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Comparison of the Effects of Iron-Enriched Spinach and Sugar Beet Leaf Composts with Ferrous Sulfate and Iron Sequestrene on Iron Availability in the Sorghum Plant in Calcareous Soil

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Mixtures of iron salts and organic matter such as manure, compost, sewage sludge and peat have been used successfully in controlling iron chlorosis in various crops. The aim of the present study was to optimize the effectiveness of natural organic substances by enriching them with iron sulfate on iron availability in sorghum. For this purpose, spinach leaves and sugar beet leaves, as ferrophyll and iron-enriched plants, were incubated with different quantities of iron sulfate. The effects of these iron-enriched composts, iron sulfate and Sequestrene 138 on iron availability in sorghum in calcareous soil were studied in a greenhouse experiment. The DTPA-AB extractable iron of soil, the total iron concentration and the chlorophyll of the plant leaves were all measured. Soil applications of the iron-enriched spinach leaves and sugar beet leaves composts and iron sulfate significantly increased the DTPA-AB extractable iron of the soil, but the spinach leaves and sugar beet leaves composts had no significant effect compared to the control and Sequestrene groups. The plant analysis indicated that the spinach leaves compost significantly increased the total iron concentration in the leaves compared to the control group and even more than the Sequestrene group. The iron-enriched spinach compost increased the total iron concentration compared to the control group, but had no significant effect compared to the sequestrene group. All the iron-enriched composts had same effect as the sequestrene group on the chlorophyll levels in the leaves and significantly increased this level compared to the control group, but the spinach leaves and sugar beet leaves composts significantly decreased the level of chlorophyll in the leaves compared to the control group.

Key Words: Iron-enriched compost, Iron sulfate, Sequestrene, Sorghum, Calcareous soil.

INTRODUCTION

Iron is recognized as an essential element as early as 1845. It is essential for plant growth and is generally considered to be a micronutrient. It is necessary for the formation of chlorophyll and functions in some of enzymes of the respiratory system. Iron is an abundant element in rocks and soil, but it is also one of the most commonly deficient micronutrients[1,2]. Iron chlorosis may result from an absolute iron deficiency in the soil, but this is not a frequent occurrence. The following factors may contribute to the development of chlorosis (either alone or in combination), *i.e.*, low iron supply, calcium carbonate in soil, bicarbonate in soil or irrigation water, high pH level, over-irrigation or waterlogged conditions, high phosphate levels, high levels of heavy metals, low or high temperatures, high levels of nitrate nitrogen, imbalances in cation ratios, poor soil aeration, certain organic matter additions to soil, viruses and root damage by nematodes and other organisms[3,4]. Iron chlorosis generally occurs on calcareous soils; a high pH level and high HCO_3^- concentrations decrease the availability of iron in these soils[5,6]. The solubility of iron decreases by a factor of 1000 with each pH increase of one unit in the pH range between 4-9[7,8].

Iron deficiency is usually manifested as an interveinal chlorosis of young leaves while the veins remain green, hence the name iron-deficiency chlorosis[9]. Iron fertilizers affect the yield, quality and iron-use efficiency in crops under the iron-deficiency conditions. But the effectiveness of synthetic chelates in controlling iron chlorosis is usually better than inorganic iron sources. In general, inorganic iron transforms to an unavailable form in calcareous soil that has a high pH level[10]. In comparison to inorganic iron fertilizers, commercially available iron chelates are about five times as effective, but the expenses are 30 to 70 times higher than inorganic iron fertilizers[11]. However, on the basis of price, it is not always economically viable[12]. Thus, it is important to attempt to replace them with cheaper iron fertilizers. Organic material that has been enriched by inorganic iron compounds can be a suitable and cheap replacement for iron synthetic chelates. Mixtures of Fe salts and organic matter such as manure, compost, sewage sludge and peat have been used successfully in controlling iron chlorosis in various crops[13].

Organic matter improves iron availability by combining with iron, thereby reducing chemical fixation or the precipitation of iron as ferric hydroxide. This reduction in fixation and precipitation results in higher concentrations of iron remaining in the soil solution that is available for root absorption. Organic matter can also affect iron availability by acting as an energy source for microorganisms that use up oxygen under waterlogged conditions. When microorganisms decompose organic matter, iron that was previously tied up in organic compounds is released in forms available for plant uptake. Finally, many organic materials can also be sources of iron[14].

The aim of this study was to produce two iron-enriched composts from spinach leaves (*Spinacia oleracea* L.) (SP) and sugar beet leaves (*Beta vulgaris* L.) (SB) and to enrich them with iron sulfate ($FeSO_4 \cdot 7H_2O$). Then, using iron Sequestrene 138 (Fe-EDDHA), we compared their effects on iron availability in sorghum (*Sorghum bioclor* L.) in calcareous soil in a greenhouse experiment.

Spinach leaves and sugar beet leaves seem to be especially favourable because they are in the ferrophyll plant group and are rich in iron (the amount of iron in ferrophyll plants varies between 50-2000 ppm). These plants have coloured N-substances that are derived from dihydroxy phenol alanin in biogenesis. They have a large amount of oxalic acid and become enriched with carboxyl and phenolic hydroxyl groups during decomposition[15], which might form organic iron complexes *via* chelation[16]. The C/N ratio of these plants is low (spinach C/N = 5.82, sugar beet C/N = 7.48) and they decompose quickly (some of the chemical properties are presented in Table-1).

TABLE-1
CHEMICAL PROPERTIES OF SPINACH LEAVES AND SUGAR BEET LEAVES

	Organic carbon (%)	Organic matter (%)	Total nitrogen (%)	Carbon/ Nitrogen	Total iron (%)
Spinach leaves	32.76	56.35	5.36	5.82	0.0371
Sugar beet leaves	31.59	54.33	4.22	7.48	0.0232

EXPERIMENTAL

Fresh spinach leaves (2000 g) and sugar beet leaves (2000 g) were incubated with increasing amounts (0-1.28 -5.6 -6.4 % weight) of net iron as an iron sulfate source (or 0- 6.4- 12.8- 32 % weight from iron sulfate 20 %) in three replications. The incubation was carried out over 41 d in a greenhouse at a constant temperature (27ºC) and enough moisture (moisture > 60 %). After 41 d, the composts were dried at 65ºC and weighed. Organic carbon (Walkley-Black), Total nitrogen (the Kjeldahl method) and total iron (dry ashing and 2N HCl) were measured (Table-2).

This study was carried out on sorghum (as a plant that is susceptible to iron deficiency) growing in calcareous soil (soil of the Research Center of flowers and ornamentals plants of Mahalat). This soil was selected because it has a high pH level, a high $CaCO_3$ concentration and a history of exhibiting iron chlorosis in flowers and ornamentals plants. (The chemical and physical properties are presented in Table-3.) The study was carried out in a greenhouse using a completely randomized design with three replications. The amounts of applications of all the composts and iron sulfate treatments were 5 g/kg soil (0.5 % weight of the soil) and the amount of application of the Fe-EDDHA treatment was 83 mg/kg soil.

TABLE-2
CHEMICAL PROPERTIES OF PRODUCED COMPOSTS

Composts	Weight loss (%)	Organic carbon (%)	Total nitrogen (%)	C/N	Total iron (%)
Spinach leaves	95.7 ± 0.3	20.86	2.29	9.10	0.7890
SP +1.28% Fe	92.51 ± 0.9	16.77	1.97	8.50	2.7337
SP + 2.56% Fe	89.11 ± 0.5	8.39	1.08	7.79	4.9756
SP + 6.4% Fe	85.38 ± 0.7	6.44	0.95	6.82	10.1337
Sugar beet leaves	95.37 ± 0.4	27.78	3.23	8.61	0.5340
SB + 1.28% Fe	91.16 ± 0.7	19.70	2.98	6.59	2.5742
SB + 2.56 % Fe	87.88 ± 0.8	13.26	2.05	6.47	4.8386
SB + 6.4% Fe	79.72 ± 0.6	9.10	1.49	6.10	9.1910

TABLE-3
PHYSICAL AND CHEMICAL PROPERTIES OF THE SOIL

Depth (cm)	EC × 10^3	pH	CC	OC	TN	P	K	Fe	Mn	Zn	Texture
0-30	1.63	7.73	38.8	0.55	0.06	7.9	160	3.1	16.9	1.1	Sandy-loam

CC = Calcium carbonate; OC = Organic carbon; TN = Total nitrogen

Before harvesting, the leaf chlorophyll a, b and the total chlorophyll concentration were estimated with an SPAD-502 meter (Minolta Co., Osaka, Japan) in fresh leaves. SPAD values were converted into a chlorophyll concentration (mg/cm^2) by using the calibration equations:

$$Y_1 = 1.56E - 06 + 3.33E - 04X + 9.03E - 06X^2 \quad (R^2 = 0.952, n = 100) \tag{1}$$

$$Y_2 = 5.46E - 04 + 6.89E - 05X + 3.37E - 06X^2 \quad (R^2 = 0.964, n = 100) \tag{2}$$

$$Y_3 = 5.52E - 04 + 4.04E - 04X + 1.25E - 05X^2 \quad (R^2 = 0.960, n = 100) \tag{3}$$

where Y_1 is the chlorophyll a, Y_2 is the chlorophyll b, Y_3 is the total chlorophyll concentration and X is the SPAD value in the leaves[17]. After 80 d, the top organs of the plant were harvested and weighed. The leaves were washed with tap water and three times with distilled water, dried at 65ºC and weighed. The total iron and manganese levels of the leaves were measured by dry ashing, the 2N HCl method and atomic absorption spectrophotometry (Varian Spectr. AA 220).

In the experimental soil, DTPA-AB-extractable iron was determined by the method set forth by Soltanpour and Schwab[18]. These results are presented in Table-4.

TABLE-4
EFFECT OF TREATMENTS ON THE AVERAGES OF CHLOROPHYLL a, CHLOROPHYLL b, CHLOROPHYLL a+b, TOTAL IRON AND TOTAL MANGANESE VALUES OF SORGHUM

Treatment	DTPA-AB extractable Fe	Chl a (mg cm^{-2})	Chl b (mg cm^{-2})	Chl a+b (mg cm^{-2})	Total Fe (mg kg^{-1})	Total Mn (mg kg^{-1})
F0 (Control)	2.559d	0.0264ed	0.00826ed	0.0349ed	60.6d	88.07b
F1($FeSO_4$,$7H_2O$)	66.36a	0.0277bc	0.00882bc	0.0367bc	67.6bcd	47.33c
SP (Spinach leaves)	4.455d	0.0254e	0.00792e	0.0335e	77.4a	107.8a
SP +1.28% Fe	28.186c	0.0292a	0.00914a	0.0386 a	70.4ab	59.33c
SP +2.56% Fe	45.865b	0.0287ab	0.00900ab	0.0379ab	71.03ab	58.43c
SP +6.40% Fe	65.445a	0.0294a	0.00922a	0.0389 a	69.33bc	52.33c
SB (Sugar beet leaves)	3.557d	0.0260e	0.00812e	0.0343e	60.87d	115.30a
SB +1.28% Fe	24.729c	0.0274cd	0.00859cd	0.0363cd	66.60bcd	61.60c
SB +2.56% Fe	46.413b	0.0294a	0.00922a	0.0388a	61.90cd	49.90c
SB +6.40% Fe	48.397b	0.0288ab	0.00901ab	0.0380ab	64.47bcd	49.17c
Sequestrene 138 (Fe-EDDHA)	7.833d	0.0282abc	0.00882abc	0.0372abc	69.20bc	84.40b

RESULTS AND DISCUSSION

Iron-enriched composts of spinach leaves and sugar beet leaves and iron sulfate have significantly increased ($\alpha = 0.01$) DTPA-AB extractable iron compared to the control group, but the spinach leaves and sugar beet leaves composts and Fe-EDDHA had no effect on DTPA-AB extractable iron. Assigning iron availability by means of soil analysis does not produce satisfactory results; perhaps iron deficiency symptoms in plants (young leaves chlorosis and leaf analysis) can estimate iron deficiency[16].

Spinach leaves and their iron-enriched compost significantly increased the total iron of sorghum ($\alpha = 0.01$) compared to the control group and this level was higher than Fe-EDDHA.

All the iron-enriched composts significantly increased chlorophyll levels (a, b and a+b of the fresh leaves) compared to the control group ($\alpha = 0.01$), but the spinach leaves and sugar beet leaves composts reduced them. Weak iron chlorosis symptoms were observed in the control treatment plants and these symptoms were middle to intensive in the spinach leaves and sugar beet leaves composts' treatments plants. However, the iron concentration in leaves is usually not correlated with the degree of iron chlorosis[19], it is correlated with other factors such as high levels of heavy metals and imbalances in cation ratios[3,4].

Nutrient interaction can be positive, negative or neutral[20]. It can be measured in terms of crop growth and nutrient concentrations in plant tissue. One type of interaction is between ions whose chemical properties are sufficiently similar and they compete for site absorption, transport and function on the plant root's surface or within plant tissues. Such interactions are more common between nutrients of a similar size, charge and geometry of coordination and electronic configuration[21]. Chinnery and Harding[22] have reported the antagonistic effect of manganese on the iron uptake and *vice-versa*. Sideris and Young[23] suggested that manganese instead of iron might react with porphyrin compounds, thereby inactivating them for subsequent conversion to chlorophyll. Leach and Taper[24] concluded that the optimum Fe/Mn ratios in plants ranged from 1.5 to 3.0 for the kidney bean and from 0.5 to 5.0 for the tomato. Iron deficiency developed at lower ratios and manganese toxicity at higher ratios. Significant correlations have been found between the Fe/Mn ratio and chlorophyll concentrations:

$$Y = 0.0048X + 0.0318$$
$$(\alpha = 0.01, n = 33, R^2 = 0.62)$$

where Y is the chlorophyll concentration (mg/cm^2) and X is Fe/Mn ratio in sorghum leaves.

It is expected that in the plants treatment of the spinach leaves and sugar beet leaves composts, manganese instead of iron reacts with porphyrin compounds and chlorosis symptoms, as found in present study. In all of the iron-enriched composts and iron sulfate treatments, iron supply influenced the amount of manganese absorbed by the plants and reduced this amount; no chlorosis symptoms were observed.

Conclusion

The data presented in this study showed that the application of the iron-enriched compost of spinach leaves had the same effect as Fe-EDDHA, but at present, in Iran the widespread use of spinach for producing composts is not economically viable. It is suggested in present work that using these experiments to carry out another study on ferrophyll plants (such as lettuce) and their waste matter.

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