No.

REPORT ON THE RESEARCH WORKS AT CITRUS AND VEGETABLE SEED RESEARCH CENTRE, DHAKA

II

JANUARY, 1984

JAPAN INTERNATIONAL COOPERATION AGENCY

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INTRODUCTION

The Citrus and Vegetable Seed Research Project in Bangladesh carried out since 1977 as a technical cooperation project between the government of Japan and the government of Bangladesh for the purpose of increasing citrus and vegetable production in Bangladesh through the improvement of techniques was terminated in November 1983 with an understanding of the extension of the cooperation for another five months.

As regards the results of research, the vegetable section has developed quite a number of promising varieties.. The citrus section, which generally requires time consuming research, has succeeded in finding out an improved method of grafting, collecting suitable varieties in and out of Bangladesh, and recommending a way of controling some serious local diseases.

The performance of the project is highly evaluated by both of the Bangladesh and Japanese governments.

This report has been compiled from five papers, and covers the research made on citrus, vegetable and nutritional aspects of these crops by both the Japanese and Bangladesh experts.

It is hoped that this report will prove to be useful to as many researchers concerned as possible.

January 1984

Mr. Takashi TAUCHI
Director, Agricultural Development
Cooperation Department,
Japan International Cooperation
Agency.

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Dr. Hiroshi SAKAI
Team Leader of Japanese Experts at Citrus
and Vegetable Seed Research Project.

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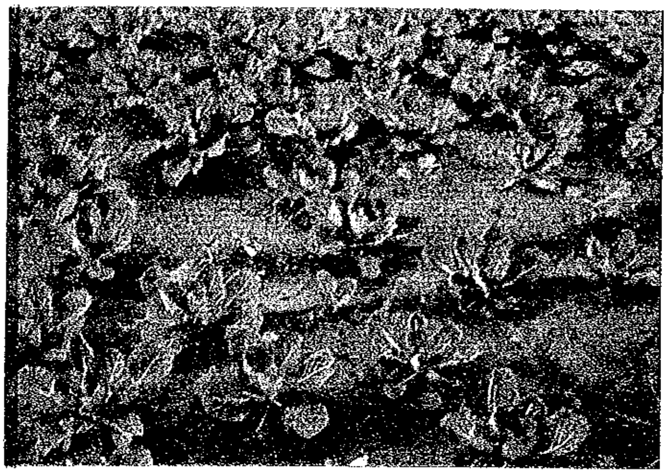
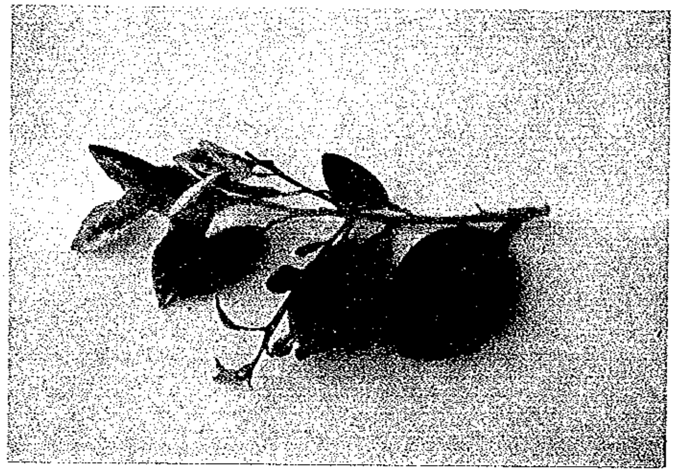


Molybdemun deficient
cabbage (1)

Molybdenum deficient
cabbage (2)

Zinc deficient citrus twig



Zinc deficiency affected
citrus tree (greening
disease also)

Wet damaged citrus tree

Introduction

People are very proud of golden land of Bnagladesh. Green leaves of rice widely cover the country in monsoon season. It is true that Bangladesh soil is considerably enriched with mineral elements such as calcium, magnesium etc. Rice is a very economical crop in Bangladesh. It can efficiently utilize nutrients from soil and water, even biologically fixed atmospheric nitrogen.

Now the scientists know that there are widely spread nutritional disorders on crops, mostly deficiencies of various nutritional elements derived from low soil fertility and poor cultural management in Bangladesh. As well known in these days, even rice, especially with high productivity is widely affected by the shortage of zinc and sulphur.

Regarding fruit trees and vegetables, there is almost no report available on their nutritional disorders. It is due to lack of advanced scietific researches. Fruit tress are grown as perennial crops in the same places for quite long years. It is very natural that their nutritional status becomes poor in improper fertilizer management. This trend is much influenced by soil factors of planted places.

Vegetables also have the similar problems, sometimes much more serious, because they are grown intensively in the same fields every season. Severe shortage of particular essential elements surely occurs, especially minor elements which are short in soil, unless they are not supplied as fertilizers. Heavy application of popular chemical fertilizers only stimulates their occurence.

Now CVSRC has been increasingly requested proper diagnosis for growth disorders, especially of vegetables from the outsides. Significant diagnosis results obtained so far on the real causes of those disorders are decribed below.

For preliminary nutrition diagnosis, the sicentists should first know about typical symptoms of deficiencies and toxicities of particular crops and soil problems of the fields where growth disorders occur. However, their mere knowledge is not useful. Their broader experience is very much needed. It is an important advice that imagination and speculation on their causes are never of use. No progress can be expected from them. Their confirmation by scientific means is the first step towards giving proper guidance to extension workers as well as farmers.

The application of particular elements in question by soil application or folia spray is one of useful means. But, because of improper application method and growth stage of application, their response is often not clear to get right diagnosis. Yield response to the application also is an important measure. But, we must know that for yield trial efforts for uniform and precise management of experimental plots are much more important than that of statistical analysis to avoid wrong conclusions. Otherwise, the trials should be repeated several seasons with wastful use of money and labour.

Therefore, chemical analysis for particular elements is the most speedy and reliable diagnostic means.

In many cases critical levels of essential elements for normal growth are unknown for particular crop plants in Bangladesh. In addition to referring to the books and publications from foreign countries, I wish to advise to send the sample plants of poor growth together with the control plants of better growth for chemical analysis, Under this condition, more precise diagnosis can be practiced in comparison with the nutritional element levels of good growth plants. In other words, particular elements which have the the highest gap between the sample plant and the control plant may be diagnosed to be growth limiting factors, together considering the results of their growth and symptom observations. The following results were obtained using CERDI analytical equipments.

1. Molybdenum deficiency in cabbage and cauliflower

Continuous and intensive cultivation of vegetable crops with heavy application of chemical fertilizers creates new deficiencies of other essential elements in low fertile soils, unless organic fertilizers such as oil cakes, compost and cowdung are not applied properly. Now cabbage, radish and some other vegetable crops are found to be seriously affected by unknown physiological disorders in the well-known vegetable growing areas. They have been more serious in very recent years.

The samples of affected cabbage plants found this season were brought for diagnosis to CVSRC, BARI by CARE, Kashimpur officers on December 8, 1982. I observed their growth status carefully and detected specific symptoms of molybdenum deficiency in terms of interveinal chlorosis and cup-shape young leaves. We also found strongly acidic soil reaction as well as very high nitrate accumulation in leaves of affected plants as seen in Table 1. Both of them are important criteria for identifying molybdenum deficiency of affected crops. Molybdenum is an essential component of nitrate reductase enzyme which reduces uptaken nitrate to ammonium form to be used for protein production in plant body. Strongly acidic soil reaction reduces the solubility of molybdenum to a higher extent.

Table 1. Soil pH of the field and nitrate and molybdenum contents in leaf of affected cabbage

Location	Soil pH	Growth status	Nitrate-nitrogen on air-dried basis		Mo
			Outer leaf ppm	Inner leaf ppm	Outer leaf ppm
Kashimpur 1	5.0	Affected	12,920	5,120	0.43
2	5.4	Affected	5,840		0.65
3	5.2	Affected	6,750		0.60
		Healthy	540		1.30
Savar	5.2	Affected	6,860	3,000	-

A folia spray of 0.03% ammonium molybdate solution was given to one of the affected fields to observe its positive effect on December 12, 1982 (S. Tasaki, Vegetable expert). All newly developed leaves of cabbage plants once affected were found to be free from the deficiency symptoms on 9th day after the spraying. Therefore, real molybdenum deficiency on those cabbage plants has been confirmed.

However, at this moment I don't know about the real cause of bright yellow, spotty appearance on affected cabbage leaf, as seen in the pictures, which is still under intensive study.

Now we wish to make the following recommendations to control molybdenum deficiency of vegetables in their intensive growing areas.

1) Grow vegetables in rotations with other kinds of crops under the condition where organic fertilizers are applied in a large quantity. Donot apply heavy amount of chemical fertilizers of acidic reaction.
2) Apply lime as well as ashes, if available.
3) Reduce basal rate of urea. Its heavy basal application causes very high accumulation of nitrate in soil. It only reduces soil pH but also forcively increases its uptake and keep very high concentration in small young plant body even for a short period. Topdress urea more often in the affected soil, as the growing plants require.
4) In phosphate deficient soil, proper application of phosphatic fertilizer increases the uptake of molybdenum from soil. Quick soil test of available phosphate is very helpful for this purpose as well as proper recommendation of TSP application for other crops. It helps increase farmers' profit and also saves very valuable resources for national economy, though the test itself is very simple.
5) For the presently affected crops, give a couple of folia spray of 0.03% solution of ammonium molybdate at the rate of 100 litres per ha.

2. Effect of high level of zinc in soil on bell pepper

I myself also tried to grow bell pepper in our kitchen garden several times until now, but I have never been successful in its fruit bearing. They say that there have been quite a few people who harvested good fruits so far in their kitchen gardens in Dhaka. What is the real cause for this interesting fact? I have tested all means which I knew to be possibly effective for improving plant nutritional status, but they were all in vain. In the meantime I happened to find out that these garden soils were heavily contaminated with zinc due to continuous sprinkling of tap water supplied through galvanized iron pipes. Bell pepper is likely to be not so tolerant to zinc toxicity.

Quite recently some bell pepper plants have failed to bear normal fruits in the Kashimpur BADC model farm. I am now requested by the CARE/Kashimpur officers to investigate their real cause along with my own interest in this problem.

In the Kashimpur farm, there were remarkable differences in growth status and fruit production between good and poor plants. The former had dark green colour, while the latter had lighter and somewhat rusty colour. As shown in Table 2., the plant analytical results show that both kinds of plants have not so different nutritional condition. The good plant, however, has slightly higher zinc content, say 77ppm which was previously thought to be a harmful level. On the other hand, it must be noticed that the poor plant has clearly higher iron content, say about 800ppm. This result may predict possible occurence of root injury. It deserves further intensive investigation.

Very high level of zinc in the plant was already detected as one of important causes which may give toxic effect on plant growth in our kitchen garden. Therefore, more detailed works including chemical analysis of other elements were done, as shown in Tab. 3. When sampled on February 11, the buds already started to fall. Some of them showed abnormal flowering. Zinc content was not very high, while iron content was very high, say over 1000ppm. When sampled on March 18, a few small fruits were developing. This was a trend against the previous result. In other words, iron content was much lower, but zinc content was much higher than those of the previous result. The plant which produced no fruit only had very high zinc content, say 136ppm. The result indicates that iron rather than zinc has more harmful effect on fruit bearing just like in Kashimpur bell pepper. However, in case of very high level of zinc any fruit cannot be produced, too.

Much clearer result on fruit bearing was observed in Mr. S. Tasaki's kitchen garden. One plant growing among dahlia plants had slender but normal growth and one big fruit. Its zinc content was fairly as high as 81ppm, but iron content was lower than 500ppm as also found in Kashimpur good plant. The other three plants having no fruit had much higher iron and zinc content. Among them the plant which showed stunt growth as well as yellowish leaf colour had 116ppm zinc and 960ppm iron. It also had highest phosphate and lowest sulfur content, indicating suspected trend of soil reduction in its root zone (Table 4).

From the foregoing studies, I notice that chemical analysis of root is now very essential. It is said that heavy metal elements usually accumulate more in root tissue. Accordingly, the separate parts of entire plant grown in our garden and also affected seriously were analyzed. As shown in Tab. 4., extraordinarily high accumulation of zinc, say 442ppm was detected in the root tissue. It is followed by side-branch and their small leaves, leaves on the main stem and young top growth in order. Iron content also showed the same trend in different parts of the plant. Manganese content was highest in main stem leaf, as followed by side-branch, root and top growth. However, it must be kept in mind that root tissue is likely to have extra iron due to soil contamination, because its perfect removal is naturally impossible. The above findings clearly indicate that root tissue should be sampled for the studies on effect of zinc accumulation which might cause root injury of bell pepper.

The plant samples were prepared in zinc contaminated soil in pots. As shown in Tab. 6., fruit-bearing normal plant had fairly high zinc content such as 87.2 and 129ppm zinc in top growth and root, respectively. However, iron content in top growth was below 300ppm, while that of the slightly affected plant was above 500ppm. Therefore, it is likely to conclude that about 500ppm iron may be critical level for normal growth as also seen in Kashimpur good plant. Generally zinc content of top growth is about 60% or a little higher of that of root including fine ones.

In the pot culture with zinc-contaminated soil, phosphatic fertilizer (TSP) application enables bell pepper to develop flowers and at the same time clearly reduces zinc content in thick root, regardless of soluble zinc content in soil. The root samples in low soluble zinc soil has averaged zinc content of 73.7ppm ranging from 58.0 to 97.5 ppm (control), while those in high soluble zinc soil has averaged zinc content of 102.6ppm ranging from 65.6 to 131.1ppm. As a result, about 100ppm zinc in thick root might be considered to be the critical level for normal flower development of bell pepper (Tab. 7).

Table 2. Nutritional status of bell pepper in Demonstration Farm, ADE Kashimpur, BADC (young top growth)

Sampled plant	Soil pH	N %	P %	K %	S %	N/S	Fe ppm	Mn ppm	Zn ppm	Cu ppm
Good plant	6.9	3.21	0.65	3.20	0.385	8.3	562	138	77.1	7.37
Poor plant	6.3	2.75	0.62	3.33	0.243	11.3	799	155	71.4	6.84

Used variety: California Wonder, sampled on February 7, 1983

Table 3. Nutritional status of bell pepper in Dr. H. Sakai's kitchen garden (young top growth)

a) Sampled on February 11, 1983

Sampled plant	P %	S %	Fe ppm	Mn ppm	Zn ppm
Small buds, no flower	0.253	0.584	1054	54.4	48.1
Fairly large buds, just fell	0.442	0.521	1139	56.1	62.3
All buds fell	0.416	0.503	621	52.6	51.9

b) Sampled on March 18, 1983

Sampled plant	P %	S %	Fe ppm	Mn ppm	Zn ppm
2 small fruits	0.350	0.521	784	78.3	85.9
Fruits just bearing	0.319	0.371	897	53.3	82.3
No fruit, slightly chlorotic	0.493	0.660	885	88.4	136.0

Table 4. Nutritional status of bell pepper in Mr. S. Tasaki's kitchen garden (young top growth)

Sampled plant	P %	S %	Fe ppm	Mn ppm	Zn ppm
Normal with one big fruit	0.528	0.845	494	53.2	81.1
Slightly affected	0.684	0.757	773	63.8	83.8
Fairly affected (virus infested ?)	0.757	0.775	796	60.3	89.2
Clearly chlorotic	0.780	0.549	960	47.9	116.2

Table 5. Nutritional status of different organs of affected bell pepper (Dr. H. Sakai's kitchen garden, sampled on March 25, 1983)

Plant part	Length cm	Weight g	N %	P %	S %	Fe ppm	Mn ppm	Zn ppm
Young top growth (small branch and leaf)		0.575	3.96	0.237	0.565	736	82.0	138.7
Main stem leaf		0.610		0.168	0.580	1450	112.7	169.4
Side branch and small leaf		0.272		0.400	0.623	2058	108.6	172.6
Branch (5 nodes)	8.0	0.184		0.294	0.628	713	41.0	87.7
Stem (10 nodes)	16.5	0.614		0.126	0.583	752	32.8	50.0
Underground stem	8.8	0.463		0.107	0.351	1000	38.9	61.5
Root	12.7	0.785		0.116	0.469	3067	86.1	441.9

Table 6. Relation in metal element content of bell pepper between young top growth and root (pot culture with zinc-contaminated soil, CVSRC/BARI)

Sampled plant	Young top growth Fe ppm	Mn ppm	Zn(a) ppm	Root Fe ppm	Mn ppm	Zn(b) ppm	a/b
Fruit bearing	282.9	313.6	87.2	(3486)	257.1	129.7	0.67
Slight wilting	526.3	343.2	90.4	(3826)	226.0	152.5	0.59
Severe wilting	1234.0	317.8	111.2	(4028)	339.7	190.4	0.58

Planted on February 17 and sampled on April 14, 1983

Table 7. Relation between zinc content of thick root and flower production of bell pepper (pot culture with zinc-contaminated soil, CVSRC/BARI)

Soil treatment	Heavily contaminated soil		Lightly contaminated soil	
	No. of flower	Zinc content of root ppm	No. of flower	Zinc content of root ppm
TSP, lime, straw	10.3	91.2	7.7	71.6
Lime, straw	0	119.3	5.5	81.5
TSP, straw	7.3	83.8	11.7	66.7
TSP, lime	9.0	77.9	8.3	61.7
Straw	1.0	131.1	9.5	-
Lime	0	120.6	7.0	79.0
TSP	8.0	65.6	9.5	58.0
Control	0	130.9	12.5	97.5

Planted on February 17 and sampled on March 25, 1983

3. Iron Level of plant leaf for the useful diagnosis of root injury

Root system has close contact with soil which supplys water and nutrients as cultural medium. It plays a very important key role for sound crop growth. However, its activity is sensitively affected by various kinds of natural and cultural conditions.

Neverthless, reliable observation of intact root system is very limited, though much required. Entire root system of fruit tree such as citrus can hardly be observed precisely, even though a small part of large root zone is digged out, and some root sample is taken for close observation of root activity.

I once made quite a pioneering contribution to the development of root activity studies by proposing a determinative method for root activity diagnosis of rice plant with a-Naphthylamine oxidation (1957). I also proposed the use of manganese to iron ratio in the upper leaf of rice shoot (straw) usually free from soil contamination in evaluating integrated root activity during growing period even after harvest and as a result finding out important growth limiting factors for further improvement of cultural practices (1966).

Both iron and manganese become well soluble in paddy soil. In most cases their content in the upper leaf is on the same level. In other words, the ratio is around 1.0. However, when the root is injured, it comes downwards below 0.1, while it increases above 10 in healthy plant in successful high yielding rice culture in Japan. That is because increased iron uptake has passive nature, so it rises through injured root system. On the other hand, manganese uptake has active nature with metabolic process of root respiration, so healthy root stimulates its uptake.

While working in Bangladesh, my major interest has been placed on plant nutrition diagnosis for finding out important growth limiting factors hampering normal crop growth essential for higher yield production. Unitl recently the results of plant chemical analysis have been used for giving adquate diagnosis of particular nutrient deficiencies and toxicities. However, in the course of my work, I experienced several cases which did not allow me to give a decisive cause of poor crop growth sample sent to me, because the sampled plant was in almost normal nutritional status.

I am now quite confident for presenting further useful guidance in detecting their real cause by applying the full use of crop chemical analysis including iron and manganese in the upper leaf of any kind of crops.

Wider availability of the chemical analysis has been convinced in various kinds of results as mentioned below.

Analytical results of various kinds of crops except rice

Wheat shoot

As seen in Table 8., INIA variety had clearly lower iron content than PABON variety, but Mn/Fe ratio had not clear difference between them. The former which grew better may have higher tolerance against wet damage. Besides, in AETI field wheat also was likely to be affected by zinc deficiency.

In the farmer's field the poor growth plant had generally better nutritional status except copper than the good growth plant. However, the former had extremely high iron content, definitely indicating wet damage due to over-irrigation.

Potato leaf

As seen in Table 9., both nitrogen and phosphorus were lower, but sulfur was higher in the affected plant than in the healthy one. The former had extremely high level of iron and at the same time severely decayed root system as compared with the latter. This fact indicates harmful effect of over-irrigation. It is quite natural that uptake of nitrogen and phosphorus was retarded under the adverse condition.

Radish leaf (a)

As seen in Table 10., both samples had farily high content of phosphorus, potassium and magnesium. It is also noticed that generally sulfur was relatively low, but zinc was very high. In comparison with both samples it is clear that extremely high level of iron may indicate root injury which was caused by wet damage. In fact the root top was observed to have some initial decay.

Radish leaf (b)

As seen in Table 11., the leaf sample had fairly high content of all kinds of nutrient elements. It shows no problem related to soil fertility. However, iron content was found to be extremely high. This fact is a very important evidence for the outbreak of root injury. It was later confirmed to occur not due to wet damage, but due to soil application of herbicide in this particular plot before transplanting for seed production.

Eggplant leaf

As seen in Table 12., in between-rows irrigation, plant growth became extraordinarily poor in low fertilizer as compared with that in high fertilizer at the later stage. Very high iron content was also found in the upper leaf sample in the former. It is, therefore, considered that low fertilizer reduced not only nutrient level in the plant, but also root tolerance against wet damage derived from over-irrigation. Special care is inevitable for avoiding over-irrigation in low fertilizer cultivation which is quite popular in Bangladesh.

Bell pepper leaf

As seen in Table 13., nitrogen, phosphorus, manganese, zinc, copper stc. were on the same level in both fruit bearing and non bearing plants. Iron and manganese were in higher status in the latter than in the former, while sulfur was in lower status. As bell pepper root is considered to be sensitive to over-irrigation, major cause for poor growth of bearing no fruit seems to be wet damage which was stimulated by both lower soil pH and high soluble zinc in the soil.

Grape leaf

As seen in Table 14., nitrogen, phosphorus, magnesium and manganese were lower, but potassium, iron, zinc and copper were higher in the affected leaf than in the healthy one. A very clear difference was found only in iron content which was extremely higher in the affected leaf. It indicates wet damage on particular trees. They were actually observed growing in the low level field which was sometimes flooded by heavy rain.

Citrus leaf

As seen in Table 15., iron content was clearly higher in the upper leaf samples which were quite yellowish and disease-infested on citrus trees after rainy season. Almost similar leaf samples also were collected from the pot trees which were kept during the same period under heavy artificial shading. This trend is considered to be derived from partial root injury.

Ordinary crops which grow under upland condition have relatively lower oxidizing power of root system, so it is easily damaged under water logged condition even for a short period of time. Once root injury occurs, iron becomes soluble in the rhizosphere, and as a result iron uptake springs up and increases its content in the upper developing leaf. This kind of trend is not applicable to manganese. Therefore, except for rice, the analysis of Mn/Fe ratio is not so important.

Generally, iron content is found fairly high, say 500ppm even in healthy leaf of plant except citrus. However, it may be considered that this level of iron content in particular tissue is not so harmful for normal metabolism, because no distinct growth retarding effect was observed in those cases. I am interested only in high iron level for diagnostic purpose. It is a very important fact that growth affected plant always have clearly higher iron level than better growth plant, though there was much less difference in other nutrient element between them.

In conclusion, the iron level of plant tissue, mostly upper leaf may be considered to be a reliable basis for diagnosing root injury.

Table 8. Analytical results of wheat shoot

(Nator AETI, Feb. 13, 1982)

Sampled plant	P %	S %	Fe ppm	Mn ppm	Zn ppm	Cu ppm	Mn/Fe
PABON less adaptive							
Sown Dec. 15	0.40	0.24	563	52.8	18.3	26.2	0.094
Sown Dec. 25	0.49	0.27	696	54.3	17.5	29.3	0.078
INIA more adaptive							
Sown Dec. 15	0.34	0.26	442	41.9	15.8	25.3	0.095
Farmer's field Good	0.29	0.20	511	74.5	26.7	27.6	0.146
Poor	0.33	0.20	1300	62.3	35.8	26.2	0.063

Table 9. Analytical results of potato leaf

(CERDI, Feb. 6, 1981)

Sampled plant	N %	P %	S %	N/S	Fe ppm	Mn ppm	Mn/Fe
Normal growth	4.95	0.37	0.157	31.5	245	52.0	0.212
Poor growth	2.42	0.29	0.189	12.8	1000	95.3	0.095

Table 10. Analytical results of radish leaf (a)

(CARE/Kashimpur, Nov. 10, 1982)

Sampled plant	P %	K %	S %	Mg %	Fe ppm	Mn ppm	Zn ppm	Mn/Fe
Good growth	0.30	2.36	0.17	1.71	553	170	75	0.307
Affected	0.38	1.47	0.11	1.29	2190	210	75	0.096

Table 11. Analytical results of radish leaf after planting for seed production (b)

(CVSRC/BARI, Feb. 4, 1981)

N %	P %	S %	N/S	Mg %	Fe ppm	Mn ppm	Zn ppm	Cu ppm	Mn/Fe
5.61	0.76	0.567	9.9	0.826	1964	96.2	137	25.6	0.049

Soil pH 6.57, EC 0.25 mυ/cm, SO_4-S 8.33ppm, 0.05N HCl soluble zinc 2.17ppm

Table 12. Analytical results of eggplant grown at different fertilizer levels.

(CVSRC/BARI, Nov. 30, 1982)

Variey	Mn ppm			Fe ppm			Zn ppm			Cu ppm		
	High	Medium	Low	High	Medium	Low	High	Medium	Low	High	Medium	Low
Rajishahi No. 3	120	84	62	616	846	1316	56	56	68	16	16	17
5	208	66	82	440	1240	2400	63	68	75	18	17	16
8	172	70	78	656	1000	1520	56	68	65	15	18	15
9	234	94	88	480	1990	2360	74	76	65	17	16	15
Khot Khotia long	198	94	24	716	666	460	65	61	50	14	-	17
Pusa Purple Long	166	78	98	500	880	1536	63	59	69	15	18	17
Pusa Kanti	208	72	82	846	930	2190	63	71	70	15	16	19
Longla	176	66	66	450	2600	1008	65	63	51	14	13	17
Banger	214	86	72	594	980	2340	65	51	74	13	16	17
Hon Naga Nasu	142	84	20	616	688	364	83	78	68	15	15	16
Kurume Naga Nasu	214	94	56	780	584	676	70	79	81	15	-	18
Taiwan Naga Nasu	176	72	56	620	604	2340	-	67	71	-	18	19
(Average)	186	80	65	610	1084	1543	66	66	67	15	16	17

Analysed with Mr. Y. Suzuki, short-term soil and fertizer expert

Table 13. Analytical results of bell pepper (California Wonder)

(CARE/Kashimpur, Feb. 7, 1983)

Sampled plant	Soil pH	N %	P %	S %	N/S	Fe ppm	Mn ppm	Zn ppm	Cu ppm	Mn/Fe
Fruit bearing	6.9	3.21	0.651	0.385	8.34	562	138	72.1	7.37	0.246
No bearing	6.3	2.75	0.616	0.243	11.3	799	155	71.4	6.84	0.194

Table 14. Analytical results on grape leaf

(Grower's field, Hyderbad, India, July 1971)

Sampled plant	N %	P %	K %	Mg %	Fe ppm	Mn ppm	Zn ppm	Cu ppm	Mn/Fe
Healthy	2.68	0.457	0.96	0.32	562	44.4	27.4	9.6	0.079
Severely affected (brown, dry tip)	2.13	0.303	1.43	0.29	1302	40.6	29.5	14.3	0.031

Table 15. Analytical results on citrus leaf

(CVSRC/BARI, Oct. 24, 1982)

Sampled plant		N %	S %	N/S	Fe ppm	Mn ppm	Zn ppm	Cu ppm	Mn/Fe
Well drained field	OKITSU-WASE	2.75	0.172	15.7	137	29.4	34.2	6.90	0.215
	KOBAYASHI-MIKAN	1.82	0.114	16.0	199	27.6	45.8	9.66	0.139
	SAMPOKAN	2.30	0.125	18.4	170	12.1	69.4	-	0.071
Poorly drained field	PUMMELO	1.90	0.131	14.5	375	43.2	33.3	6.90	0.115
	LEMON	2.35	0.160	14.7	275	58.7	66.7	6.55	0.213

4. Deficiencies of zinc, manganese and sulphur in citrus

It is well known that citrus tree is liable to be affected by deficiencies of zinc and manganese. It is also known in Bangladesh that sulphur fertility is very low in Madhupur tract area where CVSRC field also is included. Neverthless, chemical analysis of citrus leaf for these elements has never been done in Bangladesh, even at CVSRC which has one subject of leaf analysis study.

Therefore, after joining CVSRC, I have made quite intensive chemical analysis of citrus leaves sampled not only at CVSRC but also in many other locations. The results obtained so far are summerized in Table 16.

There are many leaf samples found to have deficient level of sulphur, manganese and zinc everywhere. Some leaf samples collected at CVSRC had even low level of phosphorus and magnesium. This fact indicates that low quality of cultural management has been given until recently in CVSRC field.

There are many leaf samples which showed typical zinc deficiency symptom, and some of them had really deficient level of zinc. However, in pot cultures which were supplied with fairly large amount of zinc contained in tap water, some trees showed leaf symptom of severe zinc deficiency, of course in spite of high level of zinc in their leaves. As Dr. M. Koizumi, short-term pathologist reported, they are considered to be affected by "greening disease". It might be an interesting problem to investigate how and what extent greening infection of citrus tree reduces zinc uptake from soil.

Besides, Mr. S. Kono conducted one pot culture study using 6 citrus varieties and found that soil application of sulphate and even folia spray of zinc sulphate could improve their growth to a considerable extent, as expected from leaf analytical results of low sulphur. It is now quite clear that CVSRC soil is very poor in sulphur, and sulphate should be applied as one of fertilizers in CVSRC field.

Table 16. Leaf analysis of citrus

1) Older tree, 2nd field, CVSRC

Sampling date / variety	Leaf symtoms	N %	P %	K %	S %	N/S	Ca %	Mg %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
Oct. 24												
OKITSU-WASE	Normal	2.75			0.172	15.7			137	29.4	54.2	6.90
KOBAYASHI-MIKAN	Normal	1.82			0.114	16.0			199	27.6	45.8	9.66
"	Small leaves	2.09			0.068	30.7			121	17.3	50.0	6.55
POMMELO (flat field)	Disease infested	1.90			0.131	14.5			375	43.2	33.3	6.90
SAMPOKAN		2.30			0.125	18.4			170	12.1	69.4	83.4
LEMON (flact field)	Yellowish severely infested	2.35			0.160	14.7			275	58.7	166.7	6.55
Nov. 7												
MOSAMBI	Yellowish Zn def.?				0.059				151	24.0	19.0	
Nov. 22												
Kunaunshu	Zn def.?				0.122				180	43.5	19.5	
PUMMELO	Small leaf. Zn def.?				0.182				252	15.5	18.3	
KINNOW	Mostly yellow	2.09			0.148	14.1			180	15.5	14.6	
"	Patchy yellow	2.89			0.215	13.4			248	24.8	17.1	
"	Slightly patchy yellow				0.192				243	21.7	20.7	
MOSAMBI	Large almost yellow	1.66			0.090	18.4			141	20.2	12.2	
MIHO-WASE	Actively growing twig.	2.97			0.113	26.3			126	21.7	34.1	
"	Short twig	2.29			0.137	16.7		0.68	141	34.2	14.6	
ANSEIKAN	Pale green	1.68			0.066	25.5			102	14.0	17.1	
KUNEMBO	Zn def.?	2.22			0.115	19.3			180	37.3	15.9	
LEMON (flat field)	Newly developed	2.14			0.104	20.6		0.58	136	34.2	31.7	
Dec. 2												
KINNOW	Small, severely yellow	2.06			0.131	15.7			216	19.1	21.6	
MIHO-WASE	Large, pathy yellowing	3.10			0.113	27.4			166	16.9	26.4	
March 14												
MIHO-WASE	New, light colored	3.82			0.155	24.6			(433)	46.4	27.4	
March 26												
AMANATSUKAN	New, light colored	3.08	0.129		0.181	17.0			243	69.7	32.8	
April 4												
KINNOW	New light colored	2.03	0.161	2.68	0.186	10.9		0.30	265	23.8	20.2	
SUCKERY	Healthy		0.156	3.01	0.169			0.22	407	47.6	52.0	
" on the same twig	Affected by Mg def.?		0.105	2.57	0.251			0.096	632	41.7	32.9	

2) Old leaf of older tree, 2nd field, CVSRC (August 16, 1983)

Variety	P %	K %	S %	Fe ppm	Mn ppm	Zn ppm
SEEDLESS LEMON	0.198	1.78	0.224	312	78.4	23.1
LISBON LEMON	0.188	2.15	0.137	182	57.2	(high)
HAYASHI UNSHU	0.208	2.15	0.258	253	36.0	27.6
MIYAGAWA	0.126	1.70	0.126	141	36.0	20.1
OKITSU-WASE	0.219	2.22	0.191	164	21.1	13.4
MIHO-WASE	0.206	2.00	0.215	147	55.1	49.3
KINNOW	0.186	2.22	0.141	165	19.1	24.6
JAFFA	0.146	1.48	0.198	124	10.6	19.2
KUNENBO	0.144	1.93	0.134	153	16.9	17.9
NAGPURI	0.161	3.18	0.272	241	36.0	32.8
SILVER HILL	0.153	1.78	0.238	194	59.3	20.9
RUBY RED	0.237	2.15	0.382	165	27.5	11.2
MOSAMBI	0.210	2.82	0.303	206	12.7	34.3
"	0.198	2.91	0.153	153	40.3	17.2
"	0.171	1.93	0.184	124	6.4	11.9
SHIROYANAGI NAVEL	0.151	2.00	0.191	164	21.1	13.4
HYUGANATSU	0.278	2.22	0.203	153	31.8	22.4
BENIHASSAKU	0.150	1.93	0.192	147	19.1	11.9
AMANATSUKAN	0.171	2.08	0.141	141	40.3	44.8
TANKAN	0.115	1.63	0.138	324	53.0	22.4

Sampled by Mr. S. Kono, citrus expert

3) Young tree, 2nd field, CVSRC

Sampling date / variety	Leaf symptoms	N %	P %	K %	S %	N/S	Ca %	Mg %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
Nov. 21												
OTACHIBANA	almost normal lightly thin	2.99			0.143	20.9			126	20.2	35.4	
AOSHIMAUNSHU	Large, normal Mn def.?	2.91			0.147	19.8		0.529	112	17.1	25.6	
Nov. 25												
AOSHIMAUNSHU	Large, normal	2.62			0.127	20.6			180	30.9	102.8	
Dec. 13												
OTACHIBANA	Fine, irregu lar, chlorotic pathches	2.65			0.174	15.2				22.5	23.9	5.93
Jan. 23												
OTACHIBANA	Zn def. symptom		0.454	0.758	0.158	15.0	1.38	0.408	232	25.0	8.65	6.14
Jan. 30												
HAMLIN(SO)	Bright yellow with green major veins		0.505		0.132				(427)	62.8	17.1	13.7

4) Lemon, 1st field west side of the main building, CVSRC

Sampling date	Leaf symptoms	N %	P %	K %	S %	N/S	Ca %	Mg %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
Oct. 24												
	Active growth new leaf	1.98			0.108	18.3			138	18.2	47.2	5.17
	Active growth new leaf	1.98			0.111	17.8			143	16.6	29.2	4.48
Nov. 21												
	Small compact leaf development, intervenal chlorosis				0.127				141	79.2	18.3	
	Irregular green patches	1.37			0.196	7.0			151	12.4	23.2	
	New developed, thin colored				0.115				141	14.0	31.7	
Nov. 25												
	Irregular green patches	1.47			0.186	7.9			190	14.0	31.6	
	Irregular green patches with narrow green veins	1.66			0.179	9.3			175	15.0	25.4	
March 15												
	Newly developed light colored	2.17			0.103	21.1			(340)	14.5	18.8	

5) Pot culture, CVSRC

Sampling date variety	Leaf symptoms	N %	P %	K %	S %	N/S	Ca %	Mg %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
Nov. 1												
SEMINOL	Moderately large entire yellow	2.46			0.102	24.1		0.458	190	20.7	72.7	5.69
BATANGAI	Pale doloured with green veins	2.11			0.155	13.6			172	40.6	34.0	5.00
"	Vein clearing?				0.168				269	22.4	43.1	5.86
SWEET ORANGE	Slightly vein clearing				0.238				237	70.5	40.3	5.00
Nov. 7												
BATANGAI	Pale coloured with green veins				0.129			0.525	231	48.1	32.1	
"	Almost normal				0.177			0.719	302	75.3	69.0	
Nov. 15												
FUKUHARA	Large, newly developed	2.98			0.290	10.3			167	28.8	39.4	
"	Slightly affected	2.60			0.273	9.5			186	44.7	77.3	
SEMINOL	New large	2.36			0.238	9.9			471	27.2	87.9	
S. MATSUYAMA	Newly developed				0.158				300	36.7	(417)	
"	Slightly affected				0.153				119	46.3	42.4	
TACHIBANA	Small new leaf				0.082				110	20.8	39.4	
"	Affeted by inter-veral chlorosis, small				0.235				186	30.4	(126)	
Nov. 25												
TACHIBANA	Almost normal size, slight intervenal chlorosis				0.196				215	24.0	38.6	
FUKUHARA	Severe chlorosis								250	(high)	32.5	
March 15												
SEMINOL	New almost normal	2.51			0.135	18.6			307	29.8	39.2	
PUMMELO THAILAND	Extremely yellow	1.44			0.105	13.7			380	20.8	23.1	

6) Outside of CVSRC

Sampling date / leaf sysmptoms	N %	P %	K %	S %	N/S	Ca %	Mg %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
Dec. 1											
Nepal Sweet orange											
Almost yellowish							0.502	388	33.6	34.2	8.42
Bright intervenal chlorosis							0.504	410	35.2	35.6	(39.5)
Thin coloured chlorotic patches							0.503	321	36.9	30.1	14.7
Partially yellow, disease infested				0.312			0.504	299	31.9	32.9	11.6
Large leaf, disease infested				0.192			0.505	388	26.8	34.9	9.21
Large pale colored, vein clearing				0.167			0.505	157	40.3	11.6	3.68
Jan. 27											
Rangpur Substation Lemon											
Healthy green, old	2.39	0.55	0.82	0.192	12.4	2.45	0.65	(500)	50.0	32.7	4.1
Moderately affected	1.64	0.54	0.65	0.189	8.7	2.14	0.52	(689)	37.5	17.3	4.1
Nearly intervenal chlorotic	1.88	0.40	0.68	0.212	8.9	1.76	0.40	(470)	28.6	14.4	4.1
March 30											
Chittagong Hill tracts* (averaged)	2.23	0.104	2.21	0.251	8.5		0.20	284	22.3	8.1	
August 6											
Rangpur Horticultural Extension Farm*											
SWEET ORANGE (1) old leaf		0.111		0.171				330	28.0	8.76	
very young leaf		0.126		0.130				186	21.0	7.73	
(2) old leaf		0.093		0.163				367	29.7	16.5	
young leaf		0.132		0.151				207	47.2	8.24	
PUMMELO old leaf		0.092		0.145				227	24.5	14.9	
cf. Critical level (Hunger)	2.5	0.15	1.0	0.2	15	2.0	0.3	-	30	25	-
(Deficiency)	2.0	0.10	0.5	0.13	18	1.0	0.15	60	20	15	-

* Affected by "greening disease"

( ) Probably dust-contaminated

5. Twig die back related to wet damage in monsoon season

In Bangladesh severe occurence of twig die back is one of major limiting factors against economical production of citrus. There are various causes as reported already. Quite recently Dr. M. Koizumi, short-term citrus pathologist, CVSRC has confirmed that greening disease is widely spread and highly related to twig die back. For detecting a really causal factor, it is indispensable to trace the process of its occurence from the very beginning, otherwise it might be a compound result of multiple infections and disorders.

I have been studying on the effective means of diagnosing wet damage of upland crops. Very high iron content of their upper leaf is found to be a reliable checkup.

Along this finding, I determined iron content of leaf and twig of die back affected citrus trees and found remarkably higher iron content in them, after twig die back occurred in the last dry season. The dead twig had the highest iron content, above 500ppm in 1cm long top (Table 17).

Mr. S. Kono, long-term citrus expert, CVSRC dug up the affected trees with almost entire root system and found severe root rot, especially decay of downward developing roots. This fact clearly indicateds the occcurence of wet damage.

Those results are quite understandable. Soil reduction due to very wet condition in rainy season increases soluble ferrous iron in the root zone. Root rot also increases passive uptake of iron. Iron tends to accumulate in the growing tissue, and, once accumulated, it hardly move to other tissues. Therefore, high iron content in the leaf and twig may have higher rate of iron supply, when they are developing.

In order to further study on close relationship between die back occurrence and iron accumulatiom, seasonal changes in iron content of leaf and twig of die back affected and non affected trees were determined five times during the period from April 18 to September 13, 1983. Some detailed results on P, S, Fe, Mn and Zn are shown in Table 18 and 19. The averaged values of Fe and P are summerized in Fig. 1

Iron content in twig is always lower than that in leaf as seen in Tab. 3. Phosphorus is higher in young leaf, while other elements such as S, Fe, Mn and Zn, especially Mn are higher in old leaf, as seen in Tab. 18.

In general, iron content in leaf showes a descending trend up to June, and a big difference between both trees is not seen. June result of non affected tree is only exceptional. It is very low, below 100ppm in new leaf on summer twig.

On the other hand, leaf phosphorus of affected tree was much higher than that of non affected tree, but this difference disappeared later on July 23. That is because the affected tree developed small numbers of leaf and twig, so supply of phosphorus was high enough for each leaf and twig at early stage. Therefore, it may be considered that around July 23 the affected tree achieved almost normal growth, and as a result no gap between both kinds of trees was found.

From the above-mentioned results, no leaf and twig with very high iron content has been observed so far even in monsoon season in varietal stock field. It might be due to intensive practices of soil drainage improvement (mohr drainage and sand mixing) and better management such as sufficient fertilizer application. Hence, twig die back may not occur in the coming dry season*. It, of course, needs further observation.

However, on the contrary, in poorly drained field of lemon the leaves turned to yellowish and severely disease infested just like last season. Some of them started to drop already. The analytical results of affected twigs are shown in Table 20. Leaf iron had a remarkable rise, and 1cm long top of twig also showed fairly high iron content. Besides, 1 to 5cm part from the top of twig had much lower iron content than its 1cm long top. That is to say that iron may accumulate in the top of twig much more next to leaf, when iron uptake is forced to increase. At the present stage of continuous observations the twig might have still increasing trend of iron content. In other words, it is getting higher, as root rot proceeds later on.

For the reference, one trial on forced uptake of iron using branch cut of lemon and ferrous citrate solution was conducted (Table 21). At the concentration of 0.01M, very severe toxicity symptom on the entire leaf together with extremely high iron content in leaf and twig, over 1000ppm. It may be a very particular case, so the result is out of question. At the concentra tion of 0.005M, leaf colour faded, and the lower one dropped. Leaf iron was on the level of 500ppm. It was higher than that of twig like in ordinary cases, while the trend was reverse at the concentration of 0.01M. The critical toxicity level of leaf iron may be considered to be around 500ppm. This level is not so higher than those determined on leaf samples from the field. Therefore, increased iron uptake due to wet damage in monsoon season can be considered as one of the causes for twig die back of citrus trees in Bangladesh.

* In fact, no tree die back was observed to break out in the dry season, 1984.

Table 17. Analytical results of summer twig of citrus

(CVSRC/BARI, March 15, 1983)

Variety	Growth status	P %	Fe ppm	Mn ppm	Zn ppm	Mn/Fe
URECA LEMON	Weak	0.342	453	42.9	46.2	0.095
	All dead	0.311	547	60.2	38.2	0.110
MIHO-WASE	Active	0.326	307	21.5	30.1	0.070
	Weak	0.241	327	33.2	25.8	0.102
	Dead	0.210	507	29.1	40.3	0.057
OKITSU-WASE	Active	0.312	327	48.5	28.5	0.148
	Weak	0.238	413	35.3	32.3	0.085
	Dead	0.280	467	51.2	38.7	0.110
MIYAGAWA-WASE	dead	0.260	427	44.3	26.3	0.104

Table 18. Leaf analysis of non-affected citrus trees at CVSRC fields (April 21, 1983)

Variety	Age of leaf	P %	S %	Fe ppm	Mn ppm	Zn ppm
MIHO-WASE	Old	0.23	0.214	238	79.6	30.1
	Young	0.29	0.174	209	38.1	24.0
OKITSU-WASE	Old	0.24	0.207	227	80.3	25.5
	Young	0.30	0.176	169	45.6	23.0
MIYAGAWA-WASE	Old	0.34	0.237	215	100.0	24.5
	Young	0.35	0.158	209	34.7	16.8
AMANATSU	Old	0.31	0.289	273	110.9	20.9
KAN	Young	0.34	0.176	192	37.4	21.4

Table 19 Leaf analysis of non-affected citrus trees at CVSRC field (August 30, 1983)

Variety	Tree No.		P%	Fe ppm
OKITSU-WASE	1-3	Leaf	0.113	189
		Twig	0.122	138
	2-2	Leaf	0.091	138
		Twig	0.134	133
MIHO-WASE	2-1	Leaf	0.089	148
		Twig	0.122	153
	2-7	Leaf	0.097	168
		Twig	0.120	138
HYUGANATSU	2-1	Leaf	0.124	194
		Twig	0.146	182

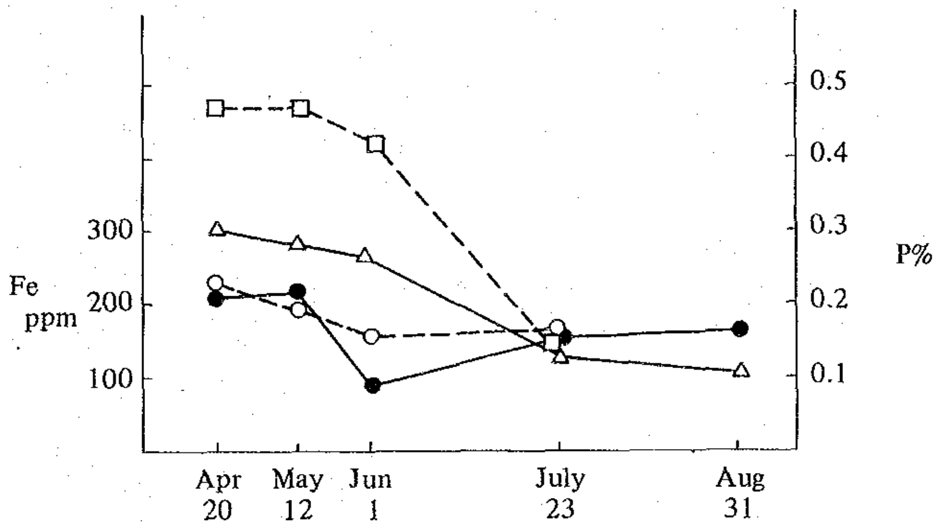


Fig. 1 Seasonal changes in iron and phosphorus content of leaf of citrus trees with (○, □) and without (●, △) dead twigs at CVSRC farm.

Table 20. Leaf and twig analysis of leaf-affected lemon tree in poorly drained CVSRC field (September 18, 1983)

Tree No.	Leaf		Twig (Top 1cm)		Twig (Top 1-5cm)	
	P	Fe	P	Fe	P	Fe
	%	ppm	%	ppm	%	ppm
2-1	0.126	389	0.151	381	0.083	221
3-2	0.091	421	0.100	299	0.068	211
7-4	0.157	405	0.207	309	0.169	153

Table 21. Forced uptake of iron in branch cut (Lemon, 11 days treatment)

Ferrous citrate Mol.	Toxicity reaction of leaf	Leaf			Twig		
		Fe	Mn	Zn	Fe	Mn	Zn
		ppm	ppm	ppm	ppm	ppm	ppm
0.01	Abundant depressed blackish spots	1040	32.9	26.1	1388	8.7	23.0
0.005	faded colour, the lower leaf dropped	520	32.9	24.2	375	6.0	31.5
0.0025	No	385	28.8	20.0	294	5.7	33.2
0	No	210	26.7	26.1	164	6.2	28.7