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Influence of micronutrient fertilizer on soybean nutrient composition

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Abstract

In order to study the effects of micronutrient fertilizers on nutrient composition of soybean, two set of experiments (pot and field) were done at the Islamic Azad University of Kermanshah province, Iran in 2010. The experimental design was a 3×3×3 factorial experiment based on Randomized Complete Block with three replicates. In the pot experiment, treatments included Zinc (0, 4, and 8 mg Zn kg^{-1} as $ZnSo_4.7H_2o$), iron (0, 4, and 8 mg Fe kg^{-1} as $FeSo_4$), and manganese (0, 15, and 30 mg Mn kg^{-1} as $MnSo_4.4H_2o$). Treatments in field experiment consisted Zn (0, 20 and 40 kg Zn ha^{-1}), Fe (0, 25 and 50 kg Fe ha^{-1}) and Mn (0, 20 and 40 kg Zn ha^{-1}) from source of $ZnSo_4$, $FeSo_4$ and $MnSo_4$, respectively. The results were shown that Zinc application had significant effects on Zn, Fe concentration in roots (P<0.01), and Zn, Fe and Mn concentration in shoots (P<0.01). Manganese in roots was not affected by zinc application. Except of shoots [Zn], iron fertilizer had significant effects on the other elements concentration in grain and root. The fertilizer treatment increased the micronutrients concentrated higher in roots compared to shoots. Zn concentrated more in roots and shoots when applied at 0 to 8 $mgkg^{-1}$. A similar trend was observed with manganese application (30 $mgkg^{-1}$ soil treatments). In the field experiment, Zinc concentrated more in seed compared to other parts of plant. While, iron application resulted [Fe] more its concentration in leaf but its further increase (> $25kg.ha^{-1}$Fe) resulted negative effects; however, the application of Mn brought down the negative effect. Mn up to 20 $kg.ha^{-1}$ had little effect on iron absorption, but its further increase reduced concentration of iron in stem, pod and grain. This study also shows that high soil concentration of manganese and iron had negative effects on zinc absorption. There was a significant and negative correlation between manganese and iron concentration in seed ($r=0.558^{**}$) and Mn and Fe concentration in leaf ($r= 0.397^{*}$).

Keyword: Fertilizer, iron, manganese, micronutrient, soybean, zinc

Introduction

Plants are at the beginning of the food chain. Therefore, improving the uptake of minerals from the soil and enhancing their transfer to and bioavailability in the edible parts of the plant will provide benefits for animal and human nutrition. Poor availability of plant nutrients limits yield in many of the world's crop production areas (Heitholt *et al*., 2003). Metals such as Zinc, iron and manganese have vital roles in plant's life cycle and very important for normal growth plants (Fageria, 2007). Zinc is considered as the most limiting factor in producing crops in different parts of the world (Mandal *et al*., 2000; Fageria & Baligar, 2005). Zn is an essential catalytic component of over 300 enzymes, including alkaline phosphatase, alcohol dehydrogenase, Cu-Zn superoxide dismutase, and carbonic anhydrase (Fox & Guerinot, 1998). Zinc plays an important role in synthesizing proteins, RNA, DNA and precursor of auxin which is essential for cell elongation (Welch, 2001; Awlad *et al.*, 2003). Also role in metabolism of nitrogen, synthesis of amino acid tryptophan, metabolism of starch, plants flowering and fruit set, increasing plant resistance to fungal disease and expanding plants roots (Fageria, 2009). For most plants, optimal Zn concentration is between 20 and 100 mg kg^{-1} tissue (Fox & Guerinot, 1998). Fe is the most limiting to agricultural production in the world (Kochian, 2000). Fe deficiency as chlorosis is a widespread problem for soybean grown on alkaline, calcareous soils (Wiersma, 2005; Lucena & Chaney, 2007; Caliskan *et al*., 2008). Iron plays an important role in nitrogen fixation and photosynthesis (Bennett, 1993). Synthesis of chlorophyll, thylakoid, and many ferrous proteins is dependent on this element (Imsande, 1998). Iron deficiency in plants is caused by factors that either inhibit its absorption and translocation or impair its utilization in metabolic processes (Fontes & Cox, 1998). Application of 2.5 mg Fe kg^{-1} soil not only increased top dry weight but also increased mean uptake of by 35.4% in different genotypes of soybean (Ghasemi-Fasaei *et al.*, 2003). Chemical characteristics of soil like pH is one of important factors in determining the degree of iron absorbance and transfer in soil and plants (Fageria, 2000). Iron solubility and availability is highly correlated to soil pH (Heitholt, 2003). Manganese is an essential element for plants growth and is identified a co- factor for nitrogen catabolism in leaves and a major factor for stabilizing nitrogen within roots and its transfer to shoots in soybean (Izaguirre-Mayoral & Sinclair, 2005). Mn plays an important role in stabilization of structural protein, the ultrastructural of choloroplasts and photosynthesis (Popelkova *et al*., 2003). Abdolsalam *et al*. (1994) reported that using iron, zinc and manganese simultaneously is more effective in increasing grain yield and produced dry matter than using them individually. The objective of this study was to: 1. Determine the effects of micronutrients fertilization on nutrient composition of soybean. 2. Evaluation of interaction between Zn, Fe and Mn in different part of the plant.

Materials and methods

Two set of experiments were done at the research field of the Islamic Azad University of Kermanshah province, Iran ($34^0 23'$ N, $47^0 8'$ E; 1351 m elevation) in 2010. The soil type of the experimental areas is silty clay, with cold and rainy winters, and hot and dry summers and

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annual average of precipitation is 478 mm. The experimental design was a 3×3×3 factorial experiment based on Randomized Complete Block with three replicates

Pot experiment

Surface soil was collected from an agricultural field and passed through a 2-mm mesh screen. The texture of the soil based on silty clay with pH 7.6 , total organic matter 1.8%, electrical conductivity (EC_e) 0.46 dsm^{-1}, total nitrogen 0.09%, available phosphorus 7.4 mg kg^{-1}, available potassium 435 mg kg^{-1}, zinc, iron and manganese 0.56, 5.1 and 3.2 mg kg^{-1}, respectively. The experiment consist of 27 treatments included of three rates of Zinc (0, 4, and 8 mg Zn kg^{-1} as $ZnSo_4.7H_2o$), three rates of iron (0, 4, and 8 mg Fe kg^{-1} as $FeSo_4$), and three rates of manganese (0, 15, and 30 mg Mn kg^{-1} as $MnSo_4.4H_2o$). All pots were fertilized with 20 mg N kg^{-1} as NH_4NO_3, 40 mg P kg^{-1} as Ca (H_2PO_4). $2H_2O$. Six seeds of soybean (cv. Williams) inoculated with *BradyRhizobium japonicum* and were sown directly in plastic pots containing 4 kg of the soil. After 48 days, plants were lifted and samples were washed in deionized water, then leaves, roots and stems were separated. For measure of dry weight samples was dried at 70 ^{0}C and 48 hours, weighed, and incinerated at 550^{0C}. For micronutrients determination, dry ash samples soluble in concentrated HNO_3 and $HCLO_4$. Zn, Fe and Mn contents were determined by Atomic Absorption Spectrometry (AAS) according to Kacar (1984).

Table1. Soil test values for the field experiment of the 0-30 cm soil layer of the experimental site

Soil properties	Value
Sand (%)	13
Clay (%)	41
Silt (%)	46
Soil texture	Silty clay
Organic matter (%)	2.3
pH	7.6
Electrical conductivity (dsm^{-1})	0.61
N (%)	0.18
P (ppm)	9.9
K (ppm)	563
Zn ($mg.kg^{-1}$)	0.71
Fe ($mg.kg^{-1}$)	6.2
Mn ($mg.kg^{-1}$)	4.3

Field experiment

Soil samples were collected from experimental area at 0-30 cm depth before basal fertilizer application to test initial soil characteristics, and soil test results are presented in Table1. 27 kg ammonium phosphate fertilizer (based on 200 $kg.ha^{-1}$) and 7 kg urea fertilizer (based on 50 $kg.ha^{-1}$) were spread evenly on the field and mixed with soil by disc. Before planting, seeds were, initially, soaked in 10% sugar solution; then each kg of seeds inoculated with 2 gr of *BradyRhizobium japonicum*. Usage amounts of fertilizers Zn (0, 20 and 40 kg Zn ha^{-1}), Fe (0, 25 and 50 kg Fe ha^{-1}) and Mn (0, 20 and 40 kg Zn ha^{-1}) from source of $ZnSo_4$, $FeSo_4$ and $MnSo_4$, respectively. Fertilizers were calculated based on plots area surface; next, were mixed with soft soil at the ratio of 1:5 and placed on furrows made manually next to the stacks. At the end of growth season, 5 plants were selected from each plot randomly. To measure concentration of elements in leaves, leaves on the most top trifoliate of the plants were used; and seeds were separated from pods. Samples (leaves, stems, pods, and seeds) washed with distilled water and were dried in the oven at 70^{0c} for 48 hours, weighed, and incinerated at 550^{0C}. Dry ash samples soluble in concentrated HNO_3 and $HCLO_4$. Zn, Fe and Mn contents were determined by Atomic Absorption Spectrometry (AAS) according to Kacar (1984).

Table 2. Analysis of variance Zn, Fe, and Mn concentration in roots and shoots of soybean (Pot experiment)

Source of variation		MS					
			Roots			Shoots	
	d.f	Zn	Fe	Mn	Zn	Fe	Mn
Block	2	47.32	10178.48	315.75	26.32	172.16	441.16
Zn	2	7475.99**	66190.96**	5.64ns	6950.42**	18532.38**	352.43**
Fe	2	16182.82**	504603.27**	348.58 **	9.96 ns	72414.89**	4627.66**
Zn×Fe	4	912.74**	51059.84**	202.79 **	13.57ns	8395.44**	537.43**
Mn	2	1733.88**	237893.87 **	24629.47**	193.87**	898.67**	95233.86**
Zn×Mn	4	328.78**	15270.66 *	66.91 ns	159.25**	48.31ns	88.15ns
Fe×Mn	4	317.00**	29807.19 **	43.43 ns	286.24**	266.17**	465.50**
Zn×Fe×Mn	8	453.16**	20146.87**	143.63 **	93.32*	157.91*	299.19**
Error	52	55.47	5075.65	33.56	37.82	69.26	46.78
Coefficient of variation (%)	-	5.63	7.98	7.66	9.51	7.98	7.19

*ns= Non significant, *significant at 5% and **=significant at1% levels of probability*

Table 3. Correlation coefficient between Zn, Fe, and Mn concentration in roots and shoots of soybean (Pot experiment)

	[Zn] root	[Fe] root	[Mn] root	[Zn] shoot	[Fe] shoot	[Mn] shoot
[Zn] root	1.00					
[Fe] root	-0.151ns	1.00				
[Mn] root	0.158ns	-0.395*	1.00			
[Zn] shoot	0.410*	0.227 ns	-0.072 ns	1.00		
[Fe] shoot	-0.615**	-0.505**	-0.272 ns	-0.303 ns	1.00	
[Mn] shoot	-0.078ns	0.541**	0.916**	-0.165 ns	-0.494**	1.00

Table 4.Mean comparison of soybean roots and shoots some of micronutrients concentration according to LSD test in %5 level (Pot experiment)

Fertilizer ($mgkg^{-1}$ soil)			Concentration ($mgkg^{-1}$)					
			Roots			Shoots		
Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn
0	0	0	152.1def	735.4 hij	50. 2 kl	62.00 fghi	59.6 ijk	48.7 i
		15	140.1fg	692.3 ij	74.2 ef	45.1 jk	51.2 jklm	117.4 e
		30	138.7 gh	637.0 j	121.6a	40.2 k	53.8 jklm	163.8 ab
	4	0	149.4efg	1176.0 a	47.3 kl	52.4 ij	165.7 cd	35.1 jk
		15	124.7ij	922.4 cdef	60.7 hij	46.6 jk	179.4 ab	100.2 f
		30	97.2 kl	659.2 ij	95.4 d	53.1 ij	159.2 cd	151.6 c
	8	0	85.1 l	965.3 cde	47.5 kl	59.1 hi	191.6 a	31.7 jk
		15	85.9 l	921.4 cdef	63.4 ghi	47.2 jk	179.4 ab	84.5 g
		30	87.4 l	838.2 fgh	117.4ab	40.7 k	165.2 cd	158.3 bc
4	0	0	164.5 bc	721.0 ij	51.4 jk	65.2 fgh	54.2 jkl	47.5 i
		15	169.7 b	700.3 ij	70.3 efg	52.4 ij	49.7 jklm	113.9 e
		30	144.3 efg	711.2 ij	100.3 cd	69.6 defg	48.6 klm	171.4 a
	4	0	127.6 hi	1204.0 a	48.3 kl	60.2 ghi	171.4 bc	30.7 jk
		15	113.3 j	1036.0 bc	67.0 fgh	60.9 ghi	154.3 de	76.3 gh
		30	100.5 k	1113.0 ab	114.3ab	71.2 cdef	140.1 f	135.9 d
	8	0	100.9 k	983.2 cde	50.9 k	65.8 fgh	76.0 gh	31.6 jk
		15	100.6 ij	873.6 efg	68.4 fgh	68.4 efgh	80.2 g	78.8 gh
		30	116.4 ij	775.6 ghi	108.4bc	52.4 ij	71.3 ghi	165.1 ab
8	0	0	183.7 a	935.1 cdef	54.9 ijk	85.0 ab	44.3 lm	39.6 ij
		15	164.20bcd	911.3 def	78.3 e	76.3 bcde	40.2 m	122.3 e
		30	173.1ab	723.0 hij	116.3 ab	88.4 a	49.1 klm	166.6 ab
	4	0	149.0 efg	1210.0 a	40.9 l	76.3 bcde	139.4 f	35.1 jk
		15	138.2 gh	995.1 cd	71.5 efg	85.4 ab	141.8 ef	79.5 gh
		30	143.4 efg	908.3 def	103.4cd	80.2 abc	128.5 f	160.8abc
	8	0	153.6 cde	1006.0bcd	53.4 jk	82.9 ab	75.2 gh	28.3 k
		15	127.8 hi	838.4 fgh	65.8fgh	80.0 abc	79.6 g	71.0 h
		30	121.3 ij	898.1 def	100.9cd	79.4 abcd	62.8 hij	123.7 e

-similar letters in each column shows non-significant difference according to LSD test in %5 level

Data analysis

Data were analyzed following analysis of variance technique with the computer package statistical software MSTATC and mean differences were adjudged by Least Significant Difference test (LSD).

Results and discussions

Pot experiment

The results of analysis of variance were shown in Table 2. Zinc application made significant effects on Zn, Fe concentration in roots ($P<0.01$), and Zn, Fe and Mn concentration in shoots ($P<0.01$). Manganese in roots was not affected by zinc application (Table 2). Except of shoots [Zn], iron fertilizer had significant effects on the other elements concentration. Manganese application had significant effects on micronutrients concentration. The interaction between Zinc, Iron, and Manganese had significant effects on Zn, Fe and Mn concentration in roots ($P<0.01$); Mn concentration in shoots ($P<0.01$), and Zn and Fe concentration in shoots ($P<0.05$). Comparison of means shows that zinc, iron, and manganese concentrations in plant organs were different under fertilizer treatments (Table 4). The micronutrients concentration was higher in roots compared with shoots in the fertilizer treatments (Fig. 1). Metals are normally found at the highest concentrations in the roots, and at the lowest concentrations in the reproductive tissues. This is because metals are sequestered into the vacuoles of root and shoot tissue and the subsequent availability of free metals in the symplast can be low. (Malan & Farrant, 1998; Shanker *et al*., 2005). In the Zinc study, concentration of Zn in roots and shoots were lower in 0 $mgkg^{-1}$treatment compared with 4 and 8 $mgkg^{-1}$ treatments (Fig. 1). On the other hand, Roots and shoots [Zn] increased with zinc application from 0 to 8 $mgkg^{-1}$. A similar trend was observed with manganese application, roots and shoots concentrated Mn more from 0 to 30 $mgkg^{-1}$ soil treatments (Fig. 1). In Fe study, Fe concentration in roots increased in 4 $mgkg^{-1}$ soil application of iron, and in excess amounts, concentration of this element was decreased. The maximum of shoots [Fe] was obtained in 8 $mg.kg^{-1}$Fe soil treatment. In the absence of Fe and Mn, roots [Zn] was the highest and with added of these elements zinc uptake by the roots was decreased. Mn application was caused that translocation of Zn from roots to shoots increased while the highest concentration of zinc in roots was observed in 8, 0, and 0 $mgkg^{-1}$ soil Zn, Fe, and Mn, respectively, and the highest shoot Zn concentration was found in 8, 0, and 30 $mgkg^{-1}$ soil treatment. Alloway (2004) reported that using manganese could enhance transfer of zinc to other organs of plants from roots. Result of pot experiment was shown that there was a negative relationship between iron and manganese in shoots concentration of these elements. Iron application could significantly reduce concentration of manganese in shoots ($P<0.01$). Ghasemi-Fasaei *et al*. (2003) reported

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Fig.1. Effects of Zinc, Iron and manganese fertilizers on Zn, Fe and Mn concentrations in roots and shoots of soybean (Pot experiment)

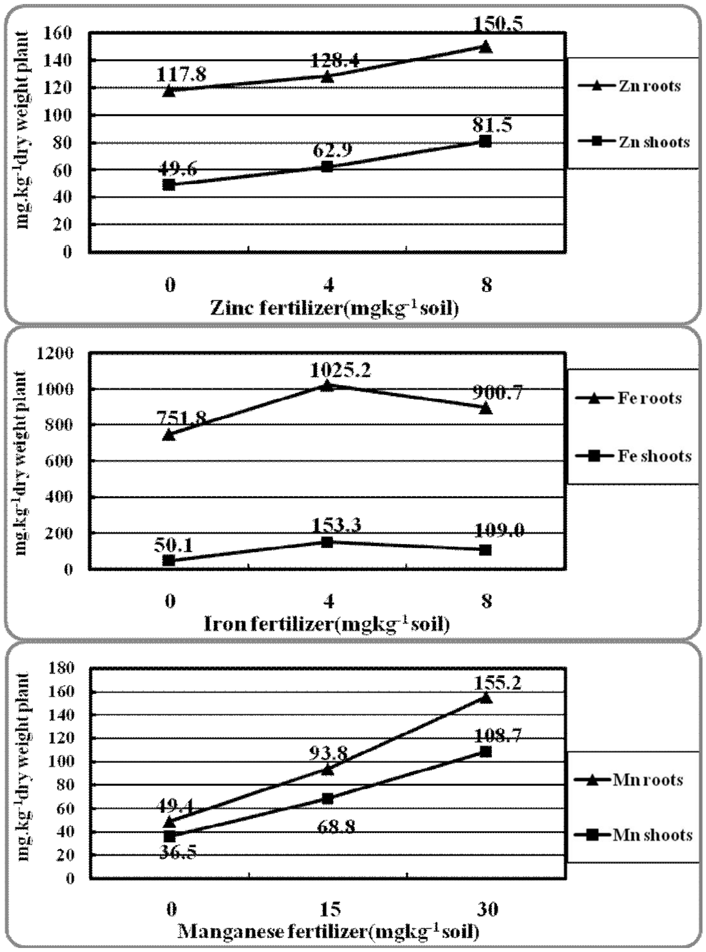

Fig.2. Effects of Zinc, Iron & manganese fertilizers on Zn, Fe and Mn concentration in leaf, stem, pod and seed (Field experiment)

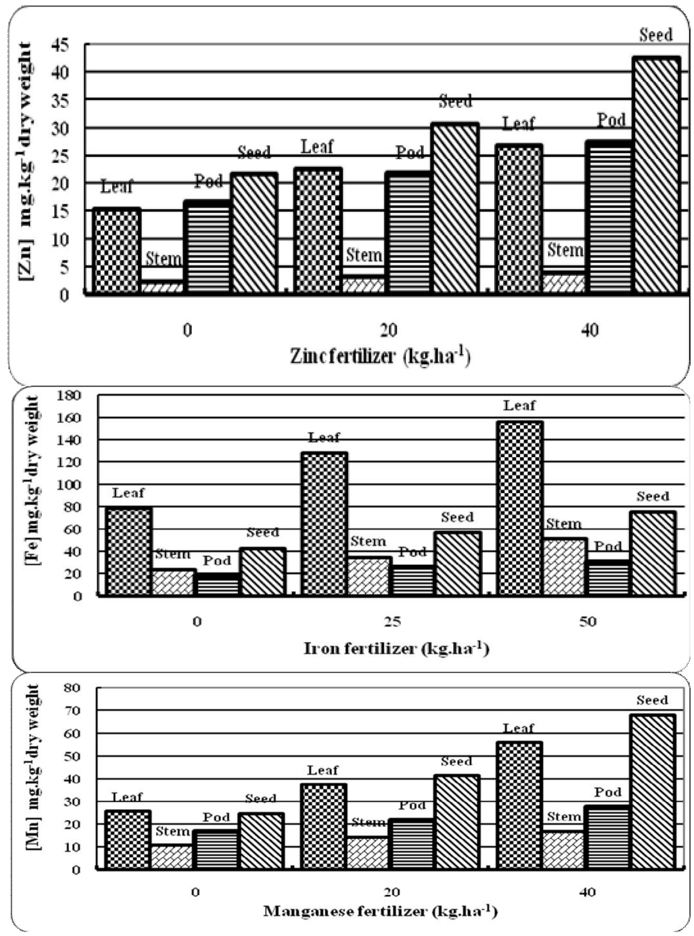

Table 5. Analysis of variance Zn, Fe, and Mn concentration in leaf, stem, pod, and seed in soybean (Field experiment)

Source of variation	d.f	Ms											
		Leaf			Stem			Pod			Seed		
		Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn
Block	2	3.50	78.91	9.07	0.37	3.55	6.13	14.08	3.38	2.71	5.00	52.84	90.29
Zn	2	901.20^{**}	1961.66^{**}	83.42^{*}	17.04^{**}	170.81^{**}	5.35^{*}	799.85^{**}	55.90^{**}	8.54^{ns}	3015.24^{**}	135.34^{**}	151.11^{**}
Fe	2	74.79^{**}	41802.89^{**}	107.49^{*}	1.84^{**}	5225.62^{**}	21.01^{**}	41.39^{**}	1180.93^{**}	15.27^{*}	59.71^{**}	7088.10^{**}	211.44^{**}
Zn×Fe	4	3.55^{ns}	412.98^{**}	37.68^{ns}	0.07^{ns}	5.47^{ns}	1.13^{ns}	11.38^{ns}	23.23^{**}	3.23^{ns}	18.65^{**}	29.38^{*}	61.60^{*}
Mn	2	12.01^{*}	2149.26^{**}	6216.34^{**}	0.60^{**}	103.00^{**}	263.34^{**}	14.54^{ns}	32.79^{**}	812.47^{**}	10.44^{*}	172.16^{**}	12783.27^{**}
Zn×Mn	4	1.64^{ns}	139.46^{ns}	7.73^{ns}	0.06^{ns}	4.03^{ns}	0.14^{ns}	0.92^{ns}	0.67^{ns}	0.44^{ns}	1.15^{ns}	1.99^{ns}	33.70^{ns}
Fe×Mn	4	2.76^{ns}	239.27^{*}	2.57^{ns}	0.14^{ns}	7.70^{ns}	0.31^{ns}	2.15^{ns}	7.27^{ns}	0.34^{ns}	5.15^{ns}	13.06^{ns}	27.04^{ns}
Zn×Fe×Mn	8	0.92^{ns}	22.53^{ns}	15.45^{ns}	0.04^{ns}	2.36^{ns}	0.60^{ns}	0.65^{ns}	1.55^{ns}	1.10^{ns}	2.81^{ns}	1.29^{ns}	12.49^{ns}
Error	52	2.83	87.16	25.94	0.09	10.41	1.52	6.62	5.21	3.82	3.25	9.52	16.80
Coefficient variation (%)	-	7.78	7.73	12.83	9.88	8.83	9.56	11.81	9.31	8.87	10.26	5.30	9.18

Ns= Non significant, * and ** = significant at 5 and 1% levels of probability, respectively

Table 6.Mean comparison of soybean leaf, stem, pod and seed some of micronutrients concentration according to LSD test in %5 level (Field experiment).

Fertilizer ($kg\ ha^{-1}$)			Concentration ($mg\ kg^{-1}$)											
			Leaf			Stem			Pod			Seed		
Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn
0	0	0	14.7 m	75.2 k	27.3 hij	2.3 ijkl	**22.** 1 hi	10.1jk	16.1 jk	17.7 h	16.1 g	21.7 hij	41.9 jk	23.2 h
		20	15.9 lm	77.6 k	37.1 defg	2.5 hijk	23.7 hi	13.9efg	16.4 ijk	18.5 h	21.7 cd	23.4 hi	42.6 jk	37.9 g
		40	16.1 lm	71.8 k	52.4 b	2.4 hijkl	21.2 i	16.3bcd	15.8 k	17.2 h	26.9 a	21.2 hij	41.3 jk	61.8 cd
	25	0	15.3 lm	121.7 hi	29.4 ghij	2.3 ijkl	33.5 ef	11.2ij	16.2 ijk	23.9 fg	17.2 efg	23.9 h	55.2 fg	23.9 h
		20	17.5 kl	124.0 ghi	39.8 de	2.5 hijk	34.7 def	14.7defg	17.4 ijk	25.2 defg	22.4 cd	21.8 hij	57.8 ef	42.7 efg
		40	16.3 lm	110.9 ij	56.2 ab	2.3 ijkl	31.6 fg	17.5abc	17.1 ijk	23.0 g	27.5 a	19.7 j	51.6 gh	67.3 bc
	50	0	14.5 m	140.8 def	23.2 j	2.0 l	48.4 c	9.1k	16.5 ijk	27.8 cde	15.2 g	20.1 j	73.2 bc	24.8 h
		20	14.2 m	145.3 def	33.8 efghi	2.2 jkl	52.0 bc	13.5fgh	17.1 ijk	28.1 cde	19.7 def	21.2 hij	76.8 ab	36.9 g
		40	14.2 m	137.4 efg	55.8 ab	2.1 kl	47.2 c	15.2def	15.3 k	26.2 cdefg	25.9 ab	20.7 ij	71.3 c	59.8 d
20	0	0	22.7 ghi	80.9f k	25.7 ij	3.3 ef	24.7 hi	11.5hij	22.1 defgh	17.9 h	17.5 efg	32.6 de	44.3 jk	27.9 h
		20	24.5 efg	84.1 k	38.4 def	3.5 def	27.2 gh	15.2def	23.5 cdefg	18.5 h	22.9 bcd	33.5 d	45.8 ij	48.7 e
		40	22.9 ghi	76.8 k	58.3 ab	3.2 fg	24.1 hi	17.9ab	22.0 efgh	18.3 h	28.5 a	31.8 de	42.7 jk	71.8 ab
	25	0	23.2 fgh	146.8 de	30.8 fghij	3.4 ef	38.5 de	11.2ij	22.6 defg	27.5 cdef	17.8 efg	31.6 de	61.7 de	27.2 h
		20	25.9 cdef	153.9 bcd	42.9 cd	3.6 cdef	39.8 d	15.7cde	23.9 cdefg	28.3 cd	23.2 bc	32.7 de	65.2 d	45.4 ef
		40	23.7 efgh	121.4 hi	61.3 a	2.8 gh	36.1 def	18.5a	21.7 fgh	26.2 cdefg	28.9 a	30.5ef	58.2 ef	77.8 a
	50	0	19.4 jk	164.4 abc	23.8 j	2.6 hij	54.2 b	10.3jk	20.2 ghij	32.8 ab	16.2 g	28.2 fg	76.7 ab	22.5 h
		20	21.1 hij	173.9 a	37.4 defg	2.8 gh	59.8 a	13.4fgh	20.4 ghi	34.6 a	20.3 cde	28.1 fg	78.2 ab	37.1 g
		40	20.3 ij	153.8 bcd	55.6 ab	2.7 hi	50.5 bc	15.8cde	18.1 hijk	29.4 bc	27.1 a	27.4 g	73.9 abc	61.3 cd
40	0	0	26.0 cde	80.7 k	24.2 j	4.0 abc	22.5 hi	10.9jk	26.1 bcde	17.1 h	17.2 efg	40.3 c	41.8 jk	25.5 h
		20	26.4 bcde	85.2 k	40.3 de	4.2 ab	23.7 hi	14.8defg	27.3 abc	15.9 h	21.8 cd	41.9 bc	43.1 jk	41.3 fg
		40	28.1 abc	73.9 k	57.9 ab	4.0 abc	22.1 hi	17.6abc	26.2 bcde	15.8 h	28.0 a	42.8 abc	40.5 k	71.8 ab
	25	0	27.6 abcd	130.4 fgh	22.6 j	3.9 bcd	33.7 ef	11.1ijk	28.5 ab	25.3 defg	17.0 fg	45.4 a	54.5 fgh	24.6 h
		20	29.7 a	138.5 efg	34.9 defgh	4.4 a	35.4 def	15.0defg	31.2 a	26.2 cdefg	21.9 cd	45.7 a	57.3 ef	42.8 efg
		40	28.9 ab	103.4 j	54.9 ab	3.7 cde	31.5 fg	16.6abcd	28.9 ab	24.5 efg	27.6 a	43.6 ab	49.9 hi	70.5 b
	50	0	25.1 defg	167.1 ab	24.8 j	3.6 cdef	48.9 c	10.1jk	26.3 bcd	32.7 ab	15.5 g	41.9 bc	75.4 abc	23.1 h
		20	25.3 defg	173.4 a	33.1 efghi	3.6 cdef	51.3 bc	13.1ghi	26.1 bcde	34.0 a	22.7 bcd	41.1 bc	78.7 a	39.5 fg
		40	24.4 efg	149.8 cde	50.3 bc	3.5 def	48.4 c	15.9bcde	25.2 bcdef	29.2 bc	28.0 a	40.7 bc	70.7 c	68.8 b

-similar letters in each column shows non-significant difference according to LSD test in %5 level

that application of iron decreased mean Mn concentration by 91%. Admittedly, transportation of iron and manganese from roots to shoots affected by antagonistic effects of these elements and thus it differ from the uptake by roots from the soil.

There was a significant and positive correlation between Zinc, Iron and manganese concentration in roots with Zn (r= 0.410*), Fe (r= 0.505**) and Mn (r= 0.916**) concentration in shoots, respectively. Also, roots and shoots [Fe] had a significant and negative correlation with the roots and shoots [Mn], respectively (Table 3).

Field experiment

The results of field experiment (Table 5 & 6) were shown that by application of micronutrients such as Zinc, Iron and Manganese increased their Concentration in different part of plant. Zinc application caused that seed [Zn] was increased more, than the other parts of plant, While using of iron was increased leaf [Fe] (Fig. 2). Antagonistic effects of iron and manganese that frequently being emphasized on in previous experiments also repeated under climatic conditions of this experiment (Chinnery & Harding 1980; Alam *et al*., 2001). Application of iron increased this element leaf, stem, pod and seed concentration (Ghasemi-Fasaei *et al*., 2003). Expending more than 25 $kg.ha^{-1}$Fe, Antagonistic effects of iron with manganese enhanced intensively. Sanchez- Raya *et al*. (1974) suggested that if iron was used in little amount, it would increase absorption and transfer of manganese by plants. In this experiment, the degree of iron absorption inhibition by manganese was higher than that of manganese absorption inhibition by iron. Manganese use highly prevented iron from being absorbed. Using manganese up to 20 $kg.ha^{-1}$had little effect on iron absorption, but an increase in its usage amount reduced stem, pod and grain concentration of iron more (Table 6). Also, application of manganese up to 20 $kg.ha^{-1}$ had a favorable effect on zinc absorption and its stem and grain concentration increased. Using zinc highly slightly reduced leaf, stem, pod and grain concentration of manganese. It appears that high concentration of zinc in soil can prevent soybean from absorbing manganese. In addition, iron use failed to affect leaf and pod concentrations of zinc Although using iron up to 25 $kg.ha^{-1}$ had no major effect on stem, grain concentrations of zinc, more amount of iron reduced pod and grain concentrations of zinc. In this study, High soil concentration of manganese and iron had negative effects on zinc absorption. Mandal *et al*. (2000) also

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Table7. Correlation coefficient between Zn, Fe, and Mn concentration in leaf, stem, pod, and seed in soybean (Field experiment)

	[Zn] leaf	[Fe] leaf	[Mn] leaf	[Zn] stem	[Fe] stem	[Mn] stem	[Zn] pod	[Fe] pod	[Mn] pod	[Zn] seed	[Fe] seed	[Mn] seed
[Zn] leaf	1.00											
[Fe] leaf	0.029^{ns}	1.00										
[Mn] leaf	0.067^{ns}	-0.397*	1.00									
[Zn] stem	0.883**	-0.053^{ns}	-0.055^{ns}	1.00								
[Fe] stem	-0.122^{ns}	0.898**	-0.119^{ns}	-0.239^{ns}	1.00							
[Mn]stem	0.161^{ns}	-0.223^{ns}	0.801**	0.097^{ns}	-0.437*	1.00						
[Zn] pod	0.827**	0.069^{ns}	-0.075^{ns}	0.865**	-0.086^{ns}	0.042^{ns}	1.00					
[Fe] pod	0.005^{ns}	0.913**	-0.150^{ns}	-0.117^{ns}	0.880**	-0.211^{ns}	0.039^{ns}	1.00				
[Mn] pod	0.157^{ns}	-0.196^{ns}	0.887**	0.056^{ns}	-0.383*	0.865**	0.006^{ns}	-0.408*	1.00			
[Zn] seed	0.918**	0.047^{ns}	-0.071^{ns}	0.883**	-0.093^{ns}	0.054^{ns}	0.870**	0.012^{ns}	0.044^{ns}	1.00		
[Fe] seed	-0.140^{ns}	0.925**	-0.152^{ns}	-0.199^{ns}	0.949**	-0.230^{ns}	-0.059^{ns}	0.896**	-0.168^{ns}	-0.292^{ns}	1.00	
[Mn] seed	0.173^{ns}	-0.206^{ns}	0.919**	0.043^{ns}	-0.148^{ns}	0.833**	0.083^{ns}	-0.145^{ns}	0.905**	0.046^{ns}	-0.558**	1.00

*Ns= Non significant; * and ** : significant at 5 and 1% levels of probability, respectively*

found that high concentration of iron and manganese in soil solution had an antagonistic effect on zinc absorption (Table 6). Application of Manganese, decreased leaf, stem, pod and grain concentration of iron in soybean, with less reduction in grain concentration comparison other part of plant; while, zinc seems to help to transfer of iron into grains from leaves. Application of zinc, increased this element concentration in grains more than this leaves. Using manganese up to 20 kg.ha^{-1}also increased zinc concentration in Leaf, stem, pod and grain. Using manganese had further effect on this elements grain concentration than that in other parts of plant. At the Field experiment was observed that micronutrients concentration in leaf had a significant and positive correlation with micronutrients concentration in seed. There was a significant and negative correlation between manganese and iron concentration in seed (r=0.558**) and Mn & Fe concentration in leaf (r= 0.397*) (Table7).

Conclusion

One component of seed quality is chemical composition, such as the concentrations of mineral elements, including micronutrients such as Zinc, Iron and Manganese. Selection of increased concentrations of essential trace elements also enhancing the nutritional value of crops (Grant *et al*., 2008). The soybean is an annual crop species, and during the growth stags, remobilization of micronutrients occurs from root to shoot (leaves and stems), and finally to seeds. The rate of these remobilized, were affected by source-sink relationships in plant, environment conditions, and by the genetic characteristics of plant. Understanding of these transportation and pattern of micronutrients distribution may lead to strategies to increase flux to seeds and result in greater Zn, Fe and Mn concentration. Waters & Sankaran (2011) stated that transfer of micronutrients from root to shoot the first of limiting factor for translocation to seeds. Also, increased flux of micronutrients from roots to shoots could possibly activate native homeostasis mechanisms that increase root uptake capacity. Plants have developed a number of transport mechanisms to control the acquisition, partitioning and deposition of the micronutrients iron, zinc and manganese (Grusak *et al*., 1999).

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