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A study on iron - chlorosis of *Citrus sinensis* (L) osb. (sweet oranges) grown in Bibile

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Abstract

Iron chlorosis is a well known agricultural problem associated with citrus plants. *Citrus sinensis* (sweet oranges) grown in Bibile showed the symptoms of iron chlorosis. Analysis of leaf samples of orange trees from two orchards showed that chlorosis is caused by a simple deficiency of iron in leaves. This deficiency is caused as a result of very low levels of soluble iron in the soil. Application of three chelating agents namely 1,2 diaminocyclohexanetetraacetic acid (CDTA), nitrilo acetic acid (NTA) and disodium salt of ethylenediamminetetraacetic acid (Na_2EDTA) to the soil under laboratory conditions resulted in an increased levels of soluble iron. CDTA was found to be more effective than the other two chelating agents in solubalization of insoluble forms of iron.

Key words : Iron chlorosis, chelating agents, soil.

1. Introduction

Iron chlorosis (chlorosis is a term used to describe marked deficiency of chlorophyll in plants) is widely recognized as one of the most important problems in plant nutrition that has plagued citrus growers in the world throughout the history of citrus growing (Walliham et al, 1954) This arises due to deficiency of iron in leaves and may be caused by lack of soluble iron in the soil. Versatile chelating agents such as polymino -polycarboxylic acids have been used with great success to keep iron in soluble form in the growth media thereby increasing its availability as a plant nutrient (Brown,1969)

Citrus sinensis (sweet oranges) grown in Bibile exhibited the symptoms of iron-chlorosis, Hence this study was undertaken to determine the extent of insolubility of iron in soil taken from two orange orchards followed by analysis of leaves to verify whether chlorosis is caused by a simple

deficiency of iron in leaves. In addition,the study was also extended to establish some effective chelating agents that would make iron soluble and cure iron deficiency.

2. Materials and methods

Study site

This study was conducted in Bibile. Two citrus orchards were selected for this study and were labeled as E_1 and E_2. Soil types and general locations of the two orchards are given in table1. Eight sweet orange trees, 5-8 feet in height, four trees from each orchard were selected randomly. These eight trees were labeled E_1P_1 to E_1P_4 and E_2 P_1 to E_2P_4

Table I. Soil types and general location of Bibile citrus orchards

Orchards	Soil group	Soil texture	Location
E_1	Reddish brown earth	Loam	Formerly Bibile group of Janatha Estate development Board, Bibile. (close to Bibile- Mahiyangana main road)
E_2	Reddish brown earth	Loam	Citrus Research Station, Department of Agriculture, Bibile. (close to Bibile- Badulla main road)

Sampling and analysis of leaves.

Leaves of similar growth size (to limit age variation) were collected from all eight trees (approximately 150 leaves from each tree) at a height of 3 to 6 feet all round from non fruiting terminals. All trees that were sampled exhibited symptoms of iron- chlorosis to some degree. Leaf samples from each tree were grouped according to the following classification based on leaf colour pattern as an index of chlorosis.

No chlorosis pattern	:	Veins and interveinal tissue green in colour
Moderate chlorosis pattern	:	interveinal tissues yellow or lighter green than veins.
Severe chlorosis pattern	:	All veins green but interveinal tissues light yellow.
Extreme chlorosis pattern	:	Green colour confined to midrib and main lateral veins or in some instances, pale yellow or white colour appeared exclusively.

Each leaf was thorughly washed with soap and tap water, and rinsed with distilled water. These leaves were dried at room temperature and then oven-dried at 55-60^{0}C for 24 hours. Dried leaf samples were ground in a stainless mill and then allowed to pass through a 0.15 mm stainless steel sieve and stored in a desiccator until chemical analysis could be performed.

A 1.0 g sample of dried and powdered leaves were weighed in to a 250 cm^3 conical flask and digested initally with 5 cm^3 of concentarted HNO_3 and thereafter with 5 cm^3 of 10 : 1: 4 mixture of concentrated HNO_3-H_2SO_4-$HClO_4$. The iron content of the digested samles were determined colorimetrically by forming iron (II)-phenanthroline complex (Jackson, 1973).

Sampling and analysis of soil

The soil from two orchards $E_1$and E_2 was used in this investigation. Soil samples were randomly collected from an area covered by a diameter of 120 cm circle around the tree at a depth of 0-15 cm and 37-45 cm. These random samples were then used to prepare two composite samples labeled as A and B from each orange tree at the aforementioned soil depths of 0-15 and 37-45 cm respectively. Soil samples composed of 15-20 soil cores which were collected using a screw type soil auger. Total of eight composite samples were taken from each orchard (E_1 and E_2). There were labeled E_1P_nA to E_1P_nB (n=1-4) and E_2P_nA to E_2P_nB (n=1-4). Each composite sample was spread out on a thick sheet of paper and removed small stones and undecomposed organic matter. Large soil aggregates were then broken up by hand and then ground by rolling gently with a wooden roller. These samples were then air dried for 24 hours and subsequently subjected to grinding sieving and quatering (Jackson,1973).

The total iron determination was carried out using 1.00 g of oven dried soil from each soil sample. A 1.00 g of sample of oven dried soil was weighed into a 250 cm^3 conical flack and digested with 20 cm^3 of concentrated HNO_3 and thereafter with 10 cm^3 of 60% $HClO_4$.The total iron content of the digested samples and soluble iron content in each soil (by extracting with ammonium acetate solution of pH=3.0) were also determined colorimetrically by forming iron(11)-phenanthroline complex.

Treatment with chelating agents

In order to determine the ability of chelating agents to convert insoluble iron to the soluble form, one representative soil sample from one orange orchard was treated with different chelating agents. The soil used was

a mixture of equal parts of top soil (E_2P_1A)and sub soil (E_2P_1B) from orchard E-2 in which orange trees were showing symptoms of iron- chlorosis. In this investigation 2.5 g 0.15 mm air-dried soil was used. Three cheating agents agents were employed in this study. There were Na_2EDTA (disodium salt of ethylenediaminetetraacetic acid),CDTA (1,2-diaminocycloh-exaneteraacetic acid), and NTA (Nitriotriacetic acid) Soil samples of 2.5 g each were placed in 13 beakers and were treated with the above mentioned chelating agents in the following manner.

1. T_1EDTA+distilled water
2. T_2EDTA+ "
3. T_3EDTA+ "
4. T_4EDTA+ "
5. T_1CDTA+ "
6. T_2CDTA+ "
7. T_3CDTA+ "
8. T_4CDTA+distilled water
9. T_1NTA+ "
10. T_2NTA+ "
11. T_3NTA+ "
12. T_4NTA+ "
13. Distilled water only

The levels of chelating agent treatment denoted T_1, T_2, T_3, and T_4 were selected as shown in table II. After applying chelating agents to the soil, they were thoroughly mixed with soil. These were then treated with 1 cm^3 of distilled water to bring the air-dried soil approximately to its moisture equivalent. The beakers were then sealed and stored in the dark at room temperature until ready for analysis The analysis was first carried after 24 hours by transferring each soil sample to a conical flask containing 15 cm^3 distilled water. The conical flask was shaken mechanically for 30 minutes.After shaking, the entire mass in the flask was transferred to a centrifuge tube with 25 cm^3 distilled water and the centrifuged (2500 rpm) for 15 minutes. The supernatant liquid was transferred into a 250 cm^3 volumetric flask and iron (III)-chelate concentration was determined by measuring the absorbance at 260 nm (Hill-cottingham, 1957).The treatmined of soil with distilled water only and subsequently extracted in the same manner as those treated with chelating agents was used as the the blank during spectrophotometric determination of iron-chelates.

Table II: Levels of chelating agent treatment

Levels of chelating agent treatment	Amount of total iron present in the 2. 5 g air dried soil (mol)	Amount of chelating agent added to the soil (mol)
T_1	1	1
T_2	1	2
T_3	1	3
T_4	1	4

*Each treatment was done in tripicate

Determination of soil pH

To a 10 g sample of soil added 25.0 cm^3 of double distilled water. The suspension was stirred at regular intervals for a period of 20 minutes. The pH of the soil water suspension was measured using a pH meter. The pH of each soil sample was measured in duplicate. The pH of citrus orchards E_1 and E_2 were 5.5 (8 samples) and5.6 (8 samples) respectively.

3. Results

Field observations indicated that almost all orange trees which were growing in both orchards showed symptoms of iron- chlorosis. The data on iron concentration of leaves of each orange tree under study as related to leaf colour pattern are given in table III and IV.

The data on total iron content and soluble iron content in the soil are presented in table Vand VI. The total iron content in both orchards were found to be high and remains virtually constant within the experimental error. Comparison of results in tables Vand VI indicates that the iron content in soil exceeds far greater than those of soluble iron (plant available iron). The value for the ratio of total iron/ soluble iron (~350) in both orchards suggest that iron present in both orchards is highly unavailable to the plants.

The behaviour of the three chelating agents was studied using using the soils from orchards E_2, soil pH=5.6 total iron content of 3.351 percent (33510 ppm) and available iron content of 0.000913 percent (91.3 ppm). The results of this investigation are given in tables VII to IX, Solubalization of iron in the presence of chelating agents is calculated as percentage recovery that is defined as.

$$\frac{\text{Solubalization of iron in the presence of chelating agent}}{\text{Insoluble iron in the soil}} \times 100$$

Measurements were made at various time intervals over a period of 90 days. The results show that the application of chelating agents increase in soluble iron content in the soil with CDTA showing greater dissolution compared to NTA and Na_2 EDTA The maximum recovery from CDTA was obtained at 90 days with treatment level T_4.

4. Discussion

Within the range of leaf samples analysed it is evident that there exist an inverse correlation between the iron content in the leaves and the degree of chlorosis.This supports the fact that chlorosis observed in orange leaves is primarily due to the simple deficiency of iron. This deficiency could be attributed to the non- availability of soluble iron in the soil.

When soils are treated with chelating agents, these form water soluble complexes with various metal cations present in the soil. The use of metal chelates as a nutritional source depends upon their stability in the soil. The stability of metal chelates depends upon several factors such as pH, nature of the metal iron and chelate , adsorption by soil particles (clay fixation) and metabolic decomposition ligands and other soil properties.

When the chelating agents CDTA, NTA and Na_2EDTA are added to soil they combine with iron to form iron (III)- chelates. The total amount of iron chelate formed,however, will not be present in the soil solution due to the subsequent adsorption of iron chelate molecules on clay particles. The adsorption of iron-chelate on soil is dependent on its clay content, the type of clay present and pH of the soil suspension. Pervious work on metal chelate reactions in soil have shown that loss of iron-chelates from soil solution results primarily from its adsorption by clay particles (Lunt et al, 1956; Hessr, 1977). As can be seen from tables VII to IX the variation of percentage recoveries of soluble iron with time may be attributed to the changes in percentage fixation of iron chelate in soil particles with time.

CDTA was found to be relatively more effective than the other two chelating agents to bring about solubalization of insoluble form of iron in soil. This can be attributed to (i) the high stability constant and (ii) the low clay sorption of Fe-CDTA complex, the latter being due to the steric hindrance of the cyclohexane ring in the Fe-CDTA molecule.

Since treatment of soil with the three cheating agent resulted in an low increase of soluble iron content, further field work is necessary to establish whether iron-chelates would offer much hope for curing iron deficiency under Bibile soil condition.

5. Conclusions

Orange trees grown in the two orchards of Bibile showed deficiency of iron in their leaves. This may be caused by the non-availability of soluble iron in the soil.Chelating agents are good reagents to solubalize iron in soil and of the three chelating agents used CDTA is more effective.

6. Acknowledgements.

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Table III. Iron concentration of orange leaves collected from orchard E_1.(mean of triplicate analysis,expressed on dry eight basis in ppm. Coefficient of variation =2-3%)

Tree	Chlorosis pattern None Concentration range	Fe content	Moderate Concentration range	Fe content	Severe Concentration range	Fe content	Extreme Concentration range	Fe content
E_1P_1		120.0		60.0		46.0		29.0
E_1P_2	120-244	160.0	55-65	57.0	34-50	50.0	15-29	24.5
E_1P_3		154.0		55.0		34.0		20.0
E_1P_4		244.0		65.0		35.0		15.0

Table IV. Iron concentration of oranges leaves collecected from orchard E_2 (Mean of triplicate analysis, expressed on dry weight basis in ppm Coefficient of variation= 2-4%)

Tree	Chlorosis pattern None Concentration range	Fe conternt	Moderate Concentration range	Fe content	Severe Concentration range	Fe content	Extreme Concentration range	Fe content
E_2P_1		90.0		56.5		45.5		14.0
E_2P_2	90-200	140	45-63	63.0	29-47.5	35.5	17.5-26	21.0
E_2P_3		185		45.0		29.0		17.5
E_2P_4		200		52.0		47.5		26.0

Table V. Total iron content of studied soils

Citrus orchard	Soil Sample	Total iron[a] (%)
E_1	E_1P_1A	3.35
	E_1P_1B	3.35
	E1P_2A	3.35
	E_1P_2B	3.35
	E_1P_3A	3.35
	E_1P_3B	3.35
	E_1P_4A	3.35
	E_1P_4B	3.35
E_2	E_2P_1A	3.36
	E_2P_1B	3.36
	E_2P_2A	3.36
	E_2P_2B	3.36
	E_2P_3A	3.36
	E_2P_3B	3.36
	E_2P_4A	3.36
	E_2P_4B	3.36

a-mean of duplicate analysis (coefficient of variation, 1%)

Table VI. Plant available iron content of studied soils

Citrus orchard	Soil Sample	Iron content[a] (ppm)
E_1	E_1P_1A	110.5
	E_1P_1B	110.5
	E_1P_2A	108.0
	E_1P_2B	108.1
	E_1P_3A	100.5
	E_1P_3B	100.4
	E_1P_4A	112.0
	E_1P_4B	112.0
E_2	E_2P_1A	91.3
	E_2P_1B	91.3
	E_2P_2A	96.5
	E_2P_2B	99.0
	E_2P_3A	97.0
	E_2P_3B	97.1
	E_2P_4A	98.0
	E_2P_4B	98.1

a - mean of triplicate analysis (coefficient of variation, 1-2%)

Table VII. Percentage recovery of soluble iron with EDTA as the chelating agent. (mean of 3 replicates)

Iron Chelate	Time of contact (in days)	Treatment levels of the chelating agent			
		T_1	T_2	T_3	T_4
		RECOVERY %			
Fe- EDTA	0	0.00	0.00	0.00	0.00
	1	0.90	0.90	0.90	0.66
	30	1.80	1.47	0.96	0.72
	60	1.38	1.00	1.20	0.60
	70	1.75	1.50	1.50	0.75
	90	1.62	1.80	1.69	1.75

Table VIII. Percentage recovery of soluble iron with CDTA as the chelating agent. (mean of 3 replicates)

Iron Chelate	Timeof contact (in days)	Treatment levels of the chelating agent			
		T_1	T_2	T_3	T_4
		RECOVERY %			
Fe - CDTA	0	0.00	0.00	0.00	0.00
	1	2.27	1.96	1.00	2.00
	30	2.65	1.75	2.00	2.50
	60	1.98	1.65	2.25	4.00
	70	3.65	3.00	4.00	4.50
	90	5.52	4.44	5.97	6.50

Table IX. Percentage recovery of soluble iron with NTA as the chelating agent. (mean of 3 replicates)

Iron Chelate	Time of contact (in days)	Treatment levels of the chelating agent			
		T_1	T_2	T_3	T_4
		RECOVERY %			
Fe - NTA	0	0.00	0.00	0.00	0.00
	1	0.23	0.27	0.27	0.48
	30	1.44	1.44	1.44	1.44
	60	1.55	1.25	1.81	1.90
	70	1.40	1.60	1.50	2.50
	90	4.02	3.90	3.92	4.20