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Review article

Nutrient deficiency symptomology in citrus: An effective diagnostic tool or just an aid for post –mortem analysis

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ABSTRACT

Nutrient responsiveness of citrus is evaluated through nutrient diagnostics based on leaf analysis, soil analysis, juice analysis, enzyme function and deficiency symptoms. Of them, deficiency symptoms are most widely used, although with a minimum practical implications on the orchard performance. Development of visible symptoms is attributed to metabolic disorders, causing changes in micromorphology before such symptoms are identifiable. The way in which symptoms develop and manifest themselves on different plant parts gives a reliable indication of the cause of nutritional disorders. But relying solely on deficiency symptoms will be too late for timely diagnosis of nutrient constraints unless complemented with both leaf and soil analysis data. Deficiency symptoms are more like doing post-mortem of decline in orchard performance than timely diagnosing the genesis of nutrient deficiency.

1. Introduction

Effect of nutrients on plant growth and development has been studied for over 350 years since the experiments of van Helmont in 1648 (Epstein, 1972). Exciting progress has been made in the past 50 years to develop and improve diagnostic techniques of identifying nutritional constraints and accordingly, the fertilizer management strategy is modified. Ericsson (1995) described three distinctly different dry matter allocation patterns with plant growth limited by nutrient availability. These patterns are: i. favouring root growth under N, P

or S as major growth constraints, ii. adversely affecting root growth under K-, Mg-, and Mn-deficiencies, and iii. no effect with shortage of Ca, Fe, and Zn. Diagnostic tools of nutrient management such as leaf analysis (Swietlik, 1996; Bell *et al*., 1997; Srivastava and Singh, 1998; 2003a; 2003b; 2003c), soil analysis (Srivastava and Singh, 2001a; 2001b; 2001c; 2002), juice analysis (Moss and Higgins, 1978; Gallasch *et al*., 1984), and to some extent, biochemical analysis (Almansa *et al*., 1994; Hellin *et al*., 1995; Srivastava and Singh 2008a; 2008b), all have been under continuous critical scrutiny and recurrent use. It is abundantly clear that no one of these alone provides complete information, except the combined use of leaf and soil analysis, which have comparatively gained some distinction (Srivastava *et al*., 2001; 2009b).

Success of current citrus nutritional research depends upon how precisely two major problems are addressed. Problem one, that manifests in the correct and timely identification of nutrient deficiency as a resultant effect of disturbed metabolic processes, especially where the occurrence of multi-nutrient deficiency is a common feature and secondly, the method to execute remedial measure, to address the deficiency of a particular nutrient (Srivastava and Singh, 2004a; 2004b; 2009a). These two efforts collectively minimize the chances of current season crop getting suffered on account of sub-optimum nutrition. There are definite limitations with the leaf analysis technique currently in use where concentration of nutrients in leaf needs to stabilize first, to collect mature leaves at a stage transition from sink to source (Foyer, 1988; Domingo *et al*., 2002; Srivastava and Singh, 2003c) before subjecting samples to chemical analysis. Physiologically, the consistency in response of fertilization is a big question mark due to cessation of auxin synthesis, during later stage of leaf and fruit development (Bustan *et al*., 1995). However, the physiological mechanisms underlying the auto inhibition of polar IAA transport is still inconclusive (Bangerth, 1989). Likewise, the influence of shortage of micronutrients viz., B, Zn, Cu, and Mo on dry matter partitioning between shoot and root is comparatively lesser understood (Srivastava, 2013b).

Of the available nutrient diagnostics tools, identifying nutritional disorders in citrus using morphological descriptors are commonly used at the orchard level (Srivastava *et al*., 1999; 2008; Srivastava 2013a; 2013c). The overall appearance of the plant and localized specific symptoms are an important aid to knowledge observers in identifying deficiencies (Srivastava and Singh, 2005). The metabolic derangements brought about by deficiencies of essential nutrients (Srivastava and Singh, 2006). Degree of deficiency is measured by the severity of symptoms and number of growth terminals affected (Srivastava and Singh, 2009b). Schematic representation given below helps a plant diagnostician to distinguish effectively at appearance of nutrient deficiencies (Fig.1).

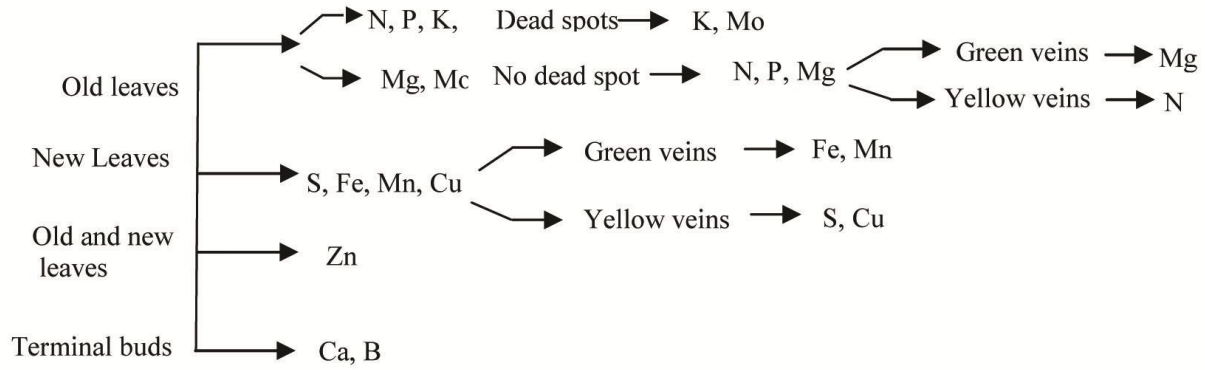

Fig. 1. Growth sites differentially used for establishing different nutrient deficiencies.

A big question mark is raised which of these nutrient diagnostic tools is/are capable of identifying nutrient constraint early in the season , and secondly , how far over traditional method of nutrient diagnosis that is based on nutrient deficiency symptoms is capable of effectively pinpointing the nutrient problem or , just an aid to post-mortem diagnosis for nutrient deficiencies. In this regard functional analysis of mineral deficiencies is one step forward early detection of nutrient constraints (Srivastava, 2009a).

2. Functional analysis of mineral deficiencies

Plants deficient in N, S or Fe accompanied by lowered chlorophyll concentration develop a similar yellow phenotypes. Mechanistically, Fe and S are highly reactive components of many enzymes, and form active electron transfer agents like Fe_2S_2, Fe_3S_4 or Fe_4S_4 clusters. Prominent enzymes containing Fe-S clusters are thioredoxin/ferredoxin reductase, ferredoxin-nitrite reductase, sulfite reductase, acotinase, succinate, phosphogluconate dehydrogenase to serve as sensors of oxidative stress (Holm *et al*., 1996; Flint and Allen, 1996). Other example of Fe-deficiency showing yellowing symptoms due to interrupted chlorophyll synthesis may be cited, even though chlorophyll chelates Mg rather than Fe. Nutrient deficiency, thus, involves degradative changes in chloroplast components and additional cellular compartments. In the light of this information, an integrative physiological approach was suggested by Imsande (1998). Biochemical marker aided nutrient constraint analysis, however, was earlier attempted but found no wide acceptance. Looking at the kind of changes that have taken place in nutritional diagnosis, this approach requires a thorough revisiting to have a blueprint of what changed between then and now. Determination of critical levels and the mineral composition of plants (nutrient balance), gives only a static view of the problem, because the biologically active form of nutrient is not determined. Additionally, many a times, the discrepancies between diagnosis of nutrient status of leaf and soil are resolved through long term field response studies. The growers till then continue to obtain sub-optimum production on account of occurrence of one or other nutrient deficiencies.

Productivity of the plant depends essentially on the nutrient balance and the biological activity. Establishment of absolute figures of normal, deficient or excess nutrient level is not real, unless the dynamic aspect of leaf nutrient concentration is considered. Studies (McIntyre, 2001; Coruzzi and Bush, 2001) have shown that nutritional status of the responsive tissues transmit signals as a regulator of gene expression and at times, that can become a limiting factor in the process of plant development. There are interesting improvements such as the determination of the nutrient evolution along the vegetative cycle, the substitution of the critical levels by the critical zone, fractionating the nutrient contents (especially the biologically active ones), and finally implementing the biochemical diagnosis. For the latter, the use of activities of specific enzymatic systems and also of metabolites concerned with photosynthesis has a good potential to improve the accuracy of nutrient constraint diagnosis over other conventional methods of nutrient diagnosis. Many studies (Alcaraz *et al*., 1986; Tavdgiridze and Putkaradze, 1991) suggested that the levels of enzymatic activity could be effectively used as an alternative diagnostic tool to leaf analysis.

Functional analysis of the nutrients is, thus, based on the examination of certain molecular compounds linked with their functional activity. Specific reference may be made to the study of the enzymatic activities directly influenced by the metabolic activity of the nutrients. For the valid use of enzymatic systems as indicators of the activity of certain nutrient element in plant tissue, it is essential for the enzymatic system to be specific for the said element. Hence, for these purposes, the choice is almost completely limited to metalloenzymes. For example, the use of the peroxidase in the diagnosis of Fe- and Mn- deficiencies (Bar-Akiva, 1961; 1964) prompted checking the utility of the method for citrus cultivars grown on differentially fertile soils. Parallel to what was observed with peroxidase, catalase, and aconitase reduced their levels of activity with Fe-deficiency and increased with Mn-deficiency, facilitated to establish the possibility of using the latter enzyme as an alternative mean of diagnosing Fe- and Mn- deficiencies (Valenzuela and Romero, 1988). In the early diagnosis of mineral deficiencies in lemon trees, aconitase has proved to be as precise as peroxidase, a specific Fe-metalloenzyme or even more so (Garcia *et al*., 1990). In the light of this background information, attempts have, therefore, been made to analyse the perspective utility of different biochemical markers suitable for diagnosing mineral deficiencies in citrus as a viable alternative to other popular methods of diagnosis like soil or leaf analysis.

Mineral deficiencies are well established causal factor(s) for sub-optimum production in citrus. Identifying nutrient constraints based on morphological symptoms or alternatively, in combination with leaf/soil analysis is often misleading, especially with reference to remediating the nutritional problems of standing crop. The task becomes further confounded by other co-factors under the conditions favouring the occurrence of multi-nutrient deficiency. Important biochemical markers for various nutrient deficiencies include: ribulose-1,5 biphosphate carboxylase (RuBPCase), nitrate reductase, and glutamate dehydrogenase for N-deficiency; citrate synthetase, aconitase, phosphoenol pyruvate kinase, and glutamic oxaloacetic transminase for P-deficiency; diesterase, acid invertase, arginine decarboxylase, and N-carbamyl putrescine aminohydrolase for K-deficiency; pyruvate kinase and succinate dehydrogenase for Ca-deficiency; and invertase for Mg-deficiency; adolase and aconitase for Fe-deficiency; catalase for Mn-deficiency (higher peroxidase activity under Mn-deficiency is differentiated from Fe-deficiency); carbonic anhydrase and nitrate reductase for Zn- deficiency; and phenylalanine ammonia lyase and

nitrate reductase for B- and Mo-deficiency, respectively. These markers have also shown some promise in establishing the physiological basis of tolerance of Satsuma mandarin (*Citrus unshiu* Marc.) against Mn- and Al-toxicities involving Mn-oxidative pathway and complexation of calmodulin protein with Al^{3+} ions, respectively.(Srivastava and Singh 2000b).The important metallo-enzyme responsible for identifying different nutrients deficiencies are further summarised (Table 1 and 2)

Table 1

Important enzyme as markers associated with different macro nutrient deficiencies in citrus.

Biochemical markers	Increase(+) or decrease (-) in activity/ concentration
N – deficiency	
- Leaf NH_3-NH_4^+, proline, total sugar	+
RuBPCase, arginine, nitrate reductase, glutamate dehydrogenase, phosphenol-pyruvate carboxylase	-
P - deficiency	
Arginine, proline, lysine, histidine, citruline, ornithine, free amino acid	+
- Ribonuclease, glutamic oxaloacetic transminase, citrate synthetase, acotinase, malic enzyme, phosphenol pyruvate carboxylase, succinate dehydrogenase	-
K - deficiency	
Cadaverine, acid invertase, lysine, histidine, L-arginine carboxylase	+
N-carbamyl putrescine amino hydrolase, pyruvate kinase	-
Ca – deficiency	
Succinate dehydrogenase, nitrate reductase, RuBPCase Pyruvate kinase	- +
Mg - deficiency	
Acid invertase, alkaline invertase	-, +

Source : Srivastava and Singh (2006); Srivastava *et al.* (2008)

Table 2

Important enzymes as markers associated with different micronutrient deficiencies in citrus.

Biochemical markers	Increase(+) or decrease (-) in activity/ concentration
Fe – deficiency	
Peroxidase, adolase, acontinase Citric acid	- +
Mn – deficiency	
Peroxidase, xylose	+
Catalase, phenyalanine, ammonia lyase, tyrosine ammonia lyase, phosphenol oxidase	-
Fe & Mn – deficiencies	
Arginine, asparagine, serine, free amino acid, shikimic acid	-
Zn – deficiency	
Arginine, proline, free amino acid	+
Carbonic anhydrase, nitrate reductase, indole acetic acid	-
B – deficiency	
Phenyalanine ammonia lyase, sugar borate complex	-
Mo – deficiency	
Peroxidase, nitrate reductase	-

Source: Srivastava and Singh (2006); Srivastava *et al.* (2008).

3. Deficiency symptoms

Visual deficiency symptoms however should be regarded as just one of several kinds of evidence of deficiency of a given nutrient element. Several factors combine to introduce considerable uncertainty in diagnosis-based on symptomology alone (Srivastava, 2012). . Different essential nutrients based on their physiological functions and biochemical behaviour, are divided into four distinct groups:

Group 1: C, H, O, N, and S. These nutrients are major constituents of organic material, involved in enzymatic processes and oxidation-reduction reactions.

Group 2: P and B. These elements are involved in energy transfer reactions and esterification with native alcohol groups in plants

Group 3: K, Ca, Mg, Mn and Cl. This group plays osmotic and ion balance roles, plus more specific functions in enzyme conformation and calalysis.

Group 4: Fe, Cu, Zn, and Mo. Present as structural chelates or metalloproteins, these elements enable electron transport by valance change.

3.1. Nitrogen deficiency

Nitrogen is absorbed in $NH_4^+$and NO_3^-It is a mobile nutrient both in soil and plant. Nitrogen is readily absorbed in NO_3^- form, but NH_4^+ form is also used. Availability is severely reduced by high soil pH, calcareousness, soil salinity/sodicity soil moisture etc.Nitrogen is involved in: i. constituent of amino acids, amides, proteins, nucleic acids, nucleotides and coenzymes, hexoseamines, etc. ii. essential for good role of cell division, growth and respiration; occurs chiefly in protein and nucleoproteins with widely varying amounts of amines, amino acids, amino sugars and others iii. most active nitrogenous compounds occur largely in the protoplasm and nuclei of plant cells (Srivastava and Singh, 1998).

3.1.1. Visual symptoms

Stagewise nutrient deficiency progresses in a following fashion (Fig.2):

Stage I: New leaves are often distinguished by general yellowing of foliage over the entire tree with absence of any distinctive yellowing pattern (Zekri, 1995a).

Stage II: Symptoms appear on older leaves and later proceed towards the younger leaves which are small in size, thin, fragile and light green in colour.

Stage III: Symptoms are characterized by light yellowish green leaves with veins slightly lighter in colour than the tissues between.

Stage IV: Mature green leaves slowly bleach to a mottled irregular green with yellow pattern turning entirely yellow which are later shed.

Stage V: Symptoms are often associated with reduced colour of fruit peel, which tends to be pale and smooth

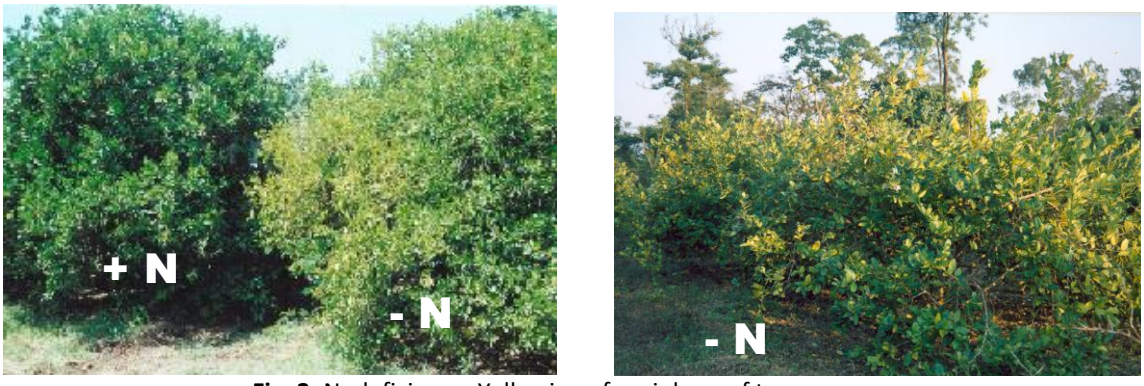

Fig. 2. N -deficiency: Yellowing of periphery of tree canopy.

3.1.2. Correction

N-deficiency symptoms can be overcome by soil application at the rate 600 g N $tree^{-1}$ $year^{-1}$ ('Nagpur' mandarin), 400 g N $tree^{-1}$ $year^{-1}$ ('Khasi' mandarin), 1000 g N $tree^{-1}$ $year^{-1}$ ('Sathgudi' sweet orange), 600-800 g N $tree^{-1}$ $year^{-1}$ (Acid Lime), 400-800 g N $tree^{-1}$ $year^{-1}$ ('Kinnow' mandarin) and 300 g N $tree^{-1}$ $year^{-1}$ ('Assam' Lemon).

While foliar N application at the rate of 1-2% and 1-1.5% urea have proved effective in Nagpur mandarin and Kinnow mandarin, respectively (Srivastava and Singh, 2003d).

3.2 Phosphorus deficiency

Phosphorus is absorbed in $H_2PO_4^-$ and HPO_4^{2-} forms. This nutrient has possesses biopolar mobility, immobile in soil and mobile in plant. It exists in three basic forms $H_2PO_4^-$, HPO_4^{2-}, H_3PO_4 with monovalent $H_2PO_4^-$ and HPO_4^{2-} form being readily available to plants. In acid soil, P is heavily fixed, while in alkaline soil, it exists in trivalent PO_4^{3-} form, not readily available to plants. Availability of P in soil is drastically reduced with salinity/sodicity, pH, calcareousness, clay texture etc.

Metabolically, P is involved in following plant processes: i. component of sugar phosphates, nucleic acids, nucleotides, coenzymes, phospholipids, phytic acid, etc. with role in reactions in which ATP is involved, ii. necessary for many life processes such as photosynthesis, synthesis and breakdown of carbohydrates, and the transfer of energy within the plant; helps plants store and use energy from photosynthesis to form seeds; develop roots, seed maturity, and resist stresses; constitutes a major part of cell nucleus and cytoplasm; involved in heredity characteristics besides playing role in cell division.

3.2.1 Visual symptoms

Seeded varieties are more prone to P-deficiency than seedless varieties (Zekri,1995a). Phosphorus deficiency visually develops in following manner (Fig. 3):

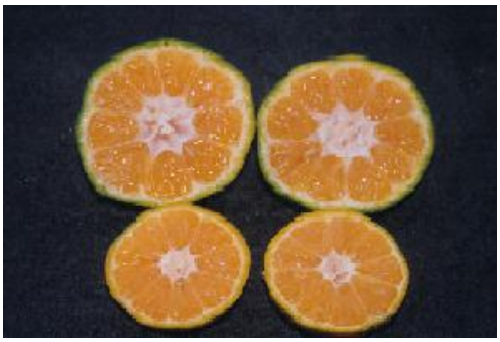

Fig. 3. P-deficiency: Thick peel and bigger hollow central core.

Stage I: Symptoms appear first on older leaves which later loose their deep green colour.
Stage II: Leaves remain small and narrow with purplish or bronze coupled with lusterless discoloration.
Stage III: Some leaves later develop necrotic areas with young leaves having reduced growth rate.
Stage IV: Roots display stunted and poorly branched characteristics.
Stage V: Fruits would be coarse and rough in texture with a coarse, thick rind and hollow central core having high acidity in proportion to total soluble solids with delayed maturity.

3.2.2. Correction

P-deficiency can be corrected by soil application of 200 g 200 g P_2O_5 $tree^{-1}year^{-1}$ ('Nagpur' mandarin, 'Kinnow' mandarin, Acid Lime and 'Jaffa' orange), 250 g P_2O_5 $tree^{-1}year^{-1}$ ('Khasi' mandarin), 100 g P_2O_5 $tree^{-1}$ $year^{-1}$ ('Mosambi sweet orange) and 400 g P_2O_5 $tree^{-1}$ $year^{-1}$ ('Sathgudi' sweet orange).

3.3. Potassium deficiency

Potassium is absorbed in K^+ form. Potassium in soil exists in dynamic equilibrium among water soluble-K, exchangeable-K, and non-exchangeable-K (Fixed-K + Mineral matrix-K). Plants use both exchangeable as well as non-exchangeable forms of K. Its availability in soil is most affected by soil moisture, clay mineralogy, pH, soil structure, wetting and drying by rainfall, temperature etc. Ammonium acetate (pH 7.0) extractable K is considered as an effective index of soil available K, while in smectite rich black soil, non-exchangeable-K also contributes to available K index.

Potassium is one of such primary nutrients which participates as cofactor in as many as 40 coenzymes because of its high mobility in plants.

i. role in stomatal movements; maintains electroneutrality in plant cells; required for many physiological functions such as formation of sugars and starch and synthesis of proteins, normal cell division and growth, ii. role in over-coming environmental stress such as frost tolerance by decreasing the osmotic potential of cell sap due to higher ratio of unsaturated to saturated fatty acids; imparting drought tolerance; regulation of internal water balance and turgidity; regulating Na influx and /or efflux at the plasmalema of root cells and iii. role in chloride exclusion behaviour through selectivity of fibrous roots for K over Na and imparts salt tolerance to cells to enable to hold the K in the vacuole against leakage when Na incurred in external medium.

3.3.1. Visual symptoms

Potassium deficiency develops in following manner:

Fig. 4. K-deficiency: undersized fruits coupled with longitudinal fruit growth.

Stage I: Symptoms begin with yellowing of tips and margins, yellow area then gets broader, necrotic areas with spotting first appearing on older leaves. Early symptoms often comprise of stunted growth, sparse foliage and somewhat bronzed and lusterless appearance of leaves with more acute deficiency.

Stage II: Leaves often wrinkle and twist, and only weak new lateral shoots emerge due to lack of mechanical strength, rendering shoots to turn S shaped (Zekri, 1995a).

Stage III: Screw type of curling towards the lower leaf surface, particularly on the lemon.

Stage IV: Reduction in fruit size with very thin smooth peel (Fig. 4) coupled with, pre-mature shedding of fruits possessing lowest acidity.While, excess K on the other hand produce very coarse texture with reduced juice content.

3.3.2. Correction

Potassium deficiency is more effectively corrected by soil application than foliar application. Its deficiency is corrected by soil application of K_2O at the rate of 300 g K_2O tree^{-1}year^{-1}('Kagzi' lime), 136 g K_2O tree^{-1}year^{-1}('Blood Red' sweet orange), 400 g K_2O tree^{-1}year^{-1}('Kinnow' mandarin), 200-500 g K_2O tree^{-1}year^{-1} ('Coorg' mandarin), 200 g K_2O tree^{-1}year^{-1} ('Sathgudi' sweet orange) and 250 g K_2O tree^{-1}year^{-1} ('Pant' lemon). While foliar spray in form of 0.5-1.0% K_2SO_4 ('Kagzi' lime) and 5% KNO_3 ('Verna' lemon) have shown good promise.(Srivastava and Singh, 2003d)

3.4. Magnesium deficiency

Magnesium is absorbed in Mg^{2+} form. It is one of the primary mobile nutrients, and active part of chlorophyll molecule. Neutral ammonium acetate extractable Mg is considered as an available index although plant available Mg is mostly associated with soil carbon exchange capacity. Soil properties such as pH, salinity/sodicity, mineralogy, soil moisture, calcareousness. etc. affect Mg availability in soil.

Magnesium holds its role in following processes: i**.** constituent of the chlorophyll molecule; and involved in photosynthesis and plays role as an activator of several enzymes; required non-specifically by large number of

enzymes involved in phosphate transfer, ii. involved in carbohydrate metabolism and synthesis of nucleic acid; related to movement of carbohydrates from leaves to upper part and stimulates P uptake and transport; and heavy application of K reduces Mg-uptake by plant roots, and iii. cultivars producing seedy fruits are more severely affected by Mg shortage than cultivars producing seedless fruits.

3.4.1. Visual symptoms

Visual symptomologies follow the number of stages.

Stage I: Symptoms first appear on mature leaves.

Stage II: Irregular yellow blotches start near the base along the mid-ribs of mature leaves that are close to fruit. These blotches later become larger and eventually coalesce to form a large area of yellow tissue to each side of the mid-rib. This yellow area enlarges untie only the tip and the base of the leaf that are green showing an inverted V-shaped (triangular) area pointed on the mid-rib (Fig. 5).

Stage III: Within the tree itself, heavily fruited limbs develop extreme Mg-deficiency symptoms that later turn completely defoliated, coupled with smaller and lower fruit yield

(Zekri ,1995b).

Fig. 5. Mg-deficiency: Formation of inverted v-shaped structure in the chlorotic background.

3.4.2. Correction

Soil application of $MgSO_4$ at the rate of 500 g $MgSO_4$ $tree^{-1}year^{-1}$('Jaffa' sweet orange and acid lime) and foliar application of $MgSO_4$ at the rate of 0.50% ('Jaffa' sweet orange) have successfully corrected Mg-deficiency.

3.5. Sulfur deficiency

Sulfur is absorbed in SO_4^{2-} form. Sulfur is one of the secondary nutrients which is highly mobile in soil and plant both. Availability in soil is severely affected by waterlogging or excess soil moisture. Sulfur has following roles in plant metabolism: i. component of cysteine, cystine, methionine, and proteins; constituent of lipoic acid, coenzymes A, thiaminepyrophosphate, glutathione,biotin, adenosine-5'- phosphosulfate and 3'-phosphoadenosine-5'- phosphosulfate, and other compounds, and ii. important for the production of amino acids proteins and chlorophyll; constituent of vitamins and some plant hormone; retards protein synthesis; affects carbohydrate metabolism and imparts hardiness and vigour to the plant(Srivastava and Singh, 2006).

2.5.1. Visual symptoms

Deficiency of sulphur is expressed in following manner:

Stage I: Symptoms appear on new growth showing younger leaves with stunted pale-green-yellow appearance with lighter veins (Zekri ,1995b).

Stage II: Trees appear stunted and pale green to yellow in color. However, such chlorosis in citrus is worst on new growth, because S dose not move readily from old to young leaves like N.

3.5.2. Correction

Gypsum application is considered most potent S-source, usually applied at the rate of 300-500 g tree^{-1} in 'Nagpur' mandarin.

3.6. Iron deficiency

Iron by plant is absorbed in Fe^{2+} form. It is one of the micronutrients becomes exceedingly mobile under waterlogged conditions; otherwise it is highly immobile in plant. In acid soils, soluble Fe could fix phosphates which are aggravated further by high water table and waterlogging. While on alkaline calcareous soils, lime induced iron chlorosis is perhaps the most researched nutritional disorder in citrus. Availability of Fe becomes less available in soils having pH beyond 7.8. High available Fe could induce Mn-deficiency.

Iron is important in plant metabolism in following manner: i. constituent of cytochromes, nonhaeme iron proteins, which are involved in photosynthesis. N_2 fixation and respiratory linked dehydrogenases and ii. involved in reduction in nitrate and sulfates and activity of peroxidases are considered biochemical index of Fe-deficiency(Srivastava *et al*., 2008).

3.6.1 Visual symptoms

Deficiency of iron is expressed in following stages:

Stage I: Interveinal white chlorosis appearing first on younger leaves.

Stage II: In some cases leaves may be completely bleached, margins and tips remain scorched.

Stage III: Appearance of paper leaves formation which visualised against sun light (Fig.6).

Stage IV: In acute cases, the leaves are reduced in size, fragile, very thin which are shed early.

Stage V: Trees die back severely on the periphery, and especially in the top. Often trees with dead top are seen with the lower limbs varying almost normal foliage (Zekri, 1995c).

Fig. 6. Fe-deficiency: Chlorosis of younger leaves with development of papery leaves.

3.6.2. Correction

Deficiency of iron is corrected more effectively through foliar application than soil application. Foliar spray of iron-chelates is likewise more useful than simple inorganic salt. Foliar spray of 0.25% of Fe-EDDHA ('Jaffa' sweet orange) and 0.50-0.75% $FeSO_4$ ('Mosambi' sweet orange, 'Kinnow' mandarin and Acid lime) have shown promising results. On the other hand, soil application of $FeSO_4$ at the rate of 50 g tree^{-1} year^{-1}('Sathgudi sweet orange), 250-300 g tree^{-1}year^{-1}('Kinnow' mandarin) and 300 g tree^{-1}year^{-1}('Kinnow' mandarin) and 22-352 g Fe-humate ('Jaffa' sweet orange) have successfully removed Fe-deficiency (Srivastava and Singh, 1998).

3.7. Manganese deficiency

Manganese is absorbed in Mn^{2+} form. Manganese is a micronutrient, highly immobile in plant and extremely mobile in acid and waterlogged soils, but absorbed in Mn^{2+} Availability of Mn in soils is drastically reduced beyond soil pH 6.5, in addition to calcareousness, salinity/sodicity, low soil moisture content etc. Higher availability of Mn in soil could induce Fe-deficiency (Srivastava *et al*., 2008).Manganese plays following roles in plant metabolism i. indispensable for the synthesis of ascorbic acid by plants; required for activity of some dehydrogenases,

decarboxylases, kinases, oxidases peroxidases and non-specifically by other divalent, cation activated enzymes and required for photosynthetic evolution of O_2. ii. Involved in production of amino acid and proteins, and role in photosynthesis and chlorophyll formation and nitrate reduction. (Srivastava and Singh , 2006)

3.7.1. Visual symptoms

Manganese deficiency develops in following manner:

Fig. 7. Mn-deficiency: Fine network of green veins in the chlorotic yellow background.

Stage I: Mottled chlorosis with green veins with yellow or white leaf web tissue, appearing first on younger leaves and later spread to older leaves (Zekri, 1995c).

Stage II: Young leaves commonly show a fine pattern or network of green veins on a lighter green background, but the pattern will not be so distinct as in Zn or Fe deficiencies because the leaf is greener (Fig.7).

Stage III: By the time, the leaves reach full size; the pattern becomes more distinct as a band of green along the mid-rib and principal lateral veins with light green areas between the veins.

Stage IV: Interveinal leaf areas develop many whitish opaque spots which give the leaf, a whitish or grey appearance.

Stage V: In more severe cases, the colour of leaf becomes dull green or yellowish green along the mid rib and main lateral veins and pale and dull for the interveinal areas.

3.7.2. Correction

Foliar spray of $MnSO_4$ is more effective than soil application. Foliar spray of $MnSO_4$ at the rate of 0.10-0.15% ('Jaffa' sweet orange), 0.25% ('Kinnow' mandarin), and 0.50% ('Coorg' mandarin) have demonstrated remunerative results. While soil application of $MnSO_4$ ranging from 50 g $tree^{-1}year^{-1}$ ('Sathgudi' sweet orange), 300 g $tree^{-1}year^{-1}$ ('Nagpur' mandarin), 292-500 g $tree^{-1}year^{-1}$ ('Jaffa' sweet orange) proved very useful in alleviating Mn-deficiency.

3.8. Copper deficiency

Copper is absorbed in Cu^{2+} form. It is another micronutrient, highly mobile in low pH soils, but usually immobile in plant. Availability of Cu in soil is dependent on pH, organic matter content, presence of Al, Mo and Fe.Copper plays following roles in plant metabolism : i. an essential component of ascorbic acid oxidase, phenolase, laccase, diamine oxidase, urease, cytochrome oxidase and galactose oxidase; ii. role in carbohydrate metabolism and chlorophyll formation; and iii. reduced water movement on account of Cu-deficiency primarily due to collapse of xylem vessels, as an indirect role.

3.8.1 Visual symptoms

Deficiency of Cu develops in following progressive manner:

Stage I : Wilting of terminal shoots, frequently followed by death. Leaf color often fades. First symptom is the formation of unusually vigorous large dark green foliage.

Fig. 8. Cu-deficiency: Gummy secretions from fruits.

Stage II: Fruit symptoms are more pronounced. Brown stained areas of hardened gum on the rind of the fruit may precede the appearance of leaf and twig symptoms.

Stage III: Fruit splitting with a part of splitting starting at the blossom end in the usual way or around the gummy stained areas. Brown stained areas on the fruits will darken and turn black by the time of half.

Stage IV: In severe case, twigs will be covered with reddish brown droplets of gums, with likely fruit splitting (Fig.8).

3.8.2. Correction

Foliar application of Cu is most popular method of alleviating Cu-deficiency. Foliar spray of $CuSO_4$ at the rate of 0.10% ('Kinnow' mandarin), 0.12% ('Jaffa' sweet orange), 0.25-0.50% ('Coorg' mandarin and 'Mosambi' sweet orange), 0.30% ('Kagzi' lime) and 0.20% Cu-EDTA ('Pant' lemon) have produced good results. Soil application of $CuSO_4$ 100 g $tree^{-1}year^{-1}$ also proved useful in 'Sathgudi' sweet orange. (Srivastava and Singh,1998; 2003c)

3.9. Zinc deficiency

Zinc by plant is absorbed in Zn^{2+} form. Zinc is the most important micronutrient of global concern, and highly deficient nutrient of equal magnitude on both acid as well as alkaline soils. It is an immobile nutrient in plant. The availability of Zn in soil is adversely affected by soil calcareousness, high P content, salinity/sodicity, overliming etc.Zinc plays following metabolic roles : i. essential constituent of alcohol dehydrogenase, glutamic dehydrogenase, lactic dehydrogenase, carbonic anhydrase regulating equilibrium between carbon dioxide, water and carbonic acid, alkaline phosphatase, carboxypeptidase-β, and other enzymes, dehydropeptidase and glycylglycine dipeptidase for protein metabolism; ii. formation of auxin and regulating water relations; and iii. adds cell membrane an integrity, stabilizes sulflahydryl groups in membrane proteins involved in ion transport (Srivastava, 2012).

3.9.1. Visual symptoms

Zinc deficiency develops in following progression:

Stage I: Young growth is first affected, which is characterised by chlorotic and nacrotic leaves, later turning into smalling multiple sprouting trenching and resetting appearance.

(Srivastava and Singh, 2003c)

Stage II: Relative amount of green and yellow tissue vary from a condition of mild Zn deficiency in which there are only small yellow splotches between the larger lateral veins to a condition characterized by green basal portion of the mid-rib and the remainder of the leaf as yellow to white (zekri,1995c).

Stage III: Whitish chlorotic streaks between veins in older leaves and whitening of upper leaves are characterized by irregular green bands along the mid-rib and main vein on a background of light yellow to almost white (Fig.9).

Stage IV: In very acute stages, the leaves are pointed abnormally narrow with the tendency to stand upright, and extremely reduced in size.

Stage V: As the deficiency progresses, the leaves are affected over the entire periphery of the tree, and the twigs become very thin and die back rapidly.

Stage VI: Profuse development of water sprouts takes place on the main branches trunk and with leaves free from deficiency symptoms.

Stage VII: Trees display a dense growth at the centre with dying appearance over its periphery.

Fig. 9. Zn-deficiency: Smalling of leaves, typical interveinal chlorosis , trenching, rossetting.

3.9.2. Correction

Like other micronutrients, foliar spray is more useful over soil fertilization. Foliar spray of $ZnSO_4$ at the rate of 0.30-0.50% ('Kinnow' mandarin), 0.5% ('Nagpur' mandarin and 'Sathgudi' sweet orange), 0.30-0.60% ('Kagzi; lime), 0.15% ('Jaffa' sweet orange),1.0% ('Coorg' mandarin) and 0.40% Zn-EDTA ('Pant' lemon) has observed very effective in combating Zn-deficiency. On the other hand, soil application of $ZnSO_4$ at the rate of 50-100 g $tree^{-1}year^{-1}$('Sathgudi' sweet orange), 300 g $tree^{-1}year^{-1}$ ('Nagpur' mandarin), 250-1000 g $tree^{-1}year^{-1}$ ('Kinnow' mandarin), 500 g $tree^{-1}year^{-1}$('Blood' red sweet orange), 294-810 g $tree^{-1}year^{-1}$ ('Pant' lemon) and 10-15 g Zn-EDTA ('Jaffa' sweet orange) have proved very effective with regard to recovery of treated plants from Zn-deficiency.

3.10. Boron deficiency

Boron is absorbed in H_3BO_3 form. Next to zinc, boron is widely deficient nutrient. It is a micronutrient mobile in soil and immobile in plant. Availability of boron in soil is reduced on account of calcareousness, salinity/sodicity, overliming. Very little is known about mineral of B in soils.Boron plays many important roles in plant metabolism : i. role in translocation of sugars from leaves, an important step towards enhanced photosynthesis. The compounds, would more easily traverse cellular membrane than would the high polar sugar molecule themselves; ii. indirect evidence for involvement of B in carbohydrate transport, borate forms complexes with certain carbohydrates but natural borate complexes in plants yet to be identified; and. iii. role in flowering, pollen tube growth N metabolism, hormone activity in addition to maintenance of Ca in soluble form. (Srivastava *et al*., 2008)

3.10.1. Visual symptoms

Boron deficiency develops through following stages:

Fig. 10. B-deficiency: Swelling of mid-rib and associated veins.

Stage I: Apical meristems blacken and die, and with breakdown of meristimatic tissue (Fig.10).

Stage II: Terminal leaves turn necrotic which shed prematurely the internodes of terminal shoots remaining shortened rosette form.

Stage III: Thickened leaves having tendency of leaves to curl downward. Younger leaves show small water soaked, spots or flecks becoming translucent as the leaves mature.

Stage IV: Flower development and seed production are usually impaired coupled with death of terminal growing point of the main stem (Zekri,1995d).

Stage V: Fruit symptoms are more reliable as hard fruit and dry due to lumps formation in the rind caused by gum impregnations. Often brown pigmented spots are seen in the white albedo portion of fruits.

3.10.2. Correction

Boron deficiency can be corrected by soil application of borax at the rate of 30 g $tree^{-1}year^{-1}$ ('Sathgudi' sweet orange), 50 g $tree^{-1}year^{-1}$ ('Coorg' mandarin) and boric acid 40-120 g $tree^{-1}year^{-1}$ ('Nagpur' mandarin). Foliar application of borax 0.20% ('Kinnow' mandarin, 'Coorg' mandarin), 0.30% ('Kagzi' lime) and boric acid 0.60% ('Mosambi' sweet orange), 0.20-0.40% ('Kagzi' lime).

3.11. Molybdenum deficiency

Molybdenum is absorbed in MoO_4^{2-} form. It is of comparatively recent addition to list of essential nutrients. Bioavailability of Mo in soils is positively correlated with soil pH. It is absorbed by plants in an ionic form (MoO_4^{2-}) which exists in an exchangeable form in soil. Molybdenum is immobile soil and mobile in plant. Availability of Mo is of lower magnitude in acid soils (Srivastava, 2012). This nutrient has multiple roles in plant metabolism: i. constituent of nitrate reductase, xanthine oxidase; assists in the formation of proteins, starch, amino acid and vitamin; and ii. Strong role in nitrogen metabolism (Srivastava and Singh, 2006).

3.11.1. Visual symptoms

Deficiency of boron develops through following stages:

Stage I: Leaf blades fail to expand further.

Stage II: Appearance of light yellow chlorosis of leaves (Fig. 11).

Stage III: As the leaves grow, yellow spots will develop deposits of brown gum on the lower leaf surface which would turn black.

Stage IV: Under severe deficiency, symptoms are found on fruits, large irregular brown, spots surrounded with yellow discoloration may develop which goes with the peel without affecting albedo (Zekri,1995d).

Fig. 11. Mo-deficiency: Formation of yellow chlorotic spots in the interveinal areas.

3.11.2. Correction

Application of ammonium molybdate at the rate of 25 g $tree^{-1}$ as soil application ('Sathgudi' sweet orange) and foliar spray at 0.08-1.0% ('Sathgudi' sweet orange and 'Kinnow' mandarin) have shown promising results as effective corrective measures.

There are defined symptoms of nutrient excess.

Nitrogen excess is expressed through vigorous vegetative growth. Thick-skinned large puffy fruit, delayed maturity, regreening likely. Juice % and quality declines. Shorter storage life. The P-excess is characterized by smaller fruit, higher % juice, thinner peels, chance of regreening. While K- excess develops thicker rough rinds and chance of regreening (Fig.12). Delayed maturity in sweet .oranges

Fig. 12. K-excess: Formation of coarse textured peel on fruits.

Fig. 13. Fe-toxicity: Accretions formed along mid-rib and veins.

Fig. 14. Al-toxicity: Formation of slimy surface on leaves.

Iron as excess formation of accretions along mid-rib and emerging lateral veins. This is also known as iron toxicity symptons .The excess of manganese develops bright yellowing on margins of old leaves dark brown tar spots on leaves. Stunted roots and shoots are formed due to copper excess. Excess of boron is featured through yellow, dead leaf tips, leaf fall and dieback with reduced fruit yield. On the Al – toxicity is characterized by stunted root growth coupled with lack of root hairs.

4. Leaf nutrient/soil fertility norms

Leaf analysis integrates all the factors that might influence nutrient availability in soil and plant uptake, and pinpoints the nutritional balance of the plant at the time of sampling. The composition of the ash of plants varied with the part analysed, with the age of the plant, and with the soil upon which the plants grew. The concept of foliar diagnosis, which included a study on the course of nutrition as reflected by its intensity (sum of percentages of NPK) and by its quality (ratio of NPK) was developed. Leaf analysis as a means of determining the nutritional requirements of plants concluded that when all other factors are constant, plant growth is a function of two

variables of nutrition, intensity and balance, and maximum growth and yield occur only upon the coincidence of optimum intensity and balance.

The use of foliar diagnosis developed rapidly, at the first in the USA. Its use later spread to South Africa, then to Israel, and later gradually to other citrus growing countries. It was proposed a tentative leaf nutrient standard for the first time using 'Valencia' sweet orange, which were intended for adoption to individual situations and varieties. Determination of the quantity of nutrients present in the whole tree provided the information on the relative amount and distribution of nutrients within the tree with leaves accumulating the highest concentration.

Leaf analysis is advantageous over soil analysis in terms of analysing the concentration of metabolically active nutrients, fixing the fertilizer requirement, verifying the occurrence of nutrient deficiency or any nutrient imbalance, and determining whether or not fertilizers applied are utilised by the plant. Soil analysis on the other hand, has certain advantages over leaf analysis that it can measure the level of immediately available nutrients in the soil (nutrient intensity), the extent to which those will be available to crop/ during the growth period (nutrient capacity), and reliable for evaluating the salinity, alkalinity or even the nutrient toxicity. Soil analysis does help in assessing the fertilizer needs, but it does not help us to evaluate the efficiency or sufficiency of nutrient uptake to ensure optimum growth and productivity.

Majority of studies have demonstrated the better correlation of fruit yield and quality with leaf analysis values than soil analysis. However, leaf analysis alone presents certain limitations. The analysis fails to identify the problem of lime-induced chlorosis, evident from absence of correlation between leaf Fe and degree of chlorosis expressed as chlorophyll content. Another limitation of leaf analysis is the fact that sampling date is recommended.

Late in the growing season, generally close to harvest. At this point, it is no longer possible to correct nutritional disorders in time to avoid negative impact on fruit yield and quality. Leaf analysis is seldom able to distinguish the metabolic (active) forms of nutrients from non-metabolic (non-active) forms. The leaf analysis norms (Table 3) and soil fertility norms (Table 4) developed for different commerical citrus cultivars in India have further helped in identifying the nutrient constraints in citrus orchards and developing the constraints based fertilizer scheduling.

Diagnosis of nutrient constraints and their management are the two pillars of an efficient fertilizer management program. Maximising quality production through constraints- based use of nutrients is a well established fact that assumes a greater significance in fertilizer responsive perennial fruit crop like citrus. A large number of diagnostic tools, namely leaf analysis, soil testing, juice analysis, and biochemical markers-aided-analysis are under continuous scrutiny, test, recurrent use, and subsequent refinement. The nutrient diagnostics that have emerged through worldover research on different commercial citrus cultivars have lacked considerably in their universal applicability due to difference in interpretation tools used in developing the nutrient diagnostic norms, besides being influenced by many other factors in the outcome of the interpretation. A diagnostic tool is considered best for both diagnostic as well as prognostic testing that minimizes the influence of these over-riding factors, and produce uniformity in diagnosis when spaced over time. Out of different diagnostic methods in practice, only leaf analysis complimented by soil analysis has made some headway. Geo-referenced soil sampling has proved to be an effective tool in defining soil variability within an orchard. Once the critical soil properties are identified, the procedural steps can be evolved to address the inconsistency in fertilization response.

The utility of conventional leaf analysis as a diagnostic tool is often cut short due to strong influence of leaf age (crop development stage). The interpretation tools used in the past such as critical nutrient concentration and sufficient range system developed by using index leaves are applicable only to specified developmental stage of crop. The researches on DRIS with citrus as test crop have shown some distinct advantages over conventional leaf analysis-based interpretation tools in order to make diagnosis possible at any stage of crop development without loosing much the precision in application of norms under diverse growing conditions. The remediation of nutrient constraints has now found a new look in terms of automated fertigation, organic cultivation using a combination of bulky organic manures, and microbial biofertilizers, site specific nutrient management as a part of precision citriculture, all to be integrated to develop a more effective INM strategy. But above all these changes, still the major challenge lies on the relationship between inconsistency in response of fertilization and modulating the quality production, because of site specific nature of yield responses.

Table 3
Leaf analysis-based nutrient diagnostics in citrus.

Nutrients	Nagpur mandarin	Khasi mandarin	Kinnow mandarin	Mosambi sweet orange	Sathgudi sweet orange
N(%)	1.70 - 2.81	1.97 - 2.56	2.28 - 2.53	1.98 - 2.57	2.01 - 2.42
P(%)	0.09 - 0.15	0.09 - 0.10	0.10 - 0.13	0.091 - 0.17	0.09 - 0.12
K(%)	1.02 - 2.59	0.99 - 1.93	1.28 - 1.63	1.33 - 1.72	1.12 - 1.82
Ca(%)	1.80 - 3.28	1.97 - 2.49	2.12 - 3.12	1.73 - 2.98	1.93 - 2.73
Mg(%)	0.43 - 0.92	0.24 - 0.48	0.32 - 0.53	0.32 - 0.69	0.36 - 0.53
Fe(ppm)	74.9 - 113.4	84.6 - 249.0	52.3 - 89.4	69.5 - 137.1	53.5 - 82.1
Mn(ppm)	54.8 - 84.6	41.6 - 87.6	41.7 - 76.3	42.2 - 87.0	48.7 - 79.3
Cu(ppm)	9.8 - 17.6	2.13 - 14.4	6.1 - 10.3	6.6 - 15.8	3.7 - 8.9
Zn(ppm)	13.6 - 29.6	16.3 - 26.6	21.3 - 28.5	11.6 - 28.7	16.5 - 23.2
Yield(kg tree^{-1})	47.7 - 117.2	31.6 - 56.3	61.8 - 140.3	76.6 - 137.9	81.2 - 145.3

Source: Srivastava and Singh (2002; 2003a; 2005; 2008b).

Table 4
Soil analysis-based nutrient diagnostics in citrus.

Nutrients	Nagpur mandarin	Khasi mandarin	Kinnow mandarin	Mosambi sweet orange	Sathgudi sweet orange
N(mg kg^{-1})	94.8 - 154.8	161.0 - 418.7	118.2 - 128.4	107.4 - 197.2	120.1 - 152.2
P(mg kg^{-1})	6.6 - 15.9	4.5 - 8.7	9.4 - 16.3	8.6 - 15.8	10.1 - 12.3
K(mg kg^{-1})	146.8 - 311.9	82.3 - 287.5	158.3 - 208.2	186.4 - 389.2	162.3 - 206.4
Fe(mg kg^{-1})	10.9 - 25.2	39.5 - 180.9	3.1 - 9.3	4.8 - 17.3	11.2 - 16.4
Mn(mg kg^{-1})	7.5 - 23.2	27.0 - 80.3	4.8 - 7.3	7.7 - 15.7	10.1 - 18.3
Cu(mg kg^{-1})	2.5 - 5.1	0.67 – 2.90	0.58 - 1.25	1.76 - 4.70	2.2 - 3.6
Zn(mg kg^{-1})	0.59 - 1.26	2.84 – 5.14	0.64 - 0.98	0.44 - 1.03	0.54 - 1.10
Yield(kg tree^{-1})	47.7 - 117.2	31.6 - 56.3	61.8 - 140.3	76.6 - 137.9	81.2 - 145.3

Source: Srivastava and Singh (2002; 2003a; 2005; 2008b).

In the light of above discussion, it is seen that most of the current nutrient diagnostic tools are meant to address the nutritional deficits of next season crop instead of addressing the nutrient constraints in current standing crop. In this regard, biochemical marker (metalloenzymes) aided diagnosis holds some promise but due to highly technical and sensitive method, this method is not so widely practiced. And, identifying nutrient constraints in citrus based on the morphological changes, as phenotypic expression, they identify the nutrient constraints so late that an orchardist is left with no option as to attend the deficiency symptoms in standing crop. It is just an aid to carry out a post-mortem diagnosis (it takes 2-3 years, e.g., zinc deficiency to manifest into some distinct morphological changes evident from chlorosis pattern). In years to come unless, some early warning system in form of, may be some nutrient probe or sensor is developed, the realm of addressing nutrient constraints in current standing crop will not succeed in concrete terms. Non-destructive method of nutrient stress diagnosis using hyperspectral analysis for proximal sensing holds the best promise, for that matter.

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