J. BIOL. ENVIRON. SCI.,
2009, 3(8), 61-65

Comparative Efficacy of Citric Acid and Fe(II) Sulfate in the Prevention of Chlorosis in Orange Trees (*Citrus sinensis* L. cv 'Darabi')

Elias Amri*, Ali Reza Shahsavar
Department of Horticulture, College of Agriculture, Shiraz University, Shiraz, Iran

ABSTRACT

A field experiment was carried out comprising foliar spray of citricric acid and Fe(II) sulfate alone and in combination with citricric acid were applied to evaluate the recovery of iron chlorosis of orange trees (*Citrus sinensis* L. cv 'Darabi' , grafted on *Citrus aurantifolia* L.) grown on a calcareous soil. The treatments were Fe(II) sulfate (500 mg Fe/L), citric acids plus Fe(II) sulfate (1000 mg/L + 500 mg Fe/L), citric acid (1000 mg/L), and distilled water as a control. A non-ionic wetting agent was used in all treatments. The use of frequent foliar sprays alleviated Fe chlorosis in orange trees. On 4 of 7 measurement dates, trees in the citric acids plus Fe(II) sulfate treatment had higher leaf chlorophyll indexes determined with a SPAD meter (SPAD values) than trees in all other treatments. Trees in the Fe(II) Sulfate treatment had higher SPAD values than trees in the citric acids and control treatments. Total leaf iron content was higher for the citric acids plus Fe(II) sulfate and Fe(II) sulfate treatments than the citric acids and control treatments. There was a difference in total leaf iron content between the citric acids and control treatments. Sprays of citric acids plus Fe(II) sulfate improved fruit size and quality compared to fruits of control trees. Sprays of Fe(II) sulfate also improved fruit quality and increase fruit size. Sprays of citric acids also improved fruit size and quality compared to fruits of control trees. These results suggest that foliar sprays with Fe could help to avoid yield and quality losses caused by Fe chlorosis in citrus orchards. Furthermore, these treatments could be done with relatively cheap materials such as solutions containing Fe(II) sulfate

Key Words: Fe(II) sulfate, citric acid, chlorophyll, chlorosis, Darabi orange.

INTRODUCTION

Orange grown on alkaline calcareous soils of becomes incipiently chlorotic. The chlorotic leaves of several crops on these soils were found to contain significantly higher amount of iron than green leaves (Mengel et al., 1994). Thus eliminating the possibility of a deficiency of iron in the soils or lack of transport there of to aerial parts as a possible cause of chlorosis in plants grown on these soils. Root iron uptake involves a reduction of iron from Fe^{3+} to Fe^{2+} at the cell membrane of epidermal root cells; this reduction is catalyzed by the enzyme (Mengel et al., 1994). After iron is reduced in the roots, it is re-oxidized back to Fe^{3+} in the apoplast where Fe^{3+} then binds with citric acid (Schmidt et al., 1999). Iron is then transported in the xylem from the roots to the leaves as ferric-citrate and re-reduced in the leaf apoplast to the Fe^{2+} form and is actively transported across the plasma membrane into the symplast where it is metabolized by the plant (De la Guardia and Alcantara, 1996; Kosegarten et al., 2001; Mengel et al., 1994; Taiz and Zeiger, 1998). Often, in calcareous soils, a sufficient quantity of iron is translocated from the roots to the leaves, but the reduction of Fe^{3+} to Fe^{2+} in the leaves is hampered by the high pH environment of the apoplast (Bruggemann et al., 1993; Gonzáles-Vallejo, 2000; Mengel et al., 1994). In calcareous soils, the correction of Fe chlorosis in trees is normally achieved by the application of Fe (III)-chelates such as Fe-EDDHA to the soil (Pestana et al., 2001; Schmidt et al., 1999). This practice has to be repeated every year because Fe is rapidly immobilized in the soil. Applying Fe treatments foliarly instead of as soil application can avoid inhibitory effects of soil bicarbonate on Fe uptake and transport to the shoot (Abadia et al., 1993). Acid treatments could release Fe immobilized within the plant by changing apoplastic pH (González-Vallejo et al., 2000; Mengel et al., 1994; Nikolic and Römheld, 1999; Pestana et al.,. 2001; Schmidt et al., 1999).

Iron chlorosis also affects leaf and Flower mineral composition (Tagliavini et al., 1995) and is responsible for signifcant decreases in yield, fruit size and fruit quality. This has been reported in several fruit trees species such as peach (Tagliavini et al., 2000) and kiwifruit (Tagliavini et al., 1995).

The overall objective of this project will be to evaluate foliar applications of weak acids (citric, ascorbic and dilute sulfuric) as cost-effective alternatives to the current standard practice of applying very expensive chelated iron to calcareous soil to prevent iron deficiency in tropical and subtropical fruit trees (Crane et al., 2007). The specific hypotheses to be tested are: 1) foliar applications of weak acids will decrease the pH of the leaf apoplast of trees grown in calcareous soils, thus increasing the bioavailability of iron and preventing or eliminating iron deficiency 2) incorporating the spraying of weak acids into an orchard management program will provide a more cost effective means of preventing iron deficiency in subtropical and tropical

* Corresponding author: Elias.Amri@gmail.com

fruit crops than the current practice of applying chelated iron to the soil. (Schaffer et al., 2007) The aim of this work was to evaluate the effectiveness of different foliar applications to alleviate Fe chlorosis in orange trees established on a calcareous soil.

MATERIALS AND METHODS

A field experiment was carried out during 2007 to 2008 in 10-year-old orange trees (*Citrus sinensis* L. cv 'Darabi' grafted on to *Citrus aurantifolia* L.), which were randomly selected in an orchard planted on a calcareous soil (35% total $CaCo_3$, 0.5% active $CaCo_3$, 3% organic matter, pH 8.1, 54% clay, 28% silt and 18% sand) located in Fasa (South of Iran). Three different foliar spray treatments were applied. The treatments were Fe(II) sulfate (500 mg Fe/L), citric acids plus Fe(II) sulfate (1000 mg/L + 500 mg Fe/L), citric acid (1000 mg/L) and distilled water as a control. A non-ionic wetting agent was used in all treatments. Treatments started on 15 August, 2007, when the diameter of the fruit was approximately 30mm, trees were treated three times during each year (August 15, 30 and 15 September in 2007 and 2008), a total of 6 times. Sprays were applied to whole tree. Treatments were applied with a manually operated sprayer by performing full wetting of the whole tree until the solution dropped from the foliage to the ground. Total leaf chlorophyll concentration was determined at the beginning and at the end of of the experiment after two week last spray application, on the trees, using a SPAD-502 apparatus (Minolta, Osaka, Japan).

At the end of the assay (30 September) at least 30 healthy, recently expanded (second and third fully developed) leaves were randomly collected per tree were washed with tap water, followed by distilled water containing a non-ionic detergent, and then with HCl (0.01 M). Finally, three rinses were carried out with distilled water. Leaves were dried at 70 C° for 48 h in an oven to determine Fe content using atomic absorption spectrometry GBC 310 (GBC Australia).

To study the effects of foliar application on fruit quality, at least 20 fruits were collected on 30 January from treated trees. Fresh weight, fruit diameter, juice content, total soluble solids (TSS), citric acid concentration and total acids (TA), were determined with standard methods (Ranagana, 1977).

The effects of treatments were evaluated using analysis of variance and the means compared by Tukey's multiple test at a probability of 5% significance level, using SAS software (SAS Version 6, 4^{th} Edn).

RESULTS AND DISCUSSION

Effects of foliar sprays on total leaf chlorophyll concentration

Foliar sprays with citric acids plus Fe(II) sulfate were effective in controlling Fe chlorosis in orange trees. Changes in total leaf chlorophyll concentration during the experiment are shown in (Fig. 1). The leaf chlorophyll concentrations in the trees treated with citric acids plus Fe(II) sulfate was always greater than in the trees that other treatments and control (Fig. 1). Sprays of Fe(II) sulfate also increased leaf chlorophyll than trees that citric acid treatments and control (Fig. 1). Sprays of citric acid also increased leaf chlorophyll than trees that control trees treated with water (Fig. 1). These results suggest that in chlorotic leaves, which have a lower amount of Fe compared with green leaves, a part of the Fe is still stored outside the mesophyll cells. As citric, sulphuric and nitric acids caused some regreening it is possible that this storage may be due to high apoplastic pH, high rates of oxidation of Fe(II) or low Fe reduction activity (Legaz et al., 1992; Mengel et al., 1994; Miller et al., 1994).

Therefore, citric acid appears to improve to some extent the availability of Fe from previously unavailable internal pools in the tree. Similar fndings were obtained with acidic solutions by Tagliavini et al. (1995) in kiwifruit. The decreases in leaf chlorophyll concentrations found at the end of the experiment in all treatments (Fig. 1). were possibly due to a dilution effect, since vegetative growth in the orange trees resumed in March (Pestana et al., 2001).

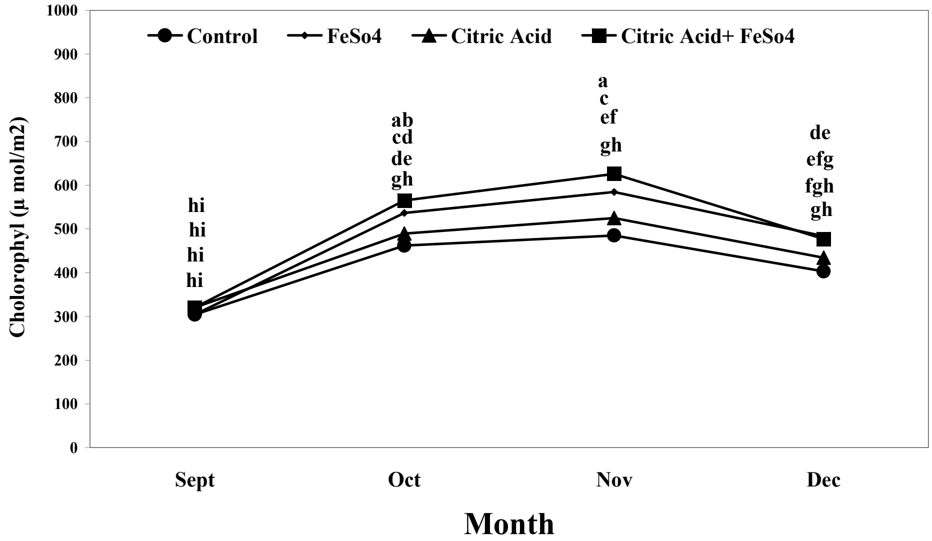

Figure 1. Changes in total leaf chlorophyll (µmol/m^{-2}) in Darabi orange trees as affected by foliar sprays. Values with the same letters are not significantly different for p = 0.05 (Tukey's multiple test).

Effects of foliar sprays on the concentrations of Fe in leaves

The citric acids plus Fe(II) Sulfate sprays significantly increased the concentrations of Fe in leaves, citric acids plus Fe(II) sulfate, due to its acidic pH, may remobilize Fe pools in chlorotic leaves as well as act as a Fe source. These simultaneous effects may explain both the regreening of the treated leaves and the increased Fe concentrations. Sprays of Fe(II) Sulfate significantly increased Fe concentrations than trees that citric acid treatments and control. Citric acid significantly increased Fe concentrations than control trees treated with water. Our data show that all acid plus iron sulfate foliar treatments tended to have higher total iron concentrations than the citric acid, Fe(II) Sulfate alone, or the control treatments (Fig. 2). Reducing the apoplastic pH of iron-chlorotic leaves with foliar applications of dilute acids such as sulfuric, citric, or nitric acid, resulted in a decrease in the leaf apoplast pH and "re-greening" of leaves of some fruit crops including kiwifruit (Tagliavini et al. 1995), orange (Pestana et al. 2002), pear (Garcia-Lavina et al. 2000) growing in calcareous soil. Preliminary research on lychee, carambola, pond apple, and avocado using weak acids with ferrous iron resulted in higher leaf ferrous iron content than leaves treated with acid only (Crane et al. 2007), or non-treated control plants.

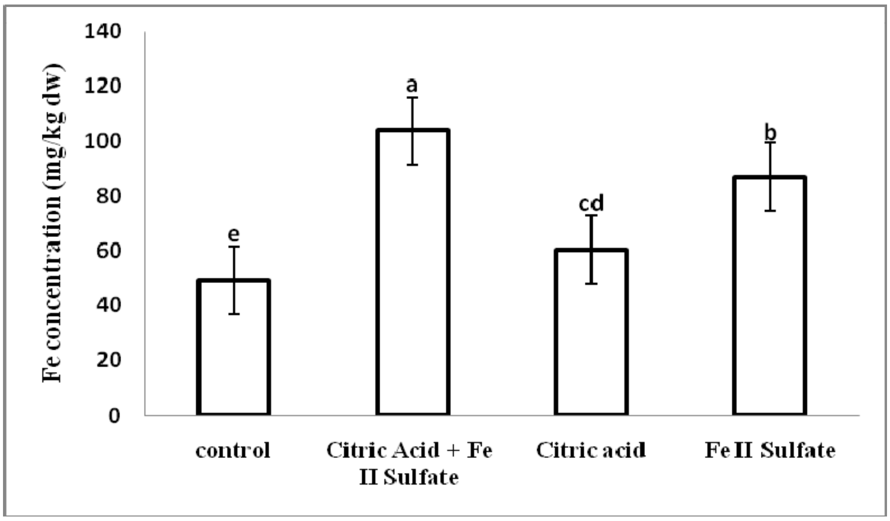

Figure 2. Fe concentration of leaves from Darabi orange trees at the end of the experiment, following different foliar applications. Values with the same letters are not significantly different for p = 0.05 (Tukey's multiple test).

Effects of foliar sprays on fruit size and quality

Foliar treatments affected both fruit quality characteristics and fruit size in Darabi orange trees (Table 1). The applications of citric acids plus Fe(II) Sulfate resulted in significantly larger fruits, with greater diameter and total juice content when compared both to the controls and to the trees treated with Fe(II) sulfate and citric acid. The citric acids plus Fe(II) sulfate sprays signifcantly decreased the concentrations of citric acid in juice compared to the control (Table 1).

Table 1. Effect of foliar applications made to control iron chlorosis on quality of Darabi orange fruits.

	control	Cit Acid + Fe II Sulfate	Citr acid	Fe Sulfate
TSS(BRIX)	8.8 cde	11.25 a	8.99 cd	10.03 b
TA(mg/l)	2.26 ef	1.00 a	1.98 cd	1.5 bc
VC(mg/l)	7.2 fg	14.88 a	11.04 cd	12.12 b
Means having the same letters within a row are not significantly different for p = 0.05 (Tukey's multiple test).				

The applications of acid plus Fe(II) Sulfate resulted in signifcantly larger fruits, with greater fresh mass, diameter and total juice content when compared both to the controls and to the trees treated with other acid treatments (Table 2).

Table 2. Effect of foliar applications made to control iron chlorosis on diameter, weight and juice weight of Darabi Orange Fruit

	control	Cit Acid + Fe II Sulfate	Citr acid	Fe Sulfate
Fruits diameter (mm)	71.75 ef	87.05 a	75.07 d	82.23 bc
Fruits weight (g)	103.8 ef	118.4 a	110.6 cd	113.2 bc
Juice weight(ml)	40 ef	61.3 a	43.3 de	54.7 bc
Means having the same letters within a row are not significantly different for p = 0.05 (Tukey's multiple test).				

CONCLUSIONS

Chelated iron can represent up to 80% of the total fertilizer cost and up to 50% of the total agricultural chemical costs for some tropical fruit crops. This project will be to evaluate foliar applications of weak acids as low cost alternatives to applying expensive chelated iron to the soil to prevent iron deficiency in tropical fruit crops (Avocado, lychee, carambola) grown in calcareous soils (Crane et al., 2007). Thus, the potential exists for the use of foliar applications of weak acids as a low-cost alternative to expensive chelated iron for preventing iron deficiency in orange trees growing on calcareous soils. These results suggest that foliar sprays with Fe could help reduce yield and quality losses caused by Fe chlorosis in citrus orchards. Furthermore, these treatments could be applied with relatively cheap materials such as solutions containing Fe(II) sulfate. Since Fe chelates were introduced, there has been little effort in the research of alternative means for controlling the chlorosis. In the present experiment, treatments were applied during fruit growth and maturation. The impact of controlling Fe chlorosis prior to fruit formation on fruit yield and quality also deserves further investigation.

REFERENCES

Abadia J. Nishio JN. Monge E. Montan L. Heras L. 1985. Mineral composition of peach affected by iron chlorosis. J. Plant Nutr.8: 697-707.

Belkhodja R. Morales F. Sanz M. Abadia A. Abadia J. 1998. Iron deficiency in peach trees: Effects on leaf chlorophyll and nutrient concentrations in flowers and leaves. Plant and Soil. 203: 257-268.

Crane J. Schaffer B. Evans E. Montas W. 2007. Effect of ascorbic acid plus ferrous sulfate on leaf greeness of carambola (*Averrhoa carambola* L.) trees. Proceedings of the Floridaa State Horticultural Society 120: 20-24.

De la Guardia MD. Alcántara E. 1996. Ferric chelate reduction by sunflower (*Helianthus annuus* L.) leaves: influence of light, oxygen, iron-deficiency and leaf age. J. Expt. Bot. 47: 669-675.

Garcia L. Fernandez PA. 2002. Foliar applications of acids with and withont FeSo4 to control Iron chlorosis in pear. Acta. Hort. 594: 217-223.

Kosgarten H. Hoffmann B. Mengel K. 2001. The paramount influence of nitrate in increasing apoplastic pH of young sunflower leaves to induce Fe deficiency chlorosis, and the re-greening effect brought about by acidic foliar sprays. J. Plant Nutr. Soil Sci. 164: 155-163.

Legaz F. Serna MD. Primo-Millo E. Martin B. 1992. Leaf Spray and Soil application of Fe-Chelates to Navelina Orange Trees. Proc. Int. Soc. Citriculture 2. : 613-617.

Mengel K. Planker R. Hoffmann B. 1994. Relationship between leaf apoplast pH and Fe chlorosis of sunflowers (*Helianthus annuus* L.). J. Plant Nutr. 17: 1053-1064.

Mengel K. 1995. Iron Availability in Plant Tissues: Iron Chlorosis on Calcareous Soils. In Iron Nutrition in Soils and Plants, J. Abadia, Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands pp. 389-397.

Miller JE. Swanepoel J. Miller D. Plessis SF. 1994. Correction of lime-induced chlorosis of citrus in the Sunday's River Valley. Subtropica 15: 18-20.

Pestana M. Varennes A. 2003. Diagnosis and correction of iron chlorosis in fruit trees: a review. Food, Agriculture & Environment 1: 46-51.

Pestana M. Correia PJ. Miguel G. 2002. Foliar treatment as a strategy to control iron chlorosis in orang trees. Acta Hort. 594: 223-229.

Ranagana S. 1977. Manual for Analysis of Fruit and Vegetable Products, Tata McGraw Hill Co. Pvt. Ltd. New Dehli pp: 94-98.

Rombola A. Bruggeman DW. Tagliavini M. 2000. Iron source affected iron reduction and re-greening of kiwifruit leaves. J. Plant Nutr. 23: 1751-1765.

Sahu MP. Sharma DD. Jam GL. Singh HG. 1987. Effects of Growth Substances, Sequestrene 138-Fe and Sulfuric Acid on Iron Chlorosis of Garden Peas (*Pisum sativum* L.). J. Hort. Sci. 62: 391-394.

Sanz M. Montan L. Carrera M. 1994. The Possibility of Using Analysis to diagnose the Nutritional Status of Pear Trees. Acta Hort. 367: 290-295.

Sanz M. Montan L. 1995. Floral Analysis: A Novel Approach for the Prognosis of Iron Defciency in Pear (*Pyrus communis* L.) and Peach(*Prunus persica* L.). In Iron Nutrition in Soils and Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 371-374.

Sanz M. Pascual J. Mach J. 1997. Prognosis and Correction of Iron Chlorosis in Peach Trees. J. Plant Nutr. 20: 1567-1572.

SAS Institute 1989. SAS=STAT User's Guide. Version 6,4th edition, Vol. 1 and 2. Statistical Analysis System Institute Inc.: Cary, NC.

Schmidt W. 1999. Review: mechanisms and regulation of reduction-based iron uptake in plants. New Phytol 141: 1-26.

Schaffer BA. Crane J. Evans E. Montas W. 2007. Foliar acid applications to prevent iron deficiency in tropical fruit crops growing in calcareous soils. Proceedings of the Floridaa State Horticultural Society 120: 198-204.

Tagliavini M. Scudellari D. Marangoni B. Tosell M. 1995. Acid-Spray regreening of kiwifruit leaves effected by lime-induced iron chlorosis. In iron nutrition in soils and plants, The Netherlands pp. 191-196.

Tagliavini M. Rombola D. Marangoni B. 1995. Response to Iron-defciency Stress of Pear and Quince Genotypes. J. Plant Nutr. 18: 2465-2482.

Tagliavini M. Abadia J. Rombola D. Abadia A. Tsipouridis C. Marangoni B. 2000. Agronomic Means for the Control of Iron Chlorosis in Deciduous Fruit Plants. J. Plant Nutr. 23: 2007-2022.

Tagliavini M. Masia A. Quartieri M. 1995. Bulk Soil and pH Rhizosphere of Peach Trees in Calcareous and Alkaline Soils as Affected by the Form of Nitrogen Fertilizers. Plant Soil. 176: 263-271.

Taiz L. Zeiger E. 1998. Mineral nutrition. In: Plant Physiology (2nd Edition). Sinauer Assoc., Inc., Publishers, Sunderland, MA. pp. 103-124.

Taiz L. Zeiger E. 1998. Assimilation of mineral nutrients. In: Plant Physiology (2nd Edition). Sinauer Assoc., Inc., Publishers, Sunderland MA. pp. 323-345.

Wallace A. Wallace GA. Cha JW. 1992. Some Modifcations in Trace Metal Toxicities and Defciencies in Plants Resulting from Interactions with other Elements and Chelating agents. The Special Case of Iron. J. Plant Nutr. 15: 1589-1598.

Wallace A. 1995. Agronomic and Horticultural Aspects of Iron and the Law of the Maximum. In Iron Nutrition in Soils and Plants, Abadia, J., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands pp. 207-216.