a 250-ml Erlenmeyer flask and 100 ml 0.5 M $NaHCO_3$ solution added. the flask closed with a rubber stopper, and shake on a shaker at 200 –300 rpm for 30 minutes. Include one flask containing all chemicals but no soil (Blank). The suspension Filtered using a Whatman No. 40 filter paper. 10 ml clear filtrate Pipetted into a 50-ml flask. the required acid added to all the unknown solutions (adding 1 ml 5 N H_2SO_4 is adequate to acidify each 10 mL $NaHCO_3$ extract to pH 5).

Statistical Analysis

Statistical analyses were done using SPSS (Version 24). One-way analysis of variance and GLM (General Linear Model) with Duncan's multiple range tests ($p<0.05$) was used. The correlation ($p<0.05$) between ions contents was calculated.

Results

The results of ANOVA showed that the effect of deficiency (except root length and Shoot length), spraying (except leaf area) and deficiency ×spraying (except the leaf area) was significant ($p<0.05$) for all growth factors according to Duncan's analysis (Table 3).

Analysis of variance showed that the difference in all mineral nutrients content between treatments, among spray of different micronutrients and treatments × spray was significant ($p<0.05$) in roots and shoots of plants and soil (Tables 4, 5, 6).

Table 3
Analysis of variance (Mean Squares) of growth factor under nitrogen deficiency and spraying by iron and zinc nutrients

	df	Root length	Shoot length	Root Dry weight	Shoot Dry weight	RWC	Leaf area
Treatment	1	0.048^{ns}	0.645^{ns}	5.134^{*}	98.031^{**}	902.224^{**}	1.321^{**}
Spray	4	100.580^{**}	43.552^{**}	5.466^{**}	41.611^{**}	76.906^{**}	0.048^{ns}
Treatment × Spray	4	64.448^{**}	27.052^{**}	6.088^{**}	22.059^{**}	66.752^{**}	0.146^{ns}
Error	20	6.303	5.855	0.740	2.892	6.205	0.083

ns,* and ** showed no significant, significant difference at 5% and 1%, significantly.

Table 4
Analysis of variance (Mean Squares) of mineral contents in roots of thymus plant one month after nitrogen deficiency and spray of micronutrients

	df	P	NO3-	Zn2+	Cu2+	Mn2+	Fe2+
Treatment	1	.169*	24.464**	.068**	.001**	.030**	5.562**
Spray	4	1.093**	14.522**	.049**	7.445E-5**	.021**	.831**
Treatment × Spray	4	.474**	4.707**	.005**	.000**	.027**	5.851**
Error	20	.022	.040	.000	1.134E-5	.000	.037

* showed significant difference at 5%.

Table 5
Analysis of variance (Mean Squares) of mineral contents in shoots of thymus plant one month after nitrogen deficiency and spray of micronutrients

	df	P	NO3-	Zn2+	Cu2+	Mn2+	Fe2+
Treatment	1	.445**	12.450**	.008*	.004**	.000*	.035*
Spray	4	2.793**	2.459**	1.394**	.001**	.001**	.085**
Treatment × Spray	4	.521**	1.171**	.023**	.001**	.001**	.124**
Error	20	.051	.018	.001	1.629E-5	3.103E-5	.006

*showed significant difference at 5%.

Table 6
Analysis of variance (Mean Squares) of soil mineral contents of thymus plant one month after nitrogen deficiency and spray of micronutrients.

	df	P	N	Zn2+	Cu2+	Mn2+	Fe2+
Treatment	1	150.549**	.000	1.031**	.183**	1.943**	2.745**
Spray	4	558.279**	.000	2.619**	.300**	1.048**	5.901**
Treatment × Spray	4	95.418**	3.053E-5	.125**	.088**	1.725**	.461**
Error	20	2.872	2.405E-6	.016	.011	.017	.023

*showed significant difference at 5%.

P content

Applying micronutrients showed no significant increase in normal plants, except for Fe^{2+} and Zn^{2+} 1 % in roots and Fe^{2+} and Zn^{2+} 2% in shoots (Fig. I). Roots and shoots of deficient plants showed significant ($p<0.05$) increase in P content by spraying micronutrients, except for Zn^{2+} 1 % in shoots. In both roots and shoots applying Fe^{2+} showed higher increase compare to Zn^{2+}.In normal and nitrogen deficiency conditions P content of soil decreased by applying micronutrients, that decrease was higher for Fe^{2+} spraying compare to Zn^{2+}.

NO^{3-} content

In roots of normal plants applying micronutrients in concentration 2 % cause

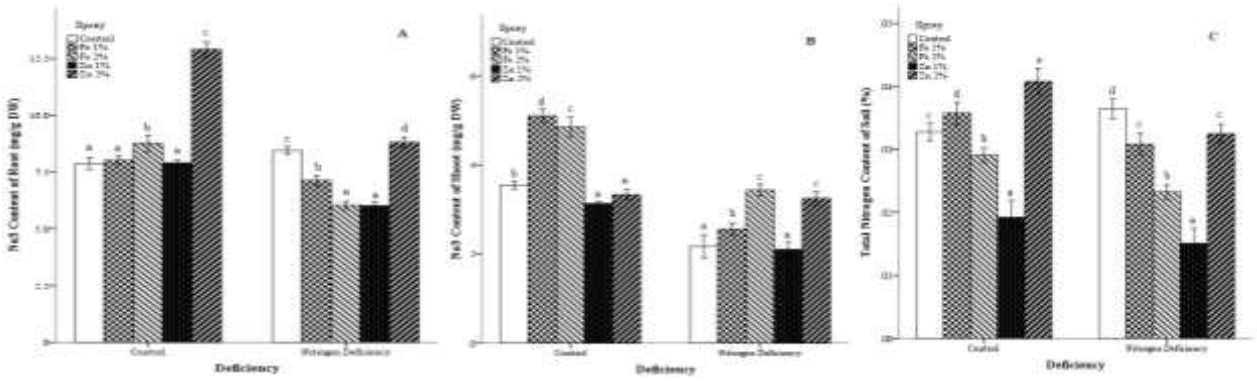

Fig. II. NO3- content of roots (A) and shoots (B) of Thymus plant and soil (C) one month after nitrogen deficiency and spray of micronutrients. Different letters above the columns indicate significant difference (P<0.05) between the treatments according to Duncan's test.

increase in NO^{3-} content, but increase in NO^{3-} content observed only by spraying Zn^{2}+ 2% in nitrogen deficiency conditions (Fig. II). About shoots applying Fe^{2+} cause increase in NO^{3-} content in normal and deficient plants, also shoots of thymus plants showed increase in NO^{3-} content by spraying Zn^{2+} 2 %. Total nitrogen content of soil decreased by applying Fe^{2+} 2 % and Zn^{2+} 1 % in normal plants, but all spraying cause decrease in total nitrogen content under deficiency conditions.

In roots and shoots of normal plants applying Fe^{2+} cause decrease in Fe^{2+} content, whereas nitrogen deficient plants showed increase in Fe^{2+} content by spraying Fe^{2+} 1% (Fig. III). Applying Zn^{2+} cause decrease in Fe^{2+} content of root in nitrogen deficient thymus plants, about shoots Zn^{2+} 2% cause decrease in Fe^{2+} content.

In normal and nitrogen deficiency conditions Fe^{2+} content of soil decreased by spraying micronutrients, that decrease was higher for Fe^{2+} spraying compare to Zn^{2+}.

Fe^{2+} content

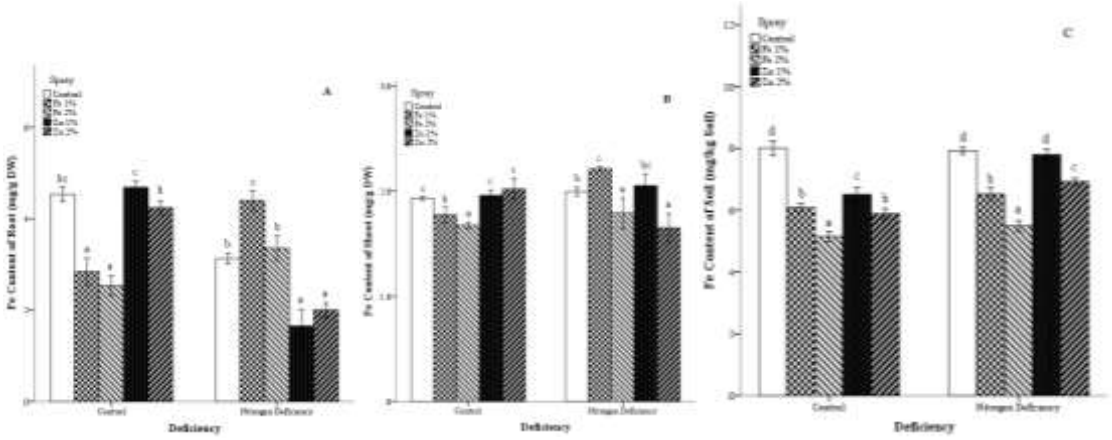

Fig. III. Fe^{2+} content of roots (A) and shoots (B) of Thymus plant and soil (C) one month after nitrogen deficiency and spray of micronutrients. Different letters above the columns indicate significant difference (P<0.05) between the treatments according to Duncan's test.

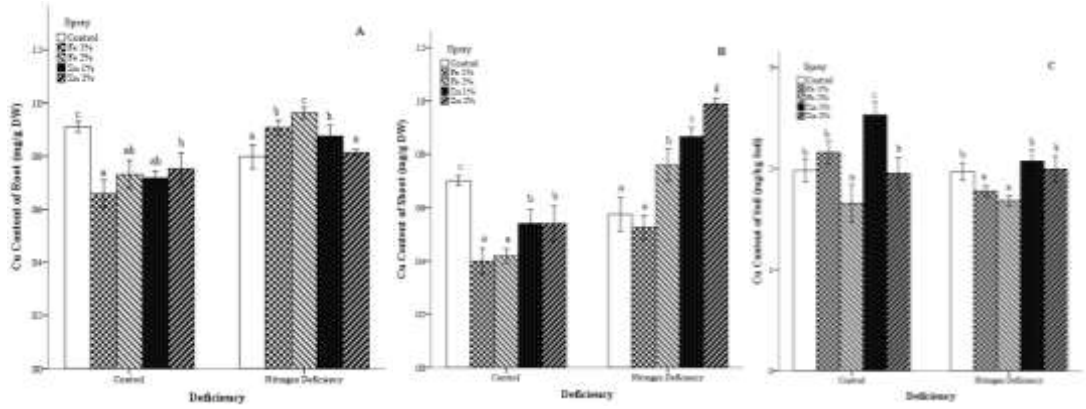

Fig. V. Cu^{2+} content of roots (A) and shoots (B) of Thymus plant and soil (C) one month after nitrogen deficiency and spray of micronutrients. Different letters above the columns indicate significant difference (P<0.05) between the treatments according to Duncan's test.

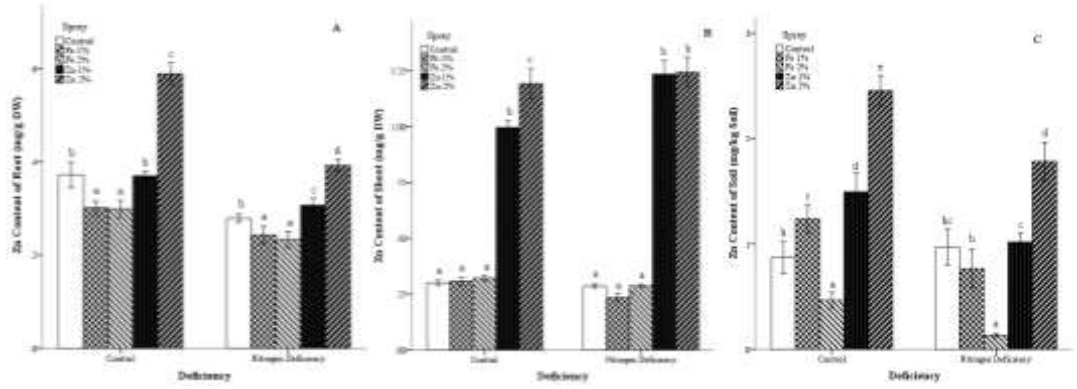

Fig. IV. Zn^{2+} content of roots (A) and shoots (B) of Thymus plant and soil (C) one month after nitrogen deficiency and spray of micronutrients. Different letters above the columns indicate significant difference (P<0.05) between the treatments according to Duncan's test

Zn^{2+} content

Zn^{2+} content increased in roots and shoots of thymus plant in normal and phosphorous deficiency conditions by spraying Zn^{2+}, treatment by Zn^{2+} 2 % showed the highest increase (Fig. IV). In roots applying Fe^{2+} cause decrease in Zn^{2+} content, but in shoots, spraying Fe^{2+} showed no significant effect compare to control.

Zn^{2+} content of soil decreased only by spraying Zn^{2+} 2 % in normal plants, other treatments cause increase. In phosphorous deficient plants also Zn^{2+} content of soil increased only by spraying Zn^{2+} 2 %.

Cu^{2+} content

Roots and shoots of normal plants showed significant (p<0.05) decrease in Cu^{2+} content by applying micronutrients (Fig. V). In nitrogen deficient plants spraying micronutrients cause increase in in Cu^{2+} content, except for Zn^{2+} 2 % in roots and Fe^{2+} 1 % in shoots.

In normal plants applying Fe^{2+} 2 % cause decrease in Cu^{2+} content of soil, but Zn^{2+} 1% cause increase. Soil of deficient plants showed decrease in Cu^{2+} content by spraying $^{Fe2+}$.

Mn^{2+} content

In normal plants applying micronutrients cause decrease in Mn^{2+} content of root, but nitrogen deficient plants showed increase in Mn^{2+} content (Fig. VI). In shoots of normal plants effect of Fe^{2+} 2 % was not significant, about deficient plants spraying Fe^{2+} 2 % and Zn^{2+} 1 % cause significant increase ($p<0.05$) in Mn^{2+} content.

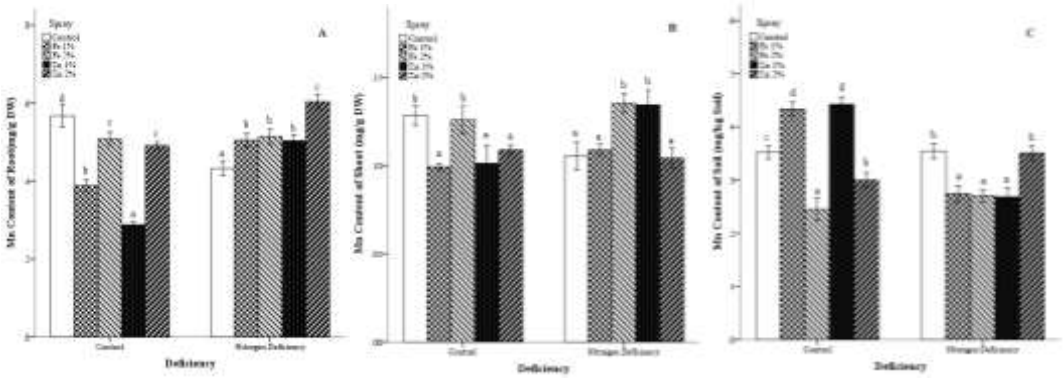

Fig. VI. Mn^{2+} content of roots (A) and shoots (B) of Thymus plant and soil (C) one month after nitrogen deficiency and spray of micronutrients. Different letters above the columns indicate significant difference ($P<0.05$) between the treatments according to Duncan's test.

Applying Fe^{2+} 1 % and Zn^{2+} 1 % cause increase of Mn^{2+} content in soil of normal plants, but in deficient plants all treatments showed decrease except for in Zn^{2+} 2 %.

Discussion

Several parameters affected the importance of nutrient sprays: a) properties of the composition such as pH, economic cost, solubility, b) environmental properties, for example relative temperature, humidity and light and c) physiological process of plant, determining the efficiency (Fernández and Brown, 2013).

Staal et al. (1991) reported that nitrogen absorption by the plant cause a relative increase in the absorption of other nutrients in the plant. The main effects of nitrogen are increase in metabolic activity of plant, acceleration of most processes and changes in plant absorption. Our results showed that the contents of some nutrients reduced under nitrogen deficiency conditions which was consistent with them.

Positive effects of micronutrients foliar spraying on plant growth may be because of increase in ability of plants to absorb nutrients and photosynthesis, as these play key roles in biochemical processes (Kalidasu et al., 2008).

Stimulating growth by zinc application may be related to high deficiency of zinc in soil. Besides, this result is possibly because of the role of zinc in increasing uptake of nutrients, cell division and chlorophyll content in leaves (Sotiropoulos et al., 2006). Our results are consistent with them, spraying of Zn cause increase in P and Zn content of root and shoot, in general, four possible causes have been considered responsible for Induced-Zn deficiency. These include (i) a P-Zn interaction in soil; (ii) a slower rate of translocation of Zn from the roots to shoot; (iii) a simple dilution effect on Zn concentration in plant tops due to growth responses to P; (iv) a metabolic disorder within plant cells related to an imbalance between P and Zn (Olsen et al., 1972)

Foliar fertilizer of Zn is widely applied and have been revealed to be rapidly soluble and available, and their cost are usually lower than soil application. Foliar fertilizer of Zn promoted plant vigor, production and fruit set in apple (Wojcik, 2007).

The enhancing effect of zinc application on nutrients contents of plants may be attributed to the favorable effect of zinc on metabolism and biological activity and their stimulating influence on translocation and accumulation of different nutrients (Marschner, 1995).

In peach and apple has been showed that the combination of nitrogen with zinc in 1-year-old peach trees following late season foliar application cause high yield (Sanchez et al., 2006). In present study application of Zn 2 % cause increase in NO^{3-} content of thymus root and in shoots of deficient plants. There was a significant correlation ($p<0.05$, $r>0.9$) between Zn and NO^{3-} content of root.

Result of Rehm and Albert (2006) revealed that spraying of FeSO4 or Fe-EDTA are efficient than Fe-chlorosis soil application in wheat. In our study application of Fe cause increase in Fe and Cu content in nitrogen deficient plants. Also there was a significant correlation ($p<0.05$, $r>0.9$) between Fe and P content of soil.

An antagonistic impact of Fe with Zn, Cu and Mn was reported, since the concentration of Fe was high in pistachio seedlings with Zn deficiency. Also soil application of Zn fertilizer decreased (11–13%) of Fe content (Shahriaripour and Tajabadipour, 2010). Present results verified them, in nitrogen deficient plants application of Fe decrease Zn content of root and shoot of thymus plant, also spraying of Zn reduce Fe content of root and shoot.

Laane (2018) reported that the foliar spraying of Zn or Fe increased root and shoot dry matter percentage. Also the uptake of N and P was increased. Also El-Fouly et al. (2011) was reported the improving of nutrient status by foliar application of micronutrients. Also Ravi et al. (2008) reported spraying of zinc and iron increased absorption of nitrogen, phosphorus, zinc and iron significantly. These results are in consistent with the findings of Kar and Babulkar (1998) in safflower.

In consistent with them, in present study foliar spraying of Zn and Fe cause increase in P and NO^{3-} content of thymus plants under nitrogen deficiency conditions. Also there was a significant negative correlation ($p<0.05$, $r> 0.8$) between P content of shoot and P content of soil.

Studies of Swaefy (1996) on *Mentha peperita* L. showed that the content of three nutrients (Fe, Zn and Mn) was generally increased by foliar application. Our results verified them, Fe, Zn and Cu content increased by spraying them in roots and shoots of deficient plants.

In the present study, iron and zinc spraying cause increase in root and shoot growth factors and difference in growth factors (except for leaf area) was significant under micronutrients spraying treatment, on the other hand application of micronutrients cause increase in nutrients absorption specially for nitrate and phosphorous. Therefore, it seems that micronutrients had effects on plant N content by influencing N absorption from soil.

Other reports verified our results, for example Ravi et al. (2008) reported The positive effect of zinc application on dry matter may be due to increase in nitrogen and phosphorus adsorption, auxin biosynthesis, chlorophyll concentration, phosphoenol pyruvate carboxylase activity and decrease in sodium accumulation in plant tissues. Also, spraying the plant with iron and zinc leads to increased plant photosynthesis. Therefore, more carbohydrates have been transferred to roots so that and absorption of nutrients increases and it cause the increase of plant growth (Marschner, 1995).

Previous reports verified increase in growth factors by application micronutrients. Coriander spraying by zinc and iron during growth stages, flowering and fruit formation caused significant increase in plant height, number of branches, fresh and dry weight of stem, essential oil percentage and grain yield (Said-Al Ahl et al., 2009). The height of the medicinal plant (Plantago ovate) was significantly affected by application of zinc and manganese (Ramroudi et al., 2011).

Conclusion

application of iron and zinc as spraying increase the absorption of other nutrients in thymus plants under deficiency conditions, but influence of zinc spraying was higher. It seems that foliar fertilizer decreases environmental hazard by decreasing the applying of chemical fertilizers in soils.

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