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Influence of foliar and soil applications of iron and manganese on soybean dry matter yield and iron-manganese relationship in a Calcareous soil

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Abstract

Iron (Fe) and manganese (Mn) are essential nutrients for plants. Application of high levels of either Fe or Mn is often accompanied by relatively low levels of uptake for the other nutrient. The antagonistic relationship of these nutrient elements may occur either during absorption by roots or during translocation from roots to shoot. A greenhouse study was carried out to study the effect of soil and foliar applications of Fe and Mn on yield and Fe-Mn status of soybean plant. Results showed that soil or foliar application of Fe or Mn did not influence soybean root or shoot dry matter yield (SDMY). Both soil and foliar applications of Fe significantly increased shoot Fe concentration and uptake; however, foliar application was more effective. Foliar spray of 1 % Fe sulfate improved plant Fe content and had no effect on SDMY or on shoot Mn concentration. Soil addition of Fe decreased root Mn concentration/uptake probably due to the well-known antagonistic effect of Fe on Mn absorption; whereas, foliar Fe application had no negative effect on shoot Mn status . Shoot Mn uptake was more negatively affected by soil Fe application than root Mn uptakes. Hence, reduction of root Mn absorption and translocation to shoot were the main reasons for suppressing effect of Fe on Mn nutrition. Also, high level of soil applied Mn (i.e., 30 mg Mn kg^{-1}) decreased Fe translocation from root to shoot. In conclusion, foliar Fe/ Mn applications are appropriate methods of applying these nutients for preventing yield reduction and nutrient imbalance in soybean grown on such calcareous soils.

Keywords: Iron, Manganese, Soil and Foliar Applications, Soybean, Calcareous Soil.
Abbreviations: SDMY- Shoot Dry Matter Yield; Fe-EDDHA- IronEthylenediamine Di-o- hydroxyphenylacetic Acid.

Introduction

Iron (Fe) and manganese are essential nutrient elements that play key roles in plants. Huda et al. (2009) reported that the optimal $FeSO_4$ concentration in a medium culture should be sufficient to satisfy the basic energy requirements for cell division and differentiation indication that $FeSO_4$ is one of the factors controlling the induction and growth of shoots. Soil conditions like high pH in calcareous soils, poor aeration, and accumulation of phosphorus (P) are conducive to low Fe availability and consequently Fe deficiency (Lindsay and Schwab, 1982), that being responsible for low yield and poor plant quality and economic loss to growers in some parts of the word (Mortvedt, 1991). Iron application usually increase soot dry matter yield (SDMY) of soybean (Hodgson et al., 1992) but higher levels may decrease soybean growth (Roomizadeh and Karimian, 1996). The high levels of either one of the Fe or Mn elements are often accompanied by relatively low levels of the other element, which phenomenon can be considered as indicative of a mutual antagonism between these elements, either during uptake by the roots, or during translocation from the roots to the leaves or other above ground parts (Van Der Vorm and Van Diest, 1979). In the literature, frequent mention is made of mutually antagonistic effects of Fe and Mn. Such effects may be operative inside the plant, when Fe and Mn compete for certain positions in enzyme systems (Lohnis, 1950 cited by Van Der Vorm and Van Diest, 1979). Van Der Vorm and Van Diest (1979) reported that at the soil conditions in which the Mn concentration of soil solution was low (10^{-5} mg L^{-1}), the Mn absorption by rice roots was a selective process, which means that the quantity of Mn absorbed by roots was larger than the quantity transported to the roots by mass flow. Moraghan (2004) reported that application of 2 mg Fe kg^{-1} soil as iron-ethylene diamine di-o-hydroxyphenylacetic Acid (Fe-EDDHA) increased shoot Fe concentration and uptake of soybean genotypes but had no considerable effect on SDMY. Moraghan et al., (2002) showed that soil application of 4 mg Fe kg^{-1} soil as Fe-EDDHA increased seed yield, Fe concentration or uptake but decreased seed Mn concentration or uptake of bean genotypes. Ghasemi-Fasaei et al., (2005) stated that soil or foliar application of Fe decreased chickpea SDMY and Mn concentration and uptake due to the antagonistic effect of Fe on Mn translocation from root to shoot. Ronaghi and Ghasemi-Fasaei (2008) reported that application of Fe-EDDHA did not result in significant increase in soybean SDMY probably due to the competition of Fe with Mn. Goos and Johnson (2000) showed that two foliar applications of Fe-EDDHA increased seed yield of three soybean genotypes. Ghasemi-Fasaei et al., (2003) reported that soil application of Fe-EDDHA significantly increased soybean shoot Fe concentration and uptake but decreased shoot Mn concentration due to the reduction in Mn absorption and translocation from root to shoot. The findings of Van Der Vorm and Van Diest (1979) appeared that the high level of available Fe did not have a suppressive effect on the uptake of Mn in rice plants and conversely, high levels of available Mn did not have any adverse effect on the uptake of

Fe. Nutritional imbalance in plants such as Mn, copper (Cu), or zinc (Zn) deficiency may be induced following the over-fertilization of Fe chelates due to absorption of relatively large amount of Fe (Ronaghi and Ghasemi-Fasaei, 2008). Furthermore, Havlin et al. (2005) stated that Mn deficiency may be induced by adding large quantities of Fe, provided that soil Mn is marginally deficient**.** Moosavi and Ronaghi (2010) reported that soil application of Fe did not influence SDMY of dry bean due to the fact that the Fe: Mn ratio in aerial part was higher than 0.4. Whereas, foliar spray of 2 % Fe sulfate significantly reduced it probably due to the high level of shoot Fe and Fe: Mn ratio greater than 4. They showed that Fe application decreased concentration/uptake of shoot manganese due to the antagonistic relationships between Fe and Mn. Accumulation of Fe to toxic levels, reduction of root: shoot ratio (Mordveth, 1991), reduction of Mn uptake and/or translocation from root to shoot (Roomizadeh and Karimian, 1996) could be the negative effects of Fe on Mn. Since there is no sufficient information on interactive effect of soil and foliar applications of Fe and Mn on shoot and root yield and Fe –Mn nutrition status of soybean plants, the objectives was to study the effect of soil and foliar applications of Fe and Mn on 1) shoot and root dry matter yield, 2) concentration and uptake of Fe and Mn, and 3) root and shoot Fe: Mn ratio of soybean (*Glycine max* L.), grown on a calcareous soil (fine-loamy, carbonatic, thermic, Typic Calcixerepts) of southern Iran.

Results

Root and shoot dry matter yield

Influence of Fe application

Soil or foliar application of Fe did not affect shoot or root dry matter yield (SDMY and RDMY, respectively) compared to that of control (Table 1). Root dry matter yield to SDMY ratio was not affected by Fe treatments (data not presented).

Influence of Mn application

Soil or foliar application of Mn similar to Fe, had no significant effect on SDMY or RDMY (Table 1). Either soil or foliar application of Mn did not affect RDMY to SDMY (data not presented).

Iron concentration and uptake

Influence of Fe application

Soil or foliar application of Fe did not affect the mean root Fe concentration or uptake in comparison to those of their controls (except for foliar application of 1 % Fe sulfate that significantly decreased these parameters by 15.8 and 23.3 %, respectively) (Tables 2 and 3). Soil application of 4 and 8 mg Fe kg^{-1} significantly increased shoot Fe concentration or uptake by about 1.4 and 1.5 fold; however, foliar application of 1 and 2 % Fe sulfate increased these parameters by more than 2.5, and 3.1 fold in comparison to that of control, respectively (Tables 2 and 3). Iron uptake of shoot also increased by about 39 and 56 % in response to soil application of 4 and 8 mg Fe kg^{-1} and by 2.4 and 3.1 fold with foliar application of 1 and 2 % ferrous sulfate, respectively (Table 3).

Influence of Mn application

Soil application of 15 and 30 mg Mn kg^{-1} and foliar application of 0.5 and 1 % Mn sulfate, significantly decreased root Fe concentration by 17.2, 27.7, 40.1, and 30.4 % and root Fe uptake by 18.8, 34.6, 37.5, and 25.9 % in comparison to those of controls, respectively. Whereas, shoot Fe concentration significantly decreased by 13 and 8.6 % with soil application of 30 mg Mn kg^{-1} and foliar application of 0.5 % ferrous sulfate, respectively. Shoot Fe uptake significantly decreased by about 10.6 % when 30 mg Mn kg^{-1} soil was applied, but it was not affected by other Mn treatments. (Tables 2 and 3).

Manganese concentration and uptake

Influence of Fe application

Soil application of 4 and 8 mg Fe kg^{-1} decreased root Mn concentration by 37 and 38 % and root Mn uptake by 45 and 41 % as compared to that of control, respectively; whereas foliar application of Fe had no significant effect on them (Tables 4 and 5). Soil application of 4 and 8 mg Fe kg^{-1} decreased shoot Mn concentration or uptake by 77 and 81 % as compared to that of control, respectively. Foliar application of 1 % Fe sulfate did not affect these parameters. Whereas, foliar application of 2 % Fe sulfate decreased shoot Mn concentration and uptake by about 20 %, (lower than the reductions occurred with soil Fe applications) (Tables 4 and 5).

Influence of Mn application

Root Mn concentration decreased significantly with application of 15 mg Mn Kg^{-1} soil or with foliar Mn applications. Whereas, root Mn uptake decreased only with application of 15 mg Mn kg^{-1} soil and foliar application of 0.5 % Mn sulfate (Tables 4 and 5). Application of 30 mg Mn kg^{-1} Soil increased shoot Mn uptake by 32 % as compared to that of control. Foliar application of 0.5 and 1 % Mn sulfate significantly increased shoot Mn concentration by 43 and 65 % and shoot Mn uptake by 46 and 70 %, respectively as compared to control treatments (Tables 4 and 5). The highest shoot Fe concentration and uptake were obtained with application of 2 % Fe sulfate (Tables 2 and 3).

Iron: manganese ratio

Results indicated that the mean Fe: Mn ratio of root or shoot was significantly affected by Fe and Mn applications. Soil application of Fe significantly increased root Fe: Mn ratio as compared to that of control. Whereas, Fe foliar applications did not affect the ratio (spray of 2 % Fe sulfate) or decreased it (spray of 1 % Fe sulfate) significantly (data not presented). Both soil and foliar Mn applications significantly decreased root Fe: Mn ratio (data not presented). The influence of Fe or Mn applications on the shoot Fe: Mn ratio was similar to that of root. However, Fe: Mn ratios of roots were generally greater than those of shoots. The Fe: Mn ratio varied from 6.8 to 23.7 and from 0.43 to 4.34 in roots and shoots, respectively (data not presented). The least Fe: Mn ratio of shoot (0.43) was obtained in control treatment.

Table 1. Effect of soil (mg Kg^{-1}) and foliar (%) application of Fe and Mn on root and shoot dry matter yields of soybean plant (g Pot^{-1}).

Mn levels	Fe levels					Mean
	0	4 (mg Kg^{-1})	8 (mg Kg^{-1})	1 (%)	2 (%)	
	Root dry matter yield (RDMY)					
0	1.25[†] a-f	1.41 a-e	1.12 d-f	1.17 c-f	1.55 a-c	1.30 ab
15 (mg Kg^{-1})	1.45 a-d	1.37 a-e	1.16 c-f	1.43 a-d	1.00 e-fb	1.28 ab
30 (mg Kg^{-1})	1.21 b-f	0.89 f	1.23 a-f	1.28 a-f	1.27 a-f	1.18 b
0.5 (%)	1.63 ab	1.27 a-f	1.54 a-d	1.15 c-f	1.21 b-f	1.36 a
1 (%)	1.43 a-b	1.21 b-f	1.65 a	1.29 a-f	1.47 a-d	1.41 a
Mean	1.39 a	1.23 a	1.34 a	1.26 a	1.30 a	
	Shoot dry matter yield (SDMY)					
0	6.28 a-e	6.02 b-e	6.49 a-d	5.68 de	6.19 b-e	6.13 a
15 (mg Kg^{-1})	6.08 b-e	6.87 a-c	6.61 a-d	5.88 c-e	6.63 a-d	6.42 a
30 (mg Kg^{-1})	6.41 a-e	6.64 a-d	5.96 c-e	6.78 a-c	6.14 b-e	6.39 a
0.5 (%)	6.46 a-d	6.59 a-d	6.99 ab	5.41 e	6.55 a-d	6.40 a
1 (%)	6.35 a-e	6.54 a-d	7.27 a	6.10 b-e	6.33 a-c	6.52 a
Mean	6.32 ab	6.53 a	6.66 a	5.97 a	6.37 a	

[†]: Means in each row or column followed by the same lower letters are not significantly different ($p < 0.05$) by Duncan's Multiple Range Test.

Discussion

In well-aerated soils iron occurs mostly in the form of Fe^{III} oxides or hydroxides, and thus the concentration of Fe^{3+} is very low (below 10^{-7} M) in the physiological pH range. As a consequence of the chemical equilibrium in aerated soils, chelated iron is the dominant form transported to the roots apoplast. However, in most well aerated soils the concentration of iron chelates is in the range of 10^{-8} to 10^{-7} M, which is lower than the required amount for adequate growth of plants (Lindsay and Schwab, 1982). In the present study Fe applications had no significant effect on SDMY or RDMY. The results were in close agreement with findings of Roomizadeh and Karimian (1996) who reported that application of Fe had no significant effect on SDMY of soybean plants or even decreased it due to the negative effect of Fe application on Mn nutrition and also similar with the findings of Moosavi and Ronaghi (2010) who reported that soil or foliar application of Fe did not affect SDMY or RDMY of dry bean (except for foliar application of 2 % Fe sulfate that significantly decreased SDMY probably due to the high level of shoot Fe concentration). Furthermore, the results were somehow similar to the findings of Niebur and Fehr (1981) who reported that soil application of Fe-chelate to 19 soybean genotypes increased the yield of only 7 genotypes. However, Ghasemi-Fasaei et al., (2003) stated that soil application of 2.5 mg Fe kg^{-1} soil as Fe-EDDHA on 12 soybean genotypes increased SDMY of two genotypes and application of 5 mg Fe kg^{-1} soil increased this parameter of two other genotypes. They also reported that SDMY of some genotypes decreased when Fe was added, probably due to the reduction of Mn concentration in plant tissue. Goos and Johnson (2000) stated that two foliar applications of Fe as Fe-EDTA at 1 to 2 and 4 to 5 trifoliate stages increased yields of soybeans. In the present study soil or foliar Mn application similar to Fe had no significant effect on SDMY or RDMY probably due to the fact that soil Mn test was in adequate range. Similar findings were reported by Moosavi and Ronaghi (2010) for dry bean . Furthermore, Boswell et al. (1981) reported that preplant broadcast application of Mn, as row at planting, sidedress or preplant broadcast plus foliar spray at 5.6 to 22.4 kg ha^{-1} did not significantly influence soybean yields. However, Randall et al. (1970) reported that broadcast application of 17 to 68 Kg $MnSO_4$ ha^{-1} and foliar application of 0.17 to 0.51 kg Mn-EDTA ha^{-1} resulted in higher yield and Mn content of soybean leaves. They noted that, in general, foliar treatments resulted in somewhat lower yields (although not significant at the $p \leq 0.05$) than that of soil applications. Robertson et al. (1973) concluded that the response of soybean yield to $MnSO_4$ was statistically significant ($p \leq 0.05$) and the highest yield was obtained when leaf Mn was in the sufficiency range (86 mg kg^{-1}); however, yield was lower when leaf Mn level was 119 mg kg^{-1}, possibly due to high Mn level. Gettier et al. (1985) observed that foliar application of 1.12 kg Mn ha^{-1} at early and late growth stages of soybean resulted in the greatest yield in comparison to that of control. The differences among soybean genotypes might be responsible for dissimilarities observed between our results and findings of other investigators. Foliar application of ferrous sulfate was more effective than soil application of Fe chelates in Fe supplying for soybean. The results were in close agreement to the findings of Moosavi and Ronaghi (2010) who reported that soil and foliar application of Fe significantly increased shoot Fe concentration and uptake of dry bean. Furthermore, similar to our findings, Hodgson et al. (1992) observed that Fe-chelate increased Fe concentration in soybean leaves up to 42 % as compared to control. However, Ghasemi-Fasaei et al. (2005) stated that soil application of Fe had no significant effect on shoot Fe concentration or uptake of soybean, whereas foliar application, similar to our results, significantly increased both of these parameters. Foliar or soil application of Mn decreased root Fe concentration and uptake. Shoot Fe uptake decreased with application of 30 mg Mn kg^{-1} soil. Therefore, high level of soil applied Mn (i.e., 30 mg Mn kg^{-1}) could limit Fe translocation from root to shoot of soybean.The results were in contrary to that of Moosavi and Ronaghi (2010) who stated that Mn had no significant effect on shoot Fe concentration or uptake in dry bean. Heenan and Campbell (1983) reported that Fe uptake by soybean plants grown in solution culture was independent of solution Mn concentration but increased with increasing solution Fe. Soil applications of Fe decreased root Mn concentration and uptake, probably due to the well known antagonistic effect of

Table 2. Effect of soil (mg Kg^{-1}) and foliar (%) application of Fe and Mn on root and shoot Fe concentration of soybean plant (mg kg^{-1}).

Mn levels	Fe levels					Mean
	0	4 (mg Kg^{-1})	8 (mg Kg^{-1})	1 (%)	2 (%)	
			Root			
0	1551[†] a	1354 ab	1146 b-f	1557 a	1531a	1428 a
15 (mg Kg^{-1})	1192 b-e	1231 b-d	1325 a-c	1146 b-f	1019 d-h	1183 b
30 (mg Kg^{-1})	1346 ab	965 d-i	1233 b-d	548 j	1070 c-h	1033 c
0.5 (%)	718 ij	897 f-i	989 d-h	825 hi	852 g-i	856 d
1 (%)	1114 b-g	1197 b-e	807 hi	912 f-i	939 c-i	994 c
Mean	1184 a	1129 a	1100 ab	997 b	1082 ab	
			Shoot			
0	57.0 hi	75.8 fg	81.9 fg	125 e	208 a	110 a
15 (mg Kg^{-1})	58.7 hi	76.9 fg	83.2 fg	134 de	170 b	105 ab
30 (mg Kg^{-1})	48.7 i	72.4 gh	90.8 f	125 e	140 cd	95 c
0.5 (%)	53.1 i	72.2 gh	79.8 fg	142 cd	154 bc	100 bc
1 (%)	55.1 i	71.9 gh	75.1 fg	162 b	167 b	106 ab
Mean	54.5 e	73.9 d	82.2 c	138 b	168 a	

[†]: Means in each row or column followed by the same lower letters are not significantly different ($p < 0.05$) by Duncan's Multiple Range Test.

Fe on Mn absorption by plant roots. The results were similar to the findings of Moosavi and Ronaghi (2010) who stated that soil Fe applications decreased root Mn concentration of dry bean by 17 % due to the dilution effect. However, the results were in contrary to the findings of Moraghan (1992) and Moraghan et al. (1986). Whereas, there was no significant effect of Fe foliar application on the root Mn concentration and uptake indicating that there is no evidence for preventing root Mn uptake by foliar application of Fe. Foliar Fe applications decreased shoot Mn concentration and uptake but lower than the reductions caused with soil Fe applications. The results were similar to that of Ghasemi-Fasaei et al. (2003) who reported that soil application of Fe-EDDHA caused a drastic decrease in shoot Mn concentration of 12 soybean genotypes. Shoot Mn uptake was more negatively affected by soil application of Fe than root Mn uptake indicating that, in addition to preventing Mn absorption by roots, the mechanisms by which absorbed Mn is translocated from root to shoot is more affected.Mortvedt et al. (1991) reported that the antagonistic interaction between Fe and Mn was probably due to the reduction of Mn concentration by dilution effect, reduction in root to shoot ratio, reduced Mn uptake, or toxic concentration of Fe in plant tissue. Roomizadeh and Karimian (1996) stated that Fe might interfere with Mn absorption and/or translocation from root to shoot. Foliar Mn applications caused greater increase in shoot Mn concentration and uptake than soil applications indicating that foliar Mn application is more effective in improving Mn nutrition status of soybean plants. Since foliar spray of 1 % Fe sulfate improved plant Fe content and had no negative effect either on SDMY or on shoot Mn concentration; therefore it is considered as an appropriate Fe application method for soybean. This is in agreement with findings of Moosavi and Ronaghi (2010) who concluded that foliar spray of 1 % Fe sulfate was the appropriate Fe treatment for dry bean and is also in agreement with the findings of Liebenberg (2002) who stated that Fe deficiencies can be rectified by foliar spray of a 1 % Fe sulfate solution or chelate. The influence of Fe or Mn applications on the shoot Fe: Mn ratio was similar to that of roots. However, Fe: Mn ratios of roots were generally greater than that of shoot. This is in agreement to the results of Van Der Vorm and Van Diest (1979) who reported that the Fe contents of above-ground parts of rice are far lower than those of roots. Moosavi and Ronaghi (2010) concluded that the lack of positive responses of dry bean SDMY to Fe and Mn applications was at least partially attributed to Fe: Mn ratios higher than 0.4. Ghasemi-Fasaei et al. (2005) observed that only soybean genotypes with a Fe: Mn ratio of less than 0.4 in controls responded positively to Fe-EDDHA applications. Shoot Fe:Mn ratios were generally greater than 0.4 which might be, at least partially, responsible for soybean SDMY not responding to Fe and/or Mn applications (data not presented).

Materials and methods

Soil analysis

A greenhouse experiment was carried out on a loamy calcareous soil (fine-loamy, carbonatic, thermic, Typic Calcixerepts) with pH of 7.8; electrical conductivity (EC_e) of 0.40 dS m^{-1}; calcium carbonate equivalent (CCE) of 45 %; organic matter (OM) of 1.5%; sodium bicarbonate extractable P (Olsen et al., 1954) of 4.5 mg kg^{-1} soil; DTPA-extractable Fe, Mn, Zn and Cu (Lindsay and Norvell, 1978) of 2.3, 3.7, 0.96 and 1.0 mg kg^{-1} soil, respectively. The mentioned attributes of the studied soil were measured using the standard methods.

Experimental design

The experiment was a 5 × 5 factorial arranged in a randomized complete design with 3 replicates. Treatments consisted of five Fe levels [foliar application (1 and 2 % $FeSO_4.7H_2O$), soil application (0, 4 and 8 mg Fe kg^{-1} soil of Fe-EDDHA)] and five Mn levels [foliar application (0.5 and 1 % $MnSO_4.4H_2O$), soil application (0, 15 and 30 mg Mn kg^{-1} soil as $MnSO_4.4H_2O$)].

Soil preparation and soybean planting

Each pot contained 3 kg soil. Pots were watered with distilled water to a near field capacity and maintained at this moisture level by adding water to a constant weight. For preventing any probable nutrient deficiency other than Fe and Mn, all pots received uniform application of 50 mg P kg^{-1} soil as $Ca(H_2PO_4)_2.H_2O$, 50 mg nitrogen (N) kg^{-1} soil as NH_4NO_3 (One half of N was added at planting and the other half was shoot-dressed three weeks after emergence), 3 mg Cu and 5 mg Zn kg^{-1} soil as their sulfates and in aqueous forms. Six soybean

Table 3. Effect of soil (mg Kg^{-1}) and foliar (%) application of Fe and Mn on root and shoot Fe uptake of soybean plant (µg pot^{-1}).

Mn levels	Fe levels					Mean
	0	4 (mg Kg^{-1})	8 (mg Kg^{-1})	1 (%)	2 (%)	
			Root			
0	1922[†] ab	1909 ab	1296 b-h	1811 bc	2397 a	1867 a
15 (mg Kg^{-1})	1740 b-d	1650 b-d	1551 b-f	1631 b-c	1011 e-h	1516 b
30 (mg Kg^{-1})	1672 b-d	869 gh	1510 b-f	707 h	1352 b-g	1222 c
0.5 (%)	1225 c-h	1140 d-h	1528 b-f	928 f-h	1013 e-h	1167 c
1 (%)	1590 b-e	1437 b-g	1322 b-h	1173 d-h	1390 b-g	1382 bc
Mean	1630 a	1401 ab	1442 ab	1250 b	1433 ab	
			Shoot			
0	251fg	321 e-g	373 e	503 d	910 a	472 a
15 (mg Kg^{-1})	252 fg	368 e	366 e	571 d	780 b	467 ab
30 (mg Kg^{-1})	215 g	327 ef	373 e	591 cd	603 cd	422 b
0.5 (%)	243 fg	329 ef	387 e	529 d	718 b	441 ab
1 (%)	244 fg	328 ef	383 e	683 bc	745 b	477 a
Mean	241 d	335 c	376 c	575 b	751 a	

[†]: Means in each row or column followed by the same lower letters are not significantly different ($p < 0.05$) by Duncan's Multiple Range Test.

Table 4. Effect of soil (mg Kg^{-1}) and foliar (%) application of Fe and Mn on root and shoot Mn concentration of soybean plant (mg kg^{-1}).

Mn levels	Fe levels					Mean
	0	4 (mg Kg^{-1})	8 (mg Kg^{-1})	1 (%)	2 (%)	
			Root			
0	99.9[†] cd	73.4 i-k	53.6 l	135 a	125 ab	97.4 a
15 (mg Kg^{-1})	88.1 d-h	52.3 l	62.8 kl	118 b	95.6 c-e	83.3 b
30 (mg Kg^{-1})	133 a	76.1 h-j	87.8 d-h	80.4 g-i	99.2 c-e	95.2 a
0.5 (%)	103 c	65.1 j-l	53.1 l	81.9 f-i	92.6 c-g	79.0 b
1 (%)	94 c-f	57.6 l	63.8 j-l	85.1 e-i	99.8 cd	80.1 b
Mean	104 a	64.9 b	64.2 b	99.9 a	103 a	
			Shoot			
0	133 cd	22 e	18 e	112 d	111 d	79 c
15 (mg Kg^{-1})	125 cd	26 e	22 e	139 cd	109 d	84 c
30 (mg Kg^{-1})	174 bc	27 e	21 e	143 cd	143 cd	101 bc
0.5 (%)	148 b-d	52 e	51 e	199 ab	117 cd	113 ab
1 (%)	200 ab	57 e	37 e	231 a	127 cd	131 a
Mean	156 a	37 c	30 c	165 a	122 b	

[†]: Means in each row or column followed by the same lower letters are not significantly different ($p < 0.05$) by Duncan's Multiple Range Test.

(Glycine max L.) seeds were planted about 2.5-cm deep and were thinned to three uniform stands one week after emergence.

Foliar and soil application of Fe and Mn

Four and 8 mg Fe kg^{-1} and 15 and 30 mg Mn kg^{-1} were applied to Fe or Mn treated soils before planting from Fe-EDDHA and $MnSO_4.4H_2O$ sources, respectively. Foliar Fe (1 and 2 %) and Mn (0.5 and 1 %) sulfates were applied using a hand-held sprayer 15 and 30 days after emergence. Polyoxyethylene Sorbitanmonodaurates (Tween 20) was added as a surfactant agent to Fe and Mn sulfate solutions.

Plant analysis

Eight weeks after planting shoots were harvested and roots were separated from soil by washing with a jet of water. Both plant parts were rinsed with distilled water, dried at 65° C for 48 hours, weighted, ground and dry ashed at 550°C. The ash was dissolved in 2 normal HCl and concentration of Fe and Mn was determined using atomic absorption spectrophotometer. Root or shoot Fe and Mn uptake was determined by multiplication of Fe or Mn concentration of root or shoot to their corresponding dry matter yield. The Fe: Mn ratio of root or shoot was determined as a nutritional balance criterion by dividing the amount of Fe uptake of root or shoot to their corresponding Mn uptakes. Shoot and root dry matter yield, Fe and Mn concentration, uptake, and their ratios in both parts of plants were used as plant responses.

Statistical analysis

The data were analyzed using MSTATC and EXCEL software packages and the mean value of plant responses were compared statistically using Duncan's Multiple Range Test at probability level of 0.05.

Table 5. Effect of soil (mg Kg^{-1}) and foliar (%) application of Fe and Mn on root and shoot Mn uptake of soybean plant (µg pot^{-1}).

Mn levels	Fe levels					Mean
	0	4 (mg Kg^{-1})	8 (mg Kg^{-1})	1 (%)	2 (%)	
			Root			
0	124[†] b-e	103 c-g	57.8 g	159 ab	197 a	128 a
15 (mg Kg^{-1})	129 b-d	71.9 fg	72.1 fg	168 ab	95.9 d-g	108 b
30 (mg Kg^{-1})	164 ab	68.4 fg	110 c-f	104 c-f	126 b-e	114 ab
0.5 (%)	167 ab	82.8 e-g	80.9 e-f	93.6 d-g	111 c-f	107 b
1 (%)	135 b-d	68.9 fg	105 c-f	109 c-f	147 bc	113 ab
Mean	144 a	78.9 b	85.0 b	127 a	136 a	
			Shoot			
0	596 cd	88 g	74 g	453 d-f	477 de	338 d
15 (mg Kg^{-1})	564 cd	111 g	85 g	657 b-d	467 d-f	377 cd
30 (mg Kg^{-1})	758 a-c	121 g	84 g	604 cd	654 b-d	444 bc
0.5 (%)	697 b-d	234 fg	243 e-g	741 bc	544 cd	492 ab
1 (%)	890 ab	257 e-g	187 g	976 a	566 cd	575 a
Mean	701 a	162 c	135 c	686 a	542 b	

[†]: Means in each row or column followed by the same lower letters are not significantly different ($p < 0.05$) by Duncan's Multiple Range Test.

Conclusions

Iron and Mn treatments did not affect shoot or root dry mater yield (SDMY and RDMY, respectively) probably due to the fact that Fe and Mn soil tests were in adequate ranges and shoot Fe:Mn ratios were greater than 0.4. Foliar spray of 1 % Fe sulfate improved plant Fe content and had no negative effect either on SDMY or on shoot Mn concentration; therefore it might be considered as an appropriate Fe treatment for soybean. The results indicated that the reduction of root Mn absorption and also reduction of Mn translocation from root to shoot were responsible for the suppressing effect of Fe on Mn nutrition of soybean.

Presence of Mn could negatively affect Fe absorption by roots and high level of soil applied Mn (i.e., 30 mg Mn kg^{-1}) could limit Fe translocation from root to shoot. Due to the fact that most of the shoot Fe: Mn ratios were higher than 0.4; therefore, the lack of positive response of soybean SDMY at least might be partially attributed to shoot Fe: Mn ratios of higher than 0.4. It seems that the Fe: Mn ratio of < 0.4 is a reliable indicator for prediction of soybean and dry bean (Moosavi and Ronaghi, 2010) plants responses to Fe and Mn applications. Soil application of Mn is not an effective method in preventing induced Mn reduction in soybean by Fe applications in calcareous soils. Therefore, foliar Fe or Mn applications or use of Fe-efficient genotypes of soybean remains effective and economic sound options for preventing yield loss and nutrient imbalance in plants grown on such calcareous soils.

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