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A comparison of various iron fertilizers with regard to their effects on the iron content of Satsuma tangerine plants

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In this study, an examination was made of the effects of various iron fertilizers on the iron content of the various organs of tangerine plants. To this purpose, four different iron fertilizers [Fe-Citrate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, Fe-EDTA and Fe-EDDHA (Sequestren)] were applied to Satsuma plants. At the end of the experiment, the effects of the applications on the Fe and active Fe content of the roots, stems and leaves were examined. In addition, apoplastic Fe in the leaves was determined. Results showed that the highest values of all three parameters (total, active and apoplast Fe) were provided by Fe-EDDHA. The research showed that iron applied to the leaves affected active and apoplastic content in the order Fe-EDDHA (sequestren) > Fe-EDTA > $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ > Fe-Citrate. Fe-EDTA followed the application of Fe-EDDHA. The highest total values of iron content in the roots were provided by Fe-Citrate and Fe-Sulfatin. Evaluation showed that Fe-EDDHA (sequestren) had a greater effect than the other Fe-containing fertilizers.

Key words: Satsuma tangerines (*Citrus unshiu* Marc.), iron, iron-containing fertilizers, active iron, apoplastic iron, chlorophyll.

INTRODUCTION

A shortage of iron, as well as being a cause of anaemia and imbalances in the immune system, growth and mental development in animals, is also a significant nutrition problem for plants and the soil (Black, 2003; Boccio and Iyenger, 2003). In Turkey, iron deficiency is an important problem for the soil (Eyüpoğlu et al., 1997; Cakmak et al., 1999) and in the human population (Cavdar et al., 1983).

In the world as a whole, 50% of land is arid or semi-arid, and 25% suffers from iron deficiency as a result of the presence of lime. Thus, 30% of the world's agricultural land is iron-deficient (Chen and Barok, 1982).

In the 19th century, botanical researchers began to study cell walls (Schindler, 1993). Cell walls are composed of cellulose, hemicelluloses, pectin and proteins (Sakurai and Nevins, 1997; Carpita et al., 1996). 5 to 10% of plant tissue is consist of apoplast (Pitman et

al., 1974; Grignon, 1991) and apoplast has an important effect on cation exchange capacity and passing into cells of ions easily (Sattelmacher et al., 1998).

The uptake of Ca^{2+} , Mg^{2+} and Fe^{2+} in particular take place by means of apoplastic transfer from new root tips (Clarkson and Sanderson, 1978). Of these elements, Ca^{+2} especially are transferred by the apoplastic route (Clarkson and Hanson, 1986; Sattelmacher, 2001).

High apoplastic pH in the leaves causes the immobilisation of iron (Mengel and Geurtzen, 1988; Kosegarten et al., 1999). Feeding excessive NO_3 and HCO_3 causes alkalinisation of the apoplast. This increase in the pH of the apoplast hinders Fe^{3+} - reductive in the plasma lemma and thus prevents uptake of iron in the cytoplasm (Mengel and Geurtzen, 1988). According to Yu et al. (2000), apoplastic pH in the leaves in particular prevents the uptake of Fe, Cu, Zn and Mn.

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Table 1. Results of physical and chemical analysis of experimental soil samples.

pH	% CaCO ₃	% Total salt		% O. M.		Structure			
7.62	6.0	0.04		1.09		Sandy clay			
N (%)	P* (ppm)	K** (ppm)	Ca** (ppm)	Mg** (ppm)	Na** (ppm)	Fe*** (ppm)	Cu*** (ppm)	Zn*** (ppm)	Mn*** (ppm)
0.06	0.20	252	3306	495	125	0.08	1.21	2.54	3.50

*Water-soluble P; **1 N NH₄OAC (pH = 7.0); ***0.05 M DTPA + TEA (pH = 7.2).

Table 2. Nutrient solutions and their amounts used on each pot in the experiment.

Nutrient element/ form	Amount (g)	Source
N	12	Ammonium Nitrate + MAP + (NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O
P/P ₂ O ₅	11	Super Phosphate + MAP + KH ₂ PO ₄
K/K ₂ O	8	KH ₂ PO ₄ + K ₂ SO ₄
Ca	1	Ca(NO ₃) ₂
Mg	0.5	Mg(NO ₃)
Fe	0.6	Fe
Zn	0.14	ZnSO ₄ .7H ₂ O
Mn	0.068	MnSO ₄ .4H ₂ O
Cu	0.015	CuSO ₄ .5H ₂ O
B	0.0066	H ₃ BO ₃
Mo	0.021	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O

When it is considered that iron is not transferred from old leaves to new leaves, so that the need for iron of the growing organs must be continually met by iron uptake, the most important consideration in plant production in order to prevent iron deficiency is iron enrichment by means of soil and leaf fertilizers. On this basis, the main aim of this study was to determine the most effective iron-containing fertilizer which would prevent the iron deficiency seen in Satsuma plants.

MATERIALS AND METHODS

The plant material used in the study was one-year-old Satsuma tangerine plants (*Citrus unshiu* Marc.) grafted on to Trifoliata rootstock. A mix of 9 kg of soil to 1 kg of perlite was used as a growing medium. The results of physical and chemical analysis shown in Table 1 show that soil samples were slightly alkaline (pH: 7.62) in reaction and rich in lime (Kellog, 1952; Evliya, 1964). Total percentage salt content of soil samples was low, organic material content was low, and structure was sandy-loamy (Soil Survey Staff, 1951). Iron content of the experimental soil was low (Lindsay and Norvell, 1978). In accordance with the random block experimental design, the following treatments were applied in five iterations:

1. Control,
2. Fe-Citrate,
3. FeSO₄.7H₂O,
4. Fe-EDTA,
5. Fe-EDDHA (Sequestren).

Applications to each pot through the vegetation period were as

shown in Table 2. The third and fourth leaves were taken from each pot, and 0.5 g of leaf samples were agitated for 30 min with 20 ml 1 N HCl, for the purpose of measuring active iron content of the plants on the AAS scale (Geurtzen, 1985). Total iron content was determined from samples prepared for analysis by wet burning of weighed 1 g leaf, stem and root samples in a mixture of concentrated nitric + perchloric acid (Kacar, 1972). The method of Nikolic and Romheld (2002) was modified in order to determine the amount of apoplastic iron. This was calculated according to this method by adding the solution to 1 g weighed leaf samples, after which infiltration was carried out and N₂ processing was repeated for 5 min, and then absorbance values were determined in a spectrophotometer set to a wavelength of 520 nm. Total chlorophyll content was determined by the 80% acetone method (Smith and Benitez, 1955).

RESULTS

Results of the study showed that the total amount of iron found in the leaves was higher in the Fe-Citrate, FeSO₄.H₂O, Fe-EDTA and Sequestren treatments than in the control (Table 3). The increases in the total amounts of iron in the iron treatments were statistically significant, and the amounts in the Fe-EDTA (132.84 ppm) and Sequestren (110.94 ppm) forms were twice as high as the control. The statistically highest amount of total iron was detected in the treatment of Fe-EDTA on tangerine leaves (132.84 ppm). The result obtained with the Fe-EDTA treatment was found to be within adequacy limit values (Bergmann, 1988). In a study conducted on grape

Table 3. Statistical analysis results of the experiment.

S/N	Treatments	Leaf total Fe (ppm)	Stem total Fe (ppm)	Root total Fe (ppm)	Active total Fe (ppm)	Total chlorophyll (%)	Apoplastic Fe (ppm)
1	Control	50.240 ^d	65.462 ^c	90.060 ^c	9.876 ^c	0.123 ^d	3.344 ^c
2	Fe-Citrate	62.100 ^{cd}	84.240 ^{bc}	324.600 ^a	13.050 ^b	0.287 ^c	4.678 ^c
3	FeSO ₄ .H ₂ O	69.440 ^c	101.680 ^{ab}	269.200 ^b	13.926 ^b	0.489 ^{ab}	6.654 ^c
4	Fe-EDTA	132.840 ^a	119.340 ^a	254.680 ^b	31.534 ^a	0.408 ^b	25.164 ^b
5	FeEDDHA	100.940 ^b	112.960 ^a	223.600 ^b	32.100 ^a	0.501 ^a	40.492 ^a
LSD		(1%) 12.732	(1%) 28.705	(1%) 54.033	(1%) 3.100	(1%) 0.093	(1%) 9.419

vines, it was found that the highest amounts of iron in leaves were obtained with Fe-EDDHA treatment (Saatci, 1990). Similar results were obtained with treatments with Fe-Citrate and Fe-Sulphate at 62.10 and 69.44 ppm. Treatments on these forms resulted the amounts of iron falling below the levels of adequacy (Bergmann, 1988).

When total amounts of iron in the stems of tangerine plants was examined, it was found that amounts in the control were lowest (65.46 ppm), and those of the Fe-EDTA treatment were highest (119.34 ppm) (Table 3). After the Fe-EDTA treatment, the Sequestren treatment was found to have 112.96 ppm of iron in the stems. The highest iron levels were found in the stems of Fe-EDTA and Sequestren treatments.

Lowest total amounts of iron in tangerine roots (90 ppm) were found in samples taken from the control pots, and highest amounts of iron (324.60 ppm) were found in the Fe-Citrate treatment (Table 3). In the Sequestren treatment, 223.6 ppm of iron was determined. The effect on the increase of forms of iron in the amounts of total iron in the roots was found to be statistically significant in comparison with the control. The reason why less iron was determined in the Fe-Sulphate and Fe-Citrate treatments than in other treatments is that these forms are stored in the roots and are not transported to other organs.

Amounts of active iron were found to be lowest (9.87 ppm) in the control and at their highest concentration levels (32.10 ppm) in the Sequestren treatment. The effect of iron treatments on the amounts of active iron in the leaves was found to be statistically 1% significant (Table 3). Geurtzen (1985) found that as the total of amount iron increased, the amount of active iron also increased. Chlorophyll levels, one of the important signs of iron deficiency, were highest in the sequestren treatment (0.501%) and lowest in the control (0.123%). Chlorophyll amounts determined were high in the iron treatments. The total amount of chlorophyll in the Fe-Citrate treatment (0.28 ppm) was half the average value of the other iron fertilizer treatments (Table 3). Saatci (1990) found the highest chlorophyll values in the Fe-EDDHA treatment.

Apoplastic-Fe was determined statistically as highest in the sequestren (Fe-EDDHA) treatment at 40.49 ppm and lowest in the control at 3.34 ppm (Table 3). Apoplastic-Fe

was determined at 25.16 ppm in the Fe-EDTA treatment, 4.67 ppm in the Fe-Citrate treatment, and 6.65 ppm in the FeSO₄.7H₂O treatment (Tables 3 and 4). Nikolic and Römheld (2002) found apoplastic iron levels in leaves of 46 mg/g in treatments without bicarbonate, and of 26 mg/g in treatments with bicarbonate.

CONCLUSION AND RECOMMENDATIONS

It was concluded that the Fe-EDDHA fertilizer form was statistically significantly effective in terms of active-Fe and apoplastic-Fe content. Fe-EDDHA was followed by Fe-EDTA. These findings show that it is correct to recommend the use of Fe-EDDHA and Fe-EDTA iron fertilizers.

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