

STUDIES ON THE BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS OF CITRUS DECLINE

By

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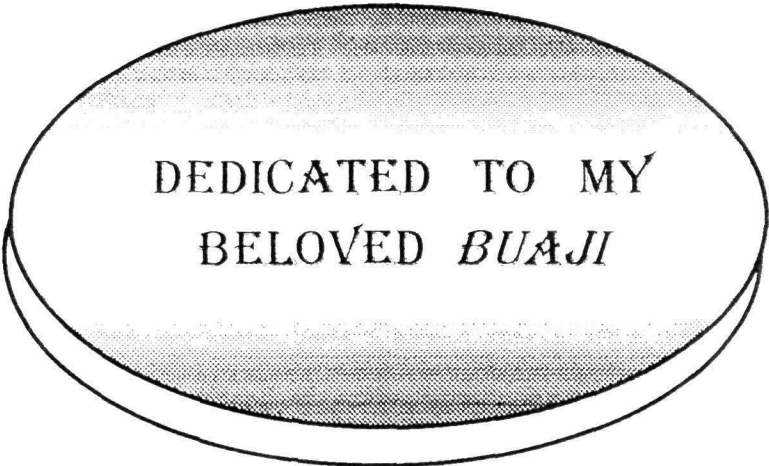
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CERTIFICATE - I

This is to certify that this dissertation entitled " **STUDIES ON THE BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS OF CITRUS DECLINE** ", submitted for the degree of Ph.D. in the subject of Horticulture to Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Mr. A. D. Huchche** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Ramesh K Arora

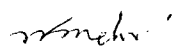
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Major Advisor

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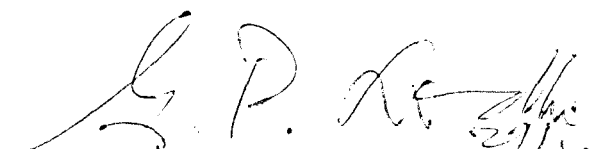
CERTIFICATE - II

This is to certify that this dissertation entitled " **STUDIES ON THE BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS OF CITRUS DECLINE** ", submitted by **Mr. A. D. Huchche** to Chaudhary Charan Singh Haryana Agricultural University, Hisar, in partial fulfillment of the requirements for the degree of Ph.D., in the subject of Horticulture has been approved by the Student's Advisory Committee.


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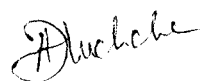
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Dated: July 27, 1999

Hisar



A. D. Huchche

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Citrus is the second most important fruit crop after grapes in area and production in the world. According to FAO (1997) statistics, the production of citrus fruits in the world touched an all time high of 93.478 million tonnes. Brazil emerged as the largest producer of citrus fruits (23.10 mt) followed by USA (15.621 mt). China (9.279 mt), India (4.55 mt) and Mexico (4.359 mt).

Various species of citrus fruits are widely cultivated in India. Among these, sweet oranges are grown commercially in Punjab, Haryana, Rajasthan, Maharashtra and Andhra Pradesh; mandarins in Assam, Karnataka, Maharashtra and Madhya Pradesh. Major producers of kagzi lime are Maharashtra, Tamil Nadu and Andhra Pradesh. However, the productivity of citrus orchards in India is 7-8 t/ha compared to 20-25 t/ha in major citrus producing countries of the world. This low productivity is due to citrus decline

which has been plaguing the citrus industry in India (Shyam Singh, 1997).

In Haryana state, the area under citrus fruits is estimated to be 4278 ha with a production of 37800 tonnes (Kartar Singh, 1998). Kinnow, a hybrid mandarin, (*Citrus nobilis* x *Citrus deliciosa*) is cultivated on a large scale for more than 35 years in India. It has achieved remarkable success in north India, Kodaikanal region of Tamil Nadu and Coorg in Karnataka. It has excelled other citrus fruits in yield performance and quality in Punjab. This variety is also totally free from granulation.

Kinnow trees perform satisfactorily in the initial years and start commercial production in the 6th to 9th years bearing 1000-1200 fruits/tree. After the 10th year the trees show slow growth with poor yields. Mandarins are particularly prone to decline. Arora et al. (1985) reported maximum incidence of decline in mandarin cultivars Honey (69.67%), Dancy (57.59%) and Kinnow (55.25%) followed by sweet orange cultivars Jaffa (50.44%) and Campbell Valencia (43.13%) and Pearl tangelo (51.37%). Within the same orchard, the healthy trees show better nutrient status than the declining trees (Sharma and Mahajan, 1990).

Declined trees do not usually die but remain impoverished and unproductive. The symptoms comprise of retarded growth of trees, appearance of chlorotic leaves, sparse foliage, dieback of twigs and in general, a sickly appearance of the tree. In most cases the trees continue to

linger on in a state of poor health for many years with a considerable reduction in fruit production. The problem of citrus decline being very widespread is causing huge losses to growers and therefore, deserves immediate attention.

Citrus decline is not a specific disease but is a symptomatic expression of many disorders in the plant. A large number of factors responsible for the citrus decline are soil pH, salt status, structure, free lime, presence of hard pan, poor drainage and soil erosion, nutrient deficiencies in calcareous soil, incompatible rootstocks, attack of insect pests and diseases, etc. (Shyam Singh, 1997). Besides these factors, specific levels of biochemical substances including enzymes play an important role in maintaining the health of the plant. The peroxidase was higher in various tristeza infected cultivars than in healthy controls (Bar-Joseph and Loebenstein, 1973).

The above mentioned factors lead to the changes in the nutritional, physiological and biochemical constituents in the plant. Keeping all these factors in view, the present studies were undertaken to find out the association of above factors to citrus decline with the following objective :

- i) To study the soil, nutritional, biochemical and physiological factors affecting the health of the Kinnow mandarin trees.

CHAPTER II

REVIEW OF LITERATURE

Citrus decline is attributed to a multiplicity of causes in different parts of citrus growing areas of the world. An overview of the research work done in various citrus fruits in citrus decline in India and abroad with reference to physiological, nutritional and biochemical aspects is taken in this chapter.

A. SOIL AND NUTRITIONAL FACTORS

Of the various factors which play a role in determining the success or failure of a citrus plantation, the condition of the soil is most important. Citrus decline is widely attributed to unfavourable soil conditions (Cheema and Bhat, 1928; Naidu and Rao 1958; Nauriyal and Chauhan 1966). Bhat (1945) and Naidu and Rao (1958) thought that a hard pan present in the subsoil was the main cause of this malady in the earstwhile Bombay and Andhra Pradesh, respectively. Failure of citrus orchards in Punjab was also reported due

to the presence of hard pans of calcium carbonate or clay (Kanwar and Randhawa, 1960).

I. Soil texture

The soil texture indicating the relative proportion of coarse and fine particles in soil plays an important role in proper root activity. The hard pan in the subsurface affects root activity and age of citrus orchards (Diware and Kolte, 1990).

Carbonate Kankar (granules) and calcium carbonate affect the physico-chemical characteristics of soil. The availability of nutrients was found to be related with CaCO_3 content in soil which leads to chlorosis in citrus (Kanwar and Randhawa, 1960; Dhingra and Kanwar, 1963; Chadda 1966; Randhawa *et al.*, 1966; Nair *et al.*, 1968; Malewar *et al.*, 1977; Mann *et al.*, 1978; El-Azhar *et al.*, 1980; Nilangekar and Patil, 1981b).

Mariakulandai and Durairaj (1958) and Malewar *et al.* (1977) reported that the poor growth leading to decline in citrus trees was due to the presence of coarse mechanical fractions whereas Srivastava *et al.* (1997) observed that some of the mandarin growing soils of Nagpur district having higher clay content (60-80%) mainly concentrated in subsurface, low coarse fragments, sand and CaCO_3 upto 150 cm depth were not suitable for Nagpur mandarin from regular flowering point of view.

The decline in citrus was also reported due to higher

clay loam content (Martin et al., 1958; Wiedenfeld et al., 1982), Changes in soil profile (Lyones and Rouse, 1979), soil variability (Haury, 1981), shallow soil (Burnett et al., 1982) and bad soil physical conditions (Ahmed and Jackson, 1965). However, Mann et al., (1978) could not find any relation between citrus decline and physico-chemical characteristics of soil.

II. Soil drainage and aeration

The high soil moisture, poor drainage and aeration and permeability are associated with high clay content of soil whereas light soils are not able to retain sufficient moisture. However, these soils have better aeration, drainage and water permeability.

Dikshit (1963) and Singh and Sharma (1975) reported that high soil moisture as well as poor aeration were the main factors associated with citrus decline. The citrus trees were also found to decline in water logged soils (Ahmed and Jackson, 1965), less water permeability and aeration in soil (Randhawa et al., 1966) and poor drainage (Kovanci et al., 1978). Nunez Moreno et al. (1994) reported slower rates of infiltration in low yielding citrus areas compared with high yielding citrus areas in Olympia, USA. The citrus blight was also found associated with moisture stress and poor drainage in soil (Burnett et al., 1982).

High water table was not present in many citrus growing areas of the country. It was only since the sixties that

it has risen within 4 to 5 feet of the surface. Citrus is sensitive to excessively moist soil conditions within its root zone and thrives best on soils that are well drained. Bhat (1995), Naik (1949) and Kanwar and Randhawa (1960) suggested that inadequate drainage was one of the important factors responsible for citrus decline in erstwhile Bombay, South India and Punjab, respectively. Excellent growth of citrus was reported in areas where the water table was below 1.5 m in all seasons (Singh *et al.*, 1962; Singh and Jawanda, 1963; Kanwar *et al.*, 1965). The effect of water table on depth and concentration of citrus roots and fruit yield was reported by Pearson and Goss (1953), Ford (1959) and Reitz and Lang (1955). It is possible to have successful citrus orchards even if the water table is within 60 cm of the surface but the table should be static. According to Chapman (1959, 1961) fluctuating water table is detrimental to orchard health. Under saturated soil conditions, aeration is generally too slow to meet the oxygen requirement of tree roots.

Poor drainage has also been reported to cause disturbances of nutritional balance in citrus trees (Haas, 1932 & 1939; Haas and Klotz, 1931). Chapman (1959) stated that iron chlorosis commonly developed on soils wherever moist soil conditions persisted over long periods.

III. Soil reaction

Soil reaction as indicated by pH has a marked influence

on soil environment, especially on the availability of plant nutrients. An ideal pH for citrus is considered to be between 5.5 to 7.5 because the soils in this pH range are more fertile and trouble free than those with higher and lower pH (Chadha *et al.*, 1970). However, a review of literature on citrus soils reveals that excellent citrus is being raised in soils ranging from highly acidic (pH 4.5) to those containing free lime (pH 8.5). On the basis of the preliminary surveys of citrus soils it was proposed that the pH of soil should not exceed 8.5 for successful growth of citrus. The citrus trees were found declining at high soil pH i.e. 9.5 and above (Bhella, 1966; Randhawa *et al.*, 1966; Nair *et al.*, 1968, Nijjar and Singh, 1971) whereas high soil acidity was a widespread constraint on yield and fruit quality in Sao Paulo, Brazil (Quaggio *et al.*, 1992).

IV. Excessive salts

Citrus trees are quite sensitive to excess of salts and are injured if the total salt concentration in the soil exceeds 1000 ppm (Rajput and Haribabu, 1985). The presence of excessive salts in any of the horizons upto a depth of 180 cm adversely affects the health of citrus plants (Nijjar and Singh, 1971; Nilangekar and Patil, 1981b). Kanwar and Dhingra (1961) found that soil salinity was one of the important causes of chlorosis in the Punjab.

The most conventionally used rootstocks in India are rough lemon followed by Rangpur lime, however, both the

rootstocks with their strains were generally found susceptible to excessive salt concentrations (upto 900 ppm chloride) in the soil (Awtar Singh, 1997).

Kanwar et al. (1965) proposed that the electrical conductivity of 1:2 soil water suspension should not be more than 0.5 mmhos/cm otherwise growth of citrus would be adversely affected. However, later it was thought that a limit of 0.8 dSm^{-1} was safe (Chadha et al., 1970). Nunez et al., 1994) observed low yielding orange orchards with EC values of 3.8 dSm^{-1} .

V. Organic matter

The soil organic fraction besides affecting fertility indirectly in many ways also has a very important direct function. It serves as a reserve of certain plant nutrients which become available through the activity of the microbial population. Findings of several workers as quoted by Hayes (1960) indicate that irrespective of the amount of nitrogen available, the trees do not flourish unless the supply of organic matter in the soil is maintained at a fairly high level.

It has been observed that soil organic matter besides carrying zinc, formed pockets of acid material in alkaline soils and retained this element in an available form (Camp and Fudge, 1945). The availability of rock phosphate which was added to the soil as fertilizer was increased by the presence of organic matter (Dalton et al., 1952). El-Azhar

et al. (1980) observed that maintaining a high level of organic matter in soil increased the citrus growth. The citrus trees generally declined due to low organic carbon (Nair et al., 1968; Nijjar and Singh, 1971; Nilangekar and Patil, 1981 a & b); Chauhan et al., 1984; Sharma et al., 1986; Pinckard, 1979).

VI. Nutritional factors

The importance of nutrition will become clear when the quantity of nutrient elements removed by citrus fruits from the soil is kept in mind. Chapman et al. (1945) reported that 18 t of citrus fruits remove 21 kg N, 5 kg P_2O_5 , 41 kg K_2O , 19 kg Ca, 3.6 kg Mg, 2.3 kg S, 45 g B, 9 g Cu, 50 g Fe, 13 g Mn and 13 g zinc. Dhatt et al. (1988) reported that a yield of 40 tonnes ha^{-1} of kinnow mandarin under Punjab conditions removed 96.0 kg N, 10.80 kg P and 78.80 kg K ha^{-1} from the soil annually. If losses due to leaching and erosion are also taken into consideration, the total annual depletion of plant food is even higher. Inadequate fertilization soon results in a decadent condition, earlier than in the case of many other important fruit trees and yield will be reduced considerably.

a. Nitrogen

If nitrogen is deficient when growth begins, the young leaves are undersized, thin and fragile and develop a very light green colour which may ultimately become yellow. Its

deficiency reduces growth rate and fruit production (Randhawa, 1970).

Soils containing 110 kg/acre of easily oxidisable nitrogen are rated low in available nitrogen and are expected to respond to supplemental applications. Mature leaves of citrus well supplied with nitrogen usually show values above 2.4% total nitrogen in the dry matter of the leaf (Chapman 1960, Embleton and Jones, 1963). There is considerable evidence to show that increasing applications of nitrogen significantly reduce the concentrations of P and K in leaves (Wallace *et al.*, 1952, Reitz and Koo, 1960; Layden, 1963), however, Smith *et al.* (1959) reported that nitrogen depressed K at low but not at high soil K levels both in leaves and roots. Deficiency of N in declined trees of citrus was widely reported (Dikshit, 1963; Kanwar *et al.*, 1963; Randhawa *et al.*, 1966; Nijjar and Singh, 1971; Mann *et al.*, 1978; Ahlawat *et al.*, 1982; Yamdagni *et al.*, 1983; Awasthi *et al.*, 1984, Coelho *et al.*, 1984; Dhillon and Dhat, 1988; Kohli *et al.*, 1997), however, Dey and Singha (1998) observed no differences in leaf N contents of healthy and declined trees of mandarin in Assam although many of the declined trees had lower leaf N content than those on healthy trees with both the trees showing sufficient levels of leaf N. On the contrary, Wutsher *et al.* (1981) reported higher leaf N concentrations in declining than in healthy grapefruit trees in South Texas.

b. Phosphorus

Young citrus trees deficient in phosphorus show reduced growth rate. The older leaves lose their deep green colour and lustre and become a faded green to bronze. Excessive leaf drop at the time of spring bloom and twig dieback are also indications of phosphorus deficiency. In Punjab, citrus soils containing less than 4 kg, 4-10 kg and greater than 10 kg/ acre sodium bicarbonate extractable phosphorus are considered low, medium and high respectively with respect to phosphorus (Randhawa, 1970). Chapman (1960) reported that the amount of phosphorus extractable with sodium bicarbonate should be more than 50 ppm in the soils supporting excellent citrus plants.

A higher level of phosphorus in soil was found to be related with citrus decline (Thorne and Wann, 1950; Kanwar and Randhawa, 1960; Nijjar and Singh, 1971; Malewar et al., 1977; Awasthi et al., 1984) whereas low to medium phosphorus was attributed to mandarin decline in Himachal Pradesh (Sharma and Mahajan, 1990) and in North Cachar Hills in Assam (Dey and Singha, 1998).

Embleton and Jones (1964) reported 0.09 to 0.11% phosphorus in leaf in deficient range, 0.12 to 0.16% in optimum and above 0.17% in excess range. The citrus trees leading to decline were observed to contain higher levels of foliar phosphorus contents (Singh and Singh, 1953; Kanwar

and Randhawa, 1960; Randhawa *et al.*, 1967; Mann *et al.*, 1979, Wutscher, 1983; Ahlawat *et al.*, 1982; Yamdagni *et al.*, 1983) whereas Sekhon *et al.* (1977), Coelho *et al.* (1984), Dhillon and Dhatt (1988) and Dey and Singha (1998) observed low foliar phosphorus levels in declining citrus trees. Perra and Perra (1981) observed increased levels of phosphorus in lemon with increasing salinity.

c. Potassium

Chlorosis and gumming of twigs, the dwarfing, curling and puckering of leaves and the premature dropping of fruit are other symptoms associated with K deficiency.

In citrus orchards of Punjab, potash application was recommended only when ammonium acetate extractable potassium (K_2O) was less than 57 kg/acre (Randhawa, 1970). Chapman *et al.* (1947) reported that citrus plants could secure sufficient potassium from a soil which had only 2.5 ppm potassium whereas 117 ppm potassium had no ill effect on the plants. A concentration of 310 ppm in the soil, however, killed the plants. Awasthi *et al.* (1984), Sharma and Mahajan (1990) in Himachal Pradesh and Dey and Singha (1998) in Assam hills observed low to medium soil potassium levels in declined mandarin orchards compared to healthy orchards.

According to Embleton and Jones (1963) leaf potassium between 0.7 and 1.1% was considered to be in the low range, between 1.2 and 1.7% to be in the optimum whereas 1.8% concentration was considered as high. A high to excess level

of leaf potassium was found to be associated with declined citrus trees (Randhawa *et al.*, 1967; Nijjar and Singh, 1971; Sekhon *et al.*, 1977; Mann *et al.*, 1979, Wutscher *et al.*, 1980; Arora *et al.*, 1981; Ahlawat *et al.*, 1982; Reddy and Sharma, 1982, Yamdagni *et al.*, 1983) whereas Coelho *et al.* (1980) in Sao Paulo, Brazil, Awasthi *et al.* (1984) and Sharma and Mahajan (1990) in Himachal Pradesh and Dhillon and Dhatt (1988) in Punjab observed low potassium levels in leaves of declined trees compared to healthy ones.

The blight affected citrus trees were also found to contain low level of potassium (Wutscher and Hardesty, 1979) while Wutscher *et al.* (1980) reported that citrus trees at one site in Texas contained lower level of potassium than healthy ones and at another site they contained higher leaf potassium levels over healthy trees.

d. Calcium

Apart from being an essential element in the plant system calcium is also a soil improving agent. Citrus trees contain more calcium than any other mineral plant nutrient.

Excess of lime in soil is harmful. Excess of calcium may be present in the soil or may result by excess application of lime. Soil with excess calcium becomes alkaline, leading to trace element deficiencies such as Fe, Zn, Cu and Mn. Phosphorus also becomes less available under such conditions. Deficient citrus trees have usually less than 1 per cent Ca in the dry matter of leaves whereas the

normal content of mature hardened leaves is from 2 per cent to as high as 7 per cent with progressive aging of leaf tissues (Chapman, 1959).

The citrus trees declined at low level of exchangeable calcium (Kanwar and Randhawa, 1960 and Kanwar and Dhingra, 1961) and at low available calcium (Nilangekar and Patil, 1981a and b, Dey and Singha, 1998). However, Coelho *et al.* (1984) in Sao Paulo, Brazil and Nunez *et al.* (1994) in semiarid north Mexico reported that high calcium content in soil was associated with citrus decline.

The calcium deficiency was observed in the declined citrus trees (Kanwar *et al.*, 1963; Sekhon *et al.*, 1977; Mann *et al.*, 1979; Prasad *et al.*, 1981; Reddy and Sharma, 1982; Dey and Singha, 1998) whereas Anderson and Calvert (1970) and Coelho *et al.* (1984) observed an increase of calcium in citrus trees by increasing severity of decline. However, Ahlawat *et al.* (1982) and Yamdagni *et al.* (1983) observed no marked difference in calcium status in the declined and healthy trees of citrus. The citrus trees suffering with blight were reported to contain lower level of calcium than healthy trees (Wutscher *et al.*, 1980a, Wutscher, 1983)

e. Magnesium

Magnesium deficiency is noticed most severely in highly acidic soils where it is leached out and does not occur ordinarily under alkaline conditions. There exists an

antagonism between K & Ca to Mg under the field conditions. Thus, the excess presence of Ca or K may lead to Mg deficiency. According to Chapman (1947), excessive P may also cause Mg deficiency although it may be found in sufficient quantity in soil. Dey and Singha (1998) observed that low fruit yields were associated with lower concentrations of available Ca in soils, low foliar Ca content and the low foliar Ca: Mg ratio in the north Cachar hills of Assam. Foliar K was regulated by the ratio of K : Ca+Mg or K: Mg in soils in north Cachar hills. Similarly foliar Ca was regulated by soil ratios of K: Mg in north Cachar and Karbi Anglong hills.

The higher level of exchangeable magnesium in soil was observed in healthy as compared to chlorotic citrus orchard (Nilangekar and Patil, 1981a; Sharma et al., 1986; Nunez et al., 1994) whereas Nilangekar and Patil (1981b) reported a marginal difference in water soluble magnesium of healthy and chlorotic citrus orchard profiles.

Randhawa et al. (1966) and Sekhon et al. (1977) reported that low magnesium was associated with the decline of citrus trees whereas Yamdagni et al. (1983) in Haryana and Sharma et al. (1986) in Jammu observed higher level of magnesium in chloritic than healthy trees. However, no marked difference in magnesium level was reported between healthy and declined citrus trees (Kanwar et al., 1963; Prasad et al., 1981; Reddy and Sharma, 1982). The blight affected citrus trees were observed to contain high level of

magnesium in their tissue (Wutscher *et al.*, 1980 a & b).

f. Zinc

Zinc is the component of number of metal enzymes including several dehydrogenases, alcohol dehydrogenase and lactic dehydrogenase. It is required for the synthesis of auxin and its concentration has inverse relation with the activity of an enzyme ribonuclease.

The availability of zinc in soil is controlled by chemical properties of soil and its deficiency affects the plant growth. Grewal *et al.* (1967) reported 0.5 ppm on the critical limit of dithizone-extractable Zn in soils of Punjab and Haryana. Leaves from trees showing deficiency symptoms had less than 15 ppm Zn on dry weight basis. The deficiency of Zn in soil was observed to cause decline in citrus (Mukherjee, 1949; Kanwar and Randhawa, 1960, Dikshit, 1963; Ahmed and Jackson, 1965; Bhella, 1966; Chadda, 1966; Randhawa *et al.*, 1967. Bandad *et al.* 1977; Ercivan and Karaca, 1979; Oxyang *et al.*, 1984; Raina 1988; Sharma and Mahajan, 1990). However, the chlorosis was not found to be associated with Zn deficiency in soil of citrus orchards (Singh and Sharma, 1975; Kovanci *et al.*, 1978 and Nilangekar and Patil, 1981b).

Low to deficient levels of foliar zinc contents have widely been reported in declined citrus trees (Randhawa *et al.*, 1967; Nair *et al.*, 1968; Anderson and Calvert, 1970, Nijjar and Singh, 1971; Singh and sharma, 1975; Malewar *et*

al., 1977; Sekhon *et al.*, 1977; Ercivan and Karaca, 1979; Mann *et al.*, 1979; Prasad *et al.*, 1981; Ahlawat *et al.*, 1982; Reddy and Sharma, 1982; Chauhan *et al.*, 1984; Sharma *et al.*, 1986 and Sharma and Mahajan, 1990) whereas higher level of zinc was also observed in declined than healthy citrus trees (Albrigo and Young, 1979, Graca and Vuuran, 1979). Kovanci *et al.* (1978) and Yamdagni *et al.* (1983) observed no marked difference in zinc content in chlorotic and healthy trees of citrus.

The higher level of foliar zinc was recorded in blight affected citrus trees (Young and Garnesey, 1978; Cohen and Wutscher, 1979, Wutscher *et al.*, 1980b; Wutscher *et al.*, 1982 a&b, Wutscher, 1983; Brlansky *et al.*, 1984; William and Albrigo, 1984 and Brlansky *et al.*, 1985). On the contrary Wutscher *et al.* (1980a) reported that zinc level decreased in citrus trees affected with blight. Under salt stress conditions Perra and Perra (1981) reported that zinc level was higher in lemon.

g. Iron

The iron plays an important role in electron transport system of the plants. A good correlation has been found between iron content and chlorophyll. It also acts as a catalyst in the enzymes found in plants in combination with protein like organic bodies.

The iron is abundantly available in upper layer of soil and its availability is related with soil conditions. Some

of the soil factors which influence iron chlorosis are excessive free lime, excessive soil moisture, excessive phosphorus, zinc, copper and manganese and possibly soil microorganisms (Randhawa, 1970).

The low availability of iron in soils was found to be associated with citrus decline (Thorne and Wann, 1950; Randhawa, 1970; Ahmed and Jackson, 1965; Chadda 1966; Nijjar and Singh, 1971). The excess of iron in soil is also the causal factor of citrus decline (Singh and Sharma, 1975; Aoba and Sekiya, 1976). Bhella (1966) reported iron deficiency in soils of declined orchards of various fruit trees. Yadav et al. (1997) observed Fe-deficiency more pronounced in monsoon flushes vis-a-vis spring flushes of Nagpur mandarin.

Deficient foliar contents of iron were associated with the decline of citrus trees (Kanwar and Randhawa, 1960; Nijjar and Singh, 1971; Bandad et al., 1977; Reddy and Sharma 1982) whereas no marked difference was found in iron level in declined and healthy trees of citrus (Singh and Sharma, 1975; Kovanci et al., 1978; Mann et al., 1979). Prasad et al. (1981) observed high foliar levels of iron in declining mandarin orchards of north-eastern India. Similarly Wutscher (1980a, 1983) observed higher foliar levels of iron in citrus trees affected with blight.

h. Manganese

The manganese in soil is an indispensable microelement

for the plant growth. Its deficiency as well as excess in soil cause growth reduction in plants.

Sherman and Harmer (1942) suggested that 3 ppm of exchangeable plus 100 ppm of reducible Mn was critical and below this, response to Mn applications could be expected. Raina (1988) observed deficient levels of Mn 2.5 ppm in the subsurface depths of citrus orchards of Dhaulakuan, Himachal Pradesh. The manganese deficiency was most common in declined citrus trees (Randhawa et al., 1967; Nair et al., 1968; Anderson and Calvert, 1970; Nijjar and Singh, 1971, Malewar et al., 1977; Sekhan et al., 1977; Reddy and Sharma, 1982, Wuwel et al., 1998) whereas manganese level was found higher in declined citrus trees (Kanwar and Randhawa, 1960; Randhawa et al., 1966; Coelho et al., 1984; Sharma et al., 1986). Kovanci et al. (1978), Mann et al. (1979) and Yamdagni et al. (1983), however, observed no marked differences in manganese content in chlorotic and healthy citrus trees.

The blight affected citrus trees were found to contain low level of manganese in their tissues (Wuscher et al., 1980a). On the contrary, William and Albrigo (1984) observed a higher level of manganese in citrus trees affected with blight as well as Perra and Perra (1981) reported higher level of manganese in lemon grown under salt stress conditions.

i. Copper

The copper is an essential microelement for the plant growth. It is an oxidising agent in soil as well as in plant systems. Analysis of citrus soil in Punjab and Haryana indicated that available copper (Cu) content of some of these soils was in the deficient range, however, leaf analysis did not reveal its deficiency. Perhaps annual Bordeaux sprays were meeting the Cu requirements of the plants (Randhawa, 1970).

Low levels of foliar copper contents were found to be associated with citrus decline (Singh and Singh, 1953; Randhawa *et al.*, 1967; Malewar *et al.*, 1977; Sekhon *et al.*, 1977 and Wuwei *et al.*; 1998) whereas Prasad *et al.*, 1981, Yamdagni *et al.*, 1983 and Coelho *et al.*, 1984 observed higher levels of copper in declined than in healthy citrus trees. On the contrary no marked differences in foliar copper contents in declined and healthy citrus trees were observed (Singh and Sharma, 1975; Kovanci *et al.*, 1978; Mann *et al.*, 1979).

Wutscher *et al.* (1980) observed lower levels of Cu at one site and higher levels at the other site in blight affected grapefruit trees in Texas. Wutscher (1983) and William and Albrigo (1984) also observed higher levels of foliar copper in blight affected citrus trees.

B. PLANT GROWTH AND YIELD

Any disorder caused by nutritional imbalance affects the plant growth like shoot growth, leaf area, leaf quality, plant water status, fresh and dry weight, flowering and fruiting.

I. Tree volume

The normal tree growth is an important feature of a good healthy plant and it varies with the nature of abnormalities in the plant. Shoot dieback and stunted growth of the plants leading to reduced tree volume as the time progresses is commonly observed in citrus decline (Chadha, 1970, Azeri, 1981; Randhawa and Srivastava, 1986). The percentage of decline varies with the age of the orchard, type of soil, nutrition, water status, etc. (Diware and Kolte, 1990). Similarly, the saline soil conditions also lead to reduced tree vigour (Furr and Ream, 1988; Patil and Bhambhota, 1978).

II. Leaf area

The leaf is an important photosynthesizing organ in a plant. The leaf area of a plant has a direct bearing on the vigour and productivity of the plant. With the reduction in leaf area the plant growth is affected severely. Reduction in leaf area in citrus was reported by Kanwar and Randhawa (1960). Blight affected citrus trees had fewer and smaller

leaves, less leaf area/tree than the healthy trees (Wutscher *et al.*, 1978; Syvertsen *et al.*, 1980). Soil salinization was also reported to affect the leaf area (Patil and Bhambhota, 1978).

The leaf area index is also a major factor determining canopy conductance and total evaporation. Canopy does not necessarily increase in proportion to leaf area index. The proportion of shaded leaves increases with increasing leaf area and these leaves have lower stomatal conductance (Jones and Cumming, 1984).

III. Stock-scion ratio

Bud union disorders have been reported from various parts of the world (Salibe and Moreira, 1961). Bridge and Youtsey (1996) reported the bud union abnormalities of rough lemon rootstock with sweet orange scion and scion/stock incompatibility was observed by Forner *et al.* (1977) in citrus. Bakhshi and Dhillon (1964) reported that a bud union crease followed in later stage by separation of outer bark was commonly met within the declining trees of some sweet orange varieties in Punjab. Apart from creasing, the declining sweet orange trees on rough lemon in Haryana also exhibit a certain degree of swelling at the union point. Whether overgrowth at the union is a symptom of incompatibility has been a highly controversial question. Goldschmidt (1956) reported that the swelling at the union though independent of abnormalities in the union structure

could be related to the degree of incompatibility of Shamanti orange on nine different rootstock trials.

IV. Fruit size and yield

The fruit size and yield are directly related to the health and vigour of the plant. The cropping and fruit quality in declined trees deteriorates seriously (Chadha *et al.*, 1970). There is a conspicuous reduction in the size of fruits, an increasing roughness and lack of shine of the rind and a decrease in juice content. Kanwar and Randhawa (1960) also reported that citrus fruits from declined trees were small and malformed whereas Burnett *et al.* (1982) observed that blight affected citrus trees produced fruits normal in appearance but reduced in size and sometimes irregular in shape.

C. PHYSIOLOGICAL CHARACTERISTICS

I. Fresh and dry weight

The fresh and dry weight of plants varies with the nature of disorder in plants. The fluctuating soil water table was associated with reduced fresh weight of trees of grapefruit (Pearson *et al.*, 1967).

The functional water status is a prerequisite for physiological processes and variation in water status affects the plant growth. Albrigo and Young (1979) and Burnett *et al.* (1982) reported that water content decreased

in blight affected declined citrus trees. On the contrary, Syvertsen *et al.* (1980) reported that there was no apparent change in water status in blight affected citrus trees whereas Wutscher *et al.* (1980) observed a significant reduction in water status of blight affected citrus trees in Florida but it did not vary between healthy and declined trees in Texas. Wutscher *et al.* (1980) and Brlansky *et al.* (1984, 1985) were of the view that water status in blight affected citrus trees reduced as the water uptake also reduced.

II. Leaf chlorophyll

The green leaves containing chlorophyll and carotenoids at the optimum level are associated with various biochemical and physiological processes in plants requiring essential elements. Carotenoids and phytol side chain of chlorophyll share with mevalonate as a common precursor in the isoprenoid pathway. The fading of leaf chlorophyll commonly named chlorosis is a good indicator of the nutritional imbalance in plant system.

The leaf chlorosis is one of the first symptoms of the nutritional status of plant leading to its decline. Shirely *et al.*, (1990) made quantitative estimation and identification of carotenoids in the citrus leaves and reported that these were qualitatively similar but quantitatively different in various species of citrus. Bhutani (1968) studied the biochemical differences in

healthy and declined citrus trees and reported reduction of chlorophyll-a and chlorophyll-b in declined chlorotic trees. Bacha et al. (1994) reported high leaf chlorophyll contents in Balady orange as a result of irrigation at four days interval. Rao et al. (1977) reported reduction in amounts of chlorophyll-a and chlorophyll-b in declined chlorotic trees. Bacha et al. (1994) reported high leaf chlorophyll contents in Balady orange as a result of irrigation at four days interval. Rao et al. (1977) reported reduction in amounts of chlorophyll-a and chlorophyll-b in declined citrus plants, whereas, Devi et al. (1997) reported low leaf chlorophyll contents in chlorosis affected citrus plants.

III. Stomatal conductance and transpiration

It is useful to distinguish between the water transpired by the leaves and all other water evaporated by crops, whether from the soil or from free water on the surface of the leaves. Only the transpired water is involved in the flux dependent lowering of leaf water potential. Rate of transpiration is affected by several factors such as radiation balance, ambient humidity and stomatal conductance.

Syvertsen and Smith (1984) reported that the leaves of apple, peach and citrus which were in the exterior canopy positions remain folded along with the midrib whereas the interior canopy leaves are often more horizontal which affect the functional reaction availability. Syvertsen et

al. (1980) observed lower stomatal conductances and lower transpiration rates in the blight affected sweet orange trees than the healthy trees. At equivalent transpirational flushes leaf water potential was much lower in blight affected trees than in healthy trees.

The stomata provide the dominant short term physiological control over evaporation whereas leaf area is equally important for long term control. A humidity response is probably involved in gradual closure of stomata during the day which has been observed in citrus (Cohen and Cohen, 1983). Ray and Sharma (1989) pointed out that it was not always easy to draw any definite conclusion regarding water status of the plants simply by measuring the pore length, width and its area but it can be assessed by means of the number of stomata per unit area.

IV. Photosynthesis

Plants exhibit considerable differences in their photosynthetic response to temperature regimes of the respective environments. For the most parts, these differences are based on selective modifications to the constituents of the photosynthetic apparatus that may limit the tolerance as well as determine the capacity of photosynthesis of specific regions of the temperature spectrum. For example, ability to sustain photosynthesis at higher temperature is likely to depend on the presence of heat stable protein synthesizing system which enables the

plants to replace or to repair components that are impaired by heat (McWilliam and Ferrar, 1974). There are also significant differences between species of higher plants in the response of chlorophyll biosynthesis to both high (Feirabend and Mikus, 1976) and to low (Smillie, 1976) temperature.

The optimum temperature for photosynthesis is between 15°C to 45°C for most of the temperate and tropical species (Pearcy, 1977) but this optimum can be shifted in either direction depending on soil moisture and/or light conditions. In the field the light and temperature surrounding the leaves can be influenced by the training system adopted.

Since the photosynthetic efficiency of the plants is associated with functional chlorophyll apparatus, the deficiency of essential minerals in this photosynthetic apparatus impairs the photosynthetic efficiency of the plant. Devi et al. (1998) reported increased chlorophyll contents and photosynthetic activity in plants treated with combinations of mineral elements Zn, Fe and Mn in sweet orange at Coimbatore.

D. BIOCHEMICAL CHARACTERISTICS

I. Enzyme activities

Tomiyama and Stahmann (1964) found acceleration and an increase in the activity of polyphenol oxidase and

peroxidase in potato tuber tissues infected by *Phytophthora infestans*. Pathogenic effects of fungus *Marasmius permicius* may be associated with auxin inactivating system (Peroxidase) (Krupasagar and Sequeiera, 1969). Bouilleva and Gaspar (1970) observed that peroxidase in extracts of Crown gall tissues of *Impatiens balsamina* was higher than in other tissues.

The peroxidase activity was higher in various tristeza infected cvs. than in healthy controls (Bar-Joseph and Loebenstein, 1973). In infected roots of Egyptian sour time, there was an increase in peroxidase activity. Pacetto and Grasso (1969) reported that there was no significant difference in the activity of polyphenol oxidase in sour orange seedlings which are susceptible to Malsecco, Moro and Torocco sweet orange seedlings which are resistant. Pilet (1961). reported that enzymatic degradation of IAA was mediated by peroxidases and other oxidative systems although there was not always a good correlation between peroxidases and IAA oxidase activity of different tissues.

The enzymatic activity was positively related to the level of the corresponding metal in the substrate and to the growth response of the trees (Bar-Akiva, 1968). Ishigavi (1971) reported that polyphenol oxidase and catalase activities were higher in the ammonia fed plants. Weinberg (1970) found that enzyme pattern of peroxidase from roots of salt grown plants was altered in that two of the five detectable isozymes migrated a little more slowly than those

in extracts from nonsaline plant tissues.

Diseased sandal leaves exhibiting spike symptoms, had a higher level of polyphenolase activity (Parthasarathi et al., 1970). Huising and Durbin (1970) observed about 1000-2000% increase in peroxidase activity in tomatoes infected with *Agrobacterium rhizogenus* than in control.

II. Starch and sugars

Carbohydrates are synthesized in the leaves and translocated to the growing sites and root system through phloem.

A marked depletion of starch in sour orange rootstock (Fawcett, 1946) and rough lemon rootstock (Chauhan, 1968) having sweet orange scion inflicted with quick decline disease was reported. Similarly starch reduction due to phloem necrosis below and above the bud union in the tristeza affected sweet orange trees was reported by Schneider (1954). Mosse (1962) observed differences in phloem starch contents in virus infected stock and scions, however, Mendel and Cohen (1967) did not find any difference in starch levels of the two components of incompatible combinations of Shamouti orange on sweet lime. Schneider (1960) observed accumulation of starch in Eureka lemon scion and its depletion in the various rootstocks used on the basis of conditions of the phloem tissue at the point of union.

Jones and Steinacker (1951) reported that the leaves

and twigs of sweet orange contained four to five times as much sugar as starch and that sugar was high in the young leaves. Sugar level reduced to a minimum level during September to November. In aged leaves this reduction is probably associated with leaf maturation, translocation and fruit growth. Beginning in December, there was a rapid increase in sugar which reached maximum in late January which is preceded by a large increase in Starch accumulation. A new growth started in April and May when there was a decrease in sugar. Sharples and Burkhort (1954) reported that considerable amount of starch accumulated in the leaves during the winter dormancy. Goldschmidt and Blumental (1956) observed that the reduced growth and vigour in certain stionic combinations of citrus was accompanied by disorder in the xylem near the bud union zone and by the starch and sugar accumulation in scion.

Mantoo (1975) observed increase in leaf sugar contents in declined sweet orange trees whereas Singh and Verma (1977) reported decrease in total, reducing and nonreducing sugars in leaves of declined citrus trees affected by tristeza and greening.

III. Phenols

Phenolic compounds include many substances such as phenolic glycosides of potential biological activity, which play an important role in plants. Pridham and Saltmarsh (1960) suggested that phenolic compounds possibly act as

soluble reserves of less common sugars such as L-rhamnose, L-arabinose etc. The total phenolic content of the bark of psorosis affected Valencia was increased. Leaves of infected trees registered a sharp increase in caffeic acid, quercetin, eriodictyol and scopoletin whereas only sinapic acid increased in the roots.

Many phenolic compounds even in the trace form are phytotoxic in nature and glycosidation greatly reduces their phytotoxicity. Almost all higher plants investigated so far have been shown to have ability to convert phenyl-alanine tyrosine to p-coumaric acid by phenylalanine ammonialyase tyrosine ammonia-lyase enzyme respectively (Young et al., 1966). The accumulation of phenols took place in all plant tissues infected by fungal, bacterial and viral diseases (Farkas and Kiraly, 1962). Monselise and Goren (1965) found reduction of hesperidin in xyloporosis as well as exocortis affected Shamauti orange and increase in the total phenolic content of bark of psorosis affected Valencia orange. Schwarz (1968) reported a fluorescent phenolic compound gentisoyl- β -D-glucose to be present in the bark and fruit albedo of greening affected citrus trees. Graca et al. (1979) observed high levels of water soluble phenols in the bark of the young tree decline trees.

IV. Proteins

The results of Wildman et al. (1969) and Takahashi and Ishii (1952) indicate that Tobacco Mosaic Vines (TMV)

multiplication may be accompanied by a concurrent decrease in the concentration of normal leaf protein. More investigations by Bawden and Kleczkowski (1957) showed that the concentration of normal protein was not consistently affected by virus infection. Commoner et al. (1955) observed that virus synthesis is associated with a net increase of proteins in TMV.

Commoner and Dietz (1952) reported that leaf tissues infected with TMV and cultured in water contained less non-protein nitrogen than comparable uninfected tissues during the time when TMV was being formed. This deficiency disappeared when virus formation ceased. The largest difference between infected and uninfected tissues occurred in ammonia content. Smaller, but significant differences in amide content were also found. The data indicated that the bulk of the N incorporated into virus protein was withdrawn from the leaf's pool of soluble N. Virus was formed from ammonia-N and non-nitrogenous carbon sources. The effect of virus infection on host N metabolism appeared to be due to the formation of virus rather than its presence. Monselise and Goren (1965) reported that total N and protein N contents of leaves and bark were found to be essentially identical for all classes of psorosis infected Valencia orange trees as compared to control.

CHAPTER III

MATERIALS AND METHODS

The present investigations were carried out during 1998-99 in a fifteen years old Kinnow mandarin orchard of Central State Farm, Hisar planted at a spacing of 6 x 6 m on rough lemon (*Citrus jambhiri*) rootstock. The Hisar is located at 29.10°N latitude and 74.4°E altitude in the semiarid area of northern India. The average rainfall of Hisar is 350 mm with maximum temperatures of 45-48°C in May and minimum temperatures of 0.6°C in December.

Ten trees each with more than 80 per cent healthy and 80 per cent declined status were earmarked in December, 1997. The declined trees were apparently in debilitated state of health as compared to vigorously growing healthy trees. There was preponderance of dead wood with many chlorotic, senescent foliage in declined trees as against a profuse lush green foliage with no apparent dead wood in healthy trees. Data was collected on various physiological and biochemical parameters at various stages of plant growth.

A. SOIL AND NUTRITIONAL ANALYSES

Various physico-chemical characteristics of soils collected from under the canopy of healthy and declined trees were studied. The soil samples were collected from five plants each of the healthy and declined trees from the area between three fourth of the radius of the tree canopy to the drip line of the tree canopy. The samples were collected with the help of the soil auger from different soil depths viz. 0-30 cm, 30-60 cm and 60-90 cm in the month of February, 1998. The soil samples were collected from four sides of the experimental tree and a composite soil sample was prepared from depth. The samples were air dried and ground with wooden pestle and mortar to pass through a 2 mm stainless steel sieve.

I. Soil physical and mechanical properties

The physical characteristics of the soil samples were determined by the standard procedures given below.

i) Sand, silt and clay

The soil saturation percentage (SP) of the soil was determined by the method of Richards (1954). Using the soil saturation percentage, the values of soil clay, silt and sand percentage were worked out by regression equation given by Dahiya et al. (1987).

$$\begin{aligned}
 \text{Silt + Clay (\%)} &= -213.74 + 68.10 \ln SP \\
 \text{Clay (\%)} &= -122.30 + 38.40 \ln SP \\
 \text{Silt (\%)} &= (\text{Silt+Clay}) - \text{clay} \\
 \text{Sand (\%)} &= 100 - (\text{Silt} + \text{Clay})
 \end{aligned}$$

where $\ln SP$ = Natural logarithm of soil saturation percentage

ii) Field capacity, wilting point and available water content

The per cent field capacity (FC), wilting point (WP) and available water content (AWC) were determined from soil saturation percentage by using the following regression equations developed by Dahiya et al. (1988a).

$$\begin{aligned}
 FC &= -66.58 + 23.24 \ln SP \\
 WP &= -38.75 + 12.53 \ln SP \\
 AWC &= -27.54 + 10.59 \ln SP
 \end{aligned}$$

where $\ln SP$ = Natural logarithm of soil saturation percentage

iii) Cation exchange capacity (CEC)

The cation exchange capacity was determined from soil saturation percentage by the regression equation developed by Dahiya et al. (1988b).

$$CEC \text{ (Meq/100g)} = -56.94 + 18.70 \ln SP$$

where $\ln SP$ = Natural logarithm of soil saturation percentage

iv) Soil pH

The soil pH was measured from the soil saturation

extract as per the procedure given by Ricahrds (1968) on pH meter.

v) Soil EC

The electrical conductivity of the soil saturation extract was measured with the help of electrical conductivity meter (Richards, 1968) and expressed as dSm^{-1} .

vi) Calcium carbonate

The calcium carbonate in soil sample was determined by the method of Puri (1949) and expressed as per cent.

vii) Organic carbon

Organic carbon was determined by the method of Walkley and Black reported by Jackson (1973) and expressed as per cent.

II. Soil nutrient analyses

Various nutrient elements in the soil extracts were analysed by the following methods and the values were compared with standards reported by various workers in table 1.

i) Available nitrogen

The available nitrogen (ppm) in soil was determined by the method of Subbiah and Asija (1956).

Table 1. Critical limits (ppm) of available nutrient elements for assessing the fertility status of soils

Nutrient element	Critical limit	Medium range	High	Reference
N	< 120	120-240	240	Subbiah & Asija (1956)
P	12.4	12.4-22.4	22.4	Olsen et al., (1954)
K	113	113-280	280	Jackson (1973)
Zn	0.8	-	-	Lindsay & Norvell (1978)
Cu	0.2	-	-	Lindsay & Norvell (1978)
Fe	4.5	-	-	Lindsay & Norvell (1978)
Mn	1.0	-	-	Lindsay & Norvell (1978)

Table 2. Leaf analysis standards for citrus in Florida (4-6 months old leaves on non-fruiting terminals)

Element	Deficient	Low	Optimum	High	Excess
N (%)	< 2.2	2.2-2.4	2.5-2.7	2.8-3.0	> 3.0
P (%)	< 0.09	0.09-0.11	0.12-0.16	0.17-0.29	> 0.3
K (%)	< 0.7	0.7-1.1	1.2-1.7	1.8-2.3	> 2.4
Ca (%)	< 1.5	1.5-2.9	3.0-4.9	5.0-6.9	> 5.0
Mg (%)	< 0.20	0.20-0.29	0.30-0.49	0.50-0.70	> 0.8
Zn (ppm)	< 17	18-24	25-100	101-300	> 300
Fe (ppm)	< 35	36-59	60-120	121-200	> 200
Mn (ppm)	< 17	18-24	25-100	101-300	> 500
Cu (ppm)	< 3	3-4	5-16	17-20	> 20

Koo et al. (1984)

ii) Available phosphorus

The available phosphorus (ppm) in soil was determined by following the procedure of Olsen *et al.* (1954).

iii) Available potassium

The soil available potassium (ppm) was determined from the soil saturation extract on flame photometer.

iv) Calcium and magnesium

The concentration of these elements in soil saturation extract was determined by 'Versenate' method of Chang and Bray (1951).

v) DTPA extractable zinc, iron, manganese and copper

The DTPA (Diethylene triamine pentaacetic acid) extract of soil was prepared (Lindsay and Norvell, 1978) and the concentration (ppm) of these micronutrients was determined with the help of inductively coupled plasma (ICP) analyser.

III. Leaf nutrient analyses

For determining leaf nutrient status seven months old healthy leaf samples from nonfruiting terminals were collected and washed with running tap water followed by 0.1% HCl and two passings through distilled water. The washed leaf samples were surface dried and then oven dried at 70°C for 48 hours. The dried samples were ground and sieved

through muslin cloth.

Digestion of plant material: 0.5 g of ground leaf sample was taken in a conical flask and 12 ml of diacid mixture (H_2SO_4 : HClO_4 in 9:1 ratio) was added to it for digestion on a hot plate by the method described by Jackson (1967) for determination of N, P, K, Ca and Mg.

For digestion of micronutrients viz. Zn, Fe, Mn and Cu, 0.5 g of ground plant tissue was taken in a conical flask and it was digested on a hot plate by adding 12 ml of diacid mixture (HNO_3 : HClO_4 in 4:1 ratio) as per the procedure described by Piper (1966). The total volume of both the aliquots for macro and microelements was made to 50 ml.

The nutrient contents in the leaf samples were determined by following methods and compared with the leaf analysis standards for citrus in Florida, as reported by Koo et al. (1984) in table 2.

i) Nitrogen

The nitrogen content was determined by the method described by Jackson (1973).

Reagents:

i) Sodium silicate (10%) solution: 10 g of sodium silicate was dissolved in 100 ml distilled water and filtered.

ii) Sodium hydroxide (10%) solution: 1.0 g sodium

hydroxide was dissolved in 100 ml distilled water and filtered.

iii) Nessler's reagent: 100 g of mercuric iodide (HgI_2) and 70 g of potassium iodide (KI) were dissolved in 400 ml of water. 100 g NaOH was dissolved in another 400 ml of water. The two solutions were mixed and the volume was made to one litre with distilled water.

Procedure: 0.2 ml of aliquot was taken into 50 ml volumetric flask. To it 1 ml of sodium silicate and 0.5 ml of 10% NaOH was added. Neck washing was done after addition of every reagent and the contents were mixed thoroughly. To it 2 ml of Nessler's reagent was added dropwise while shaking. The solution was allowed to stand for 30 minutes at room temperature and final volume was made to 50 ml. The optical density was measured at 420 nm against the reagent blank on Spectronic-20 spectrophotometer. A standard curve was prepared from graded concentrations of NH_4Cl . The nitrogen content was calculated and expressed as g/100g (%) on dry weight basis.

ii) Phosphorus

The phosphorus content was determined by vanado-molybdo phosphoric acid yellow colour method described by Jackson (1973).

iii) Potassium

Potassium content was determined from the digested extract on flame photometer. The contents were calculated and expressed in per cent on dry weight basis.

iv) Calcium and magnesium

Calcium and magnesium were determined by Versenate method (Piper, 1970).

v) Zinc, iron, manganese and copper

The digested plant samples were analysed for determining zinc, iron, manganese and copper concentrations on inductively coupled plasma (ICP) analyser and the tissue contents were expressed in ppm.

IV. PHLOEM BARK NUTRIENT ANALYSES

The phloem bark samples were collected at full bloom stage in April and at the time of leaf sampling in September for nutrient status and were analysed as per the same procedures as for leaf nutrient analysis.

B. GROWTH, YIELD AND FRUIT QUALITY

I. Tree volume

Observations on growth parameters viz. plant height, spread and stock-scion girth were recorded in January, 1998

before the spring flush. The volume of the trees was worked out using the following formula (Castle, 1983).

$$\text{Tree volume (m}^3\text{)} = 0.5236 D^2 h$$

where,

$$0.5236 = \text{Constant}$$

D = Diametrical spread of the tree canopy (m)

h = Height of the tree (m)

II. Leaf area (cm²)

Ten fully expanded leaves of 4-5 months age were collected from second to third position from the top of the shoot terminal in each of the healthy and declined trees. The leaves were washed with tap water and surface dried. The area was recorded in cm² on a leaf area meter.

III. Yield

Number of fruits per tree were recorded in both healthy and declined trees. Average weight was worked out by weighing 25 randomly picked fruits. Yield per tree was calculated by multiplying the number of fruits with the average weight.

IV. Tree efficiency

Tree efficiency (%) was worked out according to the formula given by Castle (1983).

$$\text{Tree efficiency (\%)} = \frac{\text{yield}}{\text{canopy volume}} \times 100$$

V. PHYSICO-CHEMICAL CHARACTERISTICS OF FRUITS

i) Physical characteristics

Physical characteristics like average fruit weight, fruit size with respect to length and breadth, fruit firmness in terms of pressure (kg/cm^2), per cent juice content on weight by weight basis and rind thickness (mm) were recorded.

ii) Chemical characteristics:

a. Total soluble solids (TSS) : The TSS of the representative fruit juice was determined by using hand refractometer (0-32% range) at room temperature.

b. Acid content: The titratable acidity was determined by the method given by AOAC (1990). Two milliliters of freshly extracted juice was titrated against N/10 NaOH using phenolphthalein (1%) as an indicator. The appearance of the light pink colour was taken as an end point. The acidity of the fruit was expressed as gramme of citric acid per 100 ml of juice (per cent).

c. TSS/Acid ratio: TSS/Acid ratio was calculated by dividing total soluble solids by acidity.,

d. **Ascorbic acid:** Ascorbic acid was estimated as per the method given by AOAC (1990). Two ml of fruit juice was mixed with about 2 ml of 3% metaphosphoric acid as buffer. It was titrated with 2,6-dichlorophenol indophenol dye till the light pink colour appeared. The results were expressed as mg of ascorbic acid per 100 ml of juice.

e. **Sugar content :** Sugar content in fruit juice viz. total, reducing and nonreducing sugars were estimated as per the method given by Hulme and Narain (1931). It was expressed in per cent of juice.

C. PHYSIOLOGICAL STUDIES

I. Moisture content

The fresh and dry weights of leaf and bark samples were taken to work out the moisture content (%). Moisture content was worked out by following formula.

$$\text{Moisture (\%)} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Fresh weight (g)}}$$

II. Dry matter content

The dry matter percentage was estimated according to the following formula.

$$\text{Dry matter(\%)} = \frac{\text{Dry weight (g)}}{\text{Fresh weight (g)}} \times 100$$

III. Chlorophyll and carotenoid pigments

Chlorophyll pigments viz. chlorophyll-a chlorophyll-b, total chlorophyll and carotenoids were estimated in fully expanded leaves (three months old spring flush leaves) by the method given by Welburn (1994) and expressed in terms of μ moles/g of leaf tissue.

Extraction of pigments : Four discs (of 0.28 cm^2 area) of the sample leaves were suspended in a test tube containing 15 ml of dimethyl sulphoxide (DMSO). The weight of the four discs was recorded before putting them into the test tubes. The test tubes were then placed in an oven at 60°C for four hours to facilitate the extraction of pigments.

Estimation : After the incubation period for extraction, the test tubes were cooled to room temperature and the optical density was read at 454, 645 and 665 nm on a Spectrophotometer (Spectronic-20). DMSO was used as blank.

The chlorophyll pigments were calculated by using following equations:

$$\text{Chlorophyll-a } (\mu\text{g/ml}) = 12.19 A_{665} - 3.45 A_{645}$$

$$\text{Chlorophyll-b } (\mu\text{g/ml}) = 21.99 A_{645} - 5.32 A_{665}$$

$$\text{Total chlorophyll } (\mu\text{g/ml}) = \text{chlorophyll a} + \text{chlorophyll b}$$

$$\text{Carotenoids } (\mu\text{g/ml}) = \frac{(1000 A_{454} - 2.86 \text{ Chlorophyll a} - 129.2 \text{ Chlorophyll-b})}{221}$$

221

Quantity of all the above pigments was calculated in mg g^{-1} tissue fresh weight and expressed as μ moles g^{-1} tissue

fresh weight by using the following relationships:

$$\mu \text{ moles of chlorophyll a} = \mu\text{g chlorophyll a} \times 1.119$$

$$\mu \text{ moles of chlorophyll b} = \mu\text{g chlorophyll b} \times 1.102$$

$$\mu \text{ moles of carotenoids} = \mu\text{g carotenoids} \times 1.89$$

$$\mu \text{ moles of total chlorophyll} =$$

$$\text{chlorophyll a } (\mu \text{ moles}) + \text{chlorophyll b } (\mu\text{moles})$$

IV. Leaf temperature, rate of photosynthesis, stomatal conductance and transpiration

Leaf temperature ($^{\circ}\text{C}$), rate of photosynthesis (μ moles $\text{CO}_2 \text{ m}^{-2}\text{s}^{-1}$), stomatal conductance (millimoles $\text{m}^{-2}\text{s}^{-1}$) and transpiration (millimoles $\text{m}^{-1}\text{s}^{-1}$) were simultaneously measured in the fully expanded leaves in the month of May with the help of a portable PP photosynthesis system CIRAS-I.

D. BIOCHEMICAL STUDIES

The spring flush leaves from shoot apices were sampled at bud burst (March) and full bloom (April) stages and the phloem bark samples were collected from the scion and rootstock portions from five cms above and below the stock-scion union at full bloom stage (April) and in the month of September.

I. Peroxidase enzyme activity

The peroxidase enzyme activity was determined in the leaves of spring flush shoot apices at bud burst (March)

and full bloom (April) stages.

i) Preparation of enzyme extract: The enzyme extract was prepared according to the method given by Boland et al. (1978). The fresh leaves were washed with distilled water and stored under low temperature (4-5°C). The leaves were homogenously macerated twice with three and five ml of cold 0.05 M TRIS-HCl buffer (pH 7.6) in a chilled mortar and pestle. The homogenate was centrifuged at 10000 x g for 20 minutes at 0-4°C. The supernatant obtained was used for assaying peroxidase enzyme.

ii) Enzyme assay: Peroxidase activity was assayed by the modified method of Shanon et al. (1966). The reaction mixture contained 0.2 ml of 0.2% O-dianisidine in methanol; 0.1 ml of 0.1% H₂O₂, 3.6 ml of 50 mM phosphate buffer (pH 6.5) and properly diluted aliquots of enzyme extract (0.1 ml). The reaction was initiated by the addition of H₂O₂ and the change in optical density (O.D) was noted at 430 nm on Spectronic-20 spectrophotometer.

A unit of enzyme activity was defined as a change in optical density of reaction mixture per gram of fresh weight of leaf per second.

II. Analyses of biochemical constituents

For the analysis of various sugar fractions, starch, phenols and protein contents the leaf samples of apical

shoots were collected in March, April and September and were dried at 70°C, ground and sieved through a muslin cloth. Similarly, bark samples taken from five centimeters above and below the union of stock and scion in April and September were also dried and ground to pass through muslin cloth.

i) Sugars

Extraction of sugars: 0.1 g of dried sample was taken in 5 ml of 50% ethanol, allowed to stand for ten minutes and centrifuged at 4000xg for 15 minutes. The supernatant was taken and the residue was again reextracted twice with 5 ml of 80% ethanol and centrifuged. All the above supernatants were collected and the final volume was made to 20 ml with 80% ethanol.

Estimation of sugars:

a. Total sugars

Total sugars were estimated by the method of Yemm and Willis (1954).

Reagents : Anthrone reagent (0.2%) was prepared in 70% H₂SO₄. It was freshly prepared every time and allowed to stand for 30 to 40 minutes before use under low temperature (4-5°C) in a refrigerator.

Estimation : Five ml of anthrone reagent was pipetted into test tubes and chilled in ice cold water. Ethanol was evaporated from the samples (0.2 ml) and then it was

dissolved in one ml of distilled water. The solution was layered on the anthrone reagent, cooled for five minutes and thoroughly mixed by holding the test tube in the ice cold water. The tubes were closed with cotton plug, heated in a vigorously boiling water bath for 10 minutes and then immediately cooled in ice cold water. The optical density was then recorded at 600 nm against a standard blank on Spectronic-20 spectrophotometer. The amount of sugar was calculated with reference to sucrose standard curve (20-200 μg).

b. Reducing sugars

Reducing sugars were estimated by the modified Somogy's method (Nelson, 1944 and Somogy, 1945).

Reagents:

1. Copper reagent A : It was prepared by dissolving 25 g of anhydrous Na_2CO_3 , 25 g of Rochelle salt (sodium-potassium tartrate), 200 g of NaHCO_3 and 200 g of anhydrous Na_2SO_4 in about 800 ml of distilled water and diluted to one litre.
2. Copper reagent B : 15 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was dissolved in 100 ml of distilled water containing two drops of concentrated H_2SO_4 .
3. Arsenomolybdate reagent:
 - i) 25 g of ammonium molybdate was dissolved in 450 ml of distilled water and 21 ml of Conc. H_2SO_4 was then

added with stirring.

ii) 3g of sodium hydrogen arsenate was dissolved in 25 ml of distilled water.

Both the above solutions were mixed and the volume was made to 500 ml. the solution was kept in an incubator at 37°C for 24 hours before use. This reagent was then stored in a brown glass bottle.

4. Reagent C: This reagent was prepared by mixing the copper reagent A and copper reagent B in the ratio of 25:1 before use.

Estimation of reducing sugars:

The estimation of reducing sugars was done from the above ethanol extract. The 0.2 ml of extract was taken and after evaporating ethanol, it was dissolved in 1 ml of double distilled water. To this, 1 ml of reagent C was added in each test tube. These were heated for 20 minutes in a boiling water bath and then cooled under tap water. One ml of arsenomolybdate reagent was then added, mixed thoroughly and the mixture was diluted to 10 ml. A stable blue colour was read at 520 nm on Spectronic-20 spectrophotometer.

The amount of reducing sugars was calculated with reference to a glucose standard curve (10-100 μ g).

c. Non-reducing sugars

The content of non-reducing sugars was calculated as the difference between the amount of total sugars and

reducing sugars.

ii) Starch estimation

Starch was estimated by the slightly modified method of Cleg (1956).

Extraction of starch : Residue obtained after extraction of sugars was stirred with 2.5 ml of 26% HClO_4 and was left overnight at room temperature. The contents were stirred continuously for five minutes and then occasionally for the next 15 minutes. The solution was centrifuged at 4000xg for 20 minutes, the supernatant was taken in different glass test tubes of 10 ml.

Estimation : The glucose content in the above solution was estimated by the method of Yemm and Willis (1954). Starch content was arrived at by using the relationship given below

$$\text{Starch (\%)} = \text{Glucose (\%)} \times 0.95$$

iii) Proteins

Soluble proteins were estimated by the modified method of Lowry et al. (1951).

a. Soluble proteins (in 0.3 N NaOH)

Extraction: 0.1 g of dried sample was taken in 5 ml of 50% ethanol and centrifuged at 4000xg for 15 minutes followed by another centrifugation with 5 ml of 80% ethanol. The supernatant was discarded and the residue was treated with 5

ml of 0.3N NaOH and was left for 24 hours at 30°C. The residue after 24 hours of incubation at 30°C was centrifuged at 5000 x g for 20 minutes. The supernatant was taken and volume was made 10 ml with 0.3 N NaOH.

Reagents

- i) 2% sodium carbonate solution in 0.1 N NaOH.
- ii) 0.5% copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) solution in 1% sodium citrate solution.
- iii) Alkaline copper solution: 1 ml of (ii) + 50 ml of (i). It was used within 24 hours after mixing.

Estimation of proteins: 0.1 ml of extract (containing about 20-200 μg protein) was taken in different test tubes, distilled water was added to make the volume 1 ml with blank containing 1 ml of distilled water. Three ml of alkaline copper reagent was added and tubes were shaken well. After ten minutes 0.5 ml of Folin's reagent was added, the tubes were shaken well and allowed to develop blue colour. Absorbance was read after 30 minutes of the reaction at 660 nm on Spectronic-20 spectrophotometer and the amount of protein was calculated from standard curve using bovine serum albumin.

b. Insoluble proteins (in 0.3 N NaOH)

Extraction : Insoluble proteins were estimated from the residue left after the extraction of soluble proteins by

adding 2.5 ml of 1N NaOH and keeping it overnight under low temperature. The solution was centrifuged at 5000xg for 20 minutes, supernatant was taken and used for the estimation of insoluble proteins.

Estimation : Estimation of insoluble proteins was carried out in a similar way as in case of soluble proteins.

c. Total proteins

Total proteins were arrived at by simply adding soluble and insoluble proteins.

iv) Phenols

The total phenols were estimated by the method of Amorium et al. (1977) using Folin Ciocalteu phenol reagent

Extraction of phenols: The extract obtained for total sugars was also used for the estimation of total phenols.

Reagents

- i) Folin-Ciocalteu's phenol reagent (1N)
- ii) Sodium carbonate solution (20%). It was prepared by dissolving 20 g sodium carbonate in 100 ml distilled water.

Estimation : One ml aliquot was taken from the extract and to it 4 ml distilled water was added. To this 0.5 ml Folin Ciocalteu reagent was added and mixed properly. After 5 minutes, 0.5 ml of 20% Na_2CO_3 solution was added. It was

mixed vigorously and allowed to stand for one hour. The total volume was made to 10 ml with distilled water. The optical density was read at 750 nm against reagent blank. Standard curve was prepared using graded concentrations of tannic acid. The values were expressed in g per 100 g of dry weight (%).

STATISTICAL ANALYSES

The statistical analyses of the data were done by two sampled 'T' test, two factors and three factors factorial randomized block design depending upon the nature of data.

CHAPTER IV

EXPERIMENTAL RESULTS

The present investigations entitled "Studies on biochemical and physiological aspects of citrus decline", were carried out to find out the differences with respect to various nutritional, physiological and biochemical factors affecting tree health in the healthy and declined trees of Kinnow mandarin. The findings are divided into following heads:

- A. Soil and nutritional aspects
- B. Growth, yield and fruit quality
- C. Physiological aspects
- D. Biochemical aspects

A. SOIL AND NUTRITIONAL ASPECTS

I. Soil characteristics

a. Mechanical properties

The mechanical properties of soil indicating clay, sand

and silt content along with other factors are presented in table 3. The data indicate that the soil of the experimental orchard was loamy sand with 7.14% clay, 84.18% sand and 8.68% silt with the absence of CaCO_3 content. The cation exchange capacity in the top profile (0-30 cm) was 6.39 meq/100g soil which decreased in the next 30 cm depth i.e. in the 30-60 cm depth (5.35 meq/100 g) and again increased to 6.55 meq/100 g in the 60-90 cm depth. Similarly, field capacity, wilting point and available water content showed more values in the upper (0-30 cm) and lower (60-90 cm) soil depth compared to the values in the middle soil depth (30-60 cm).

b. Soil physicochemical properties

The physicochemical properties of soils at various depths under the canopy of the healthy and declined Kinnow mandarin trees are presented in table 4.

i) Soil pH

The soil pH values differed significantly in healthy (8.45) and declined (8.30) trees irrespective of soil depth. There was no significant difference in pH values at 0-30 and 30-60 cm soil depths, however, significantly higher pH (8.53) was observed at the depth of 60-90cm. The interaction of soil pH between health status and soil depth was non-significant.

Table 3. Mechanical properties of soil profiles in the experimental orchard of Kinnow mandarin

Soil property	Soil depth (cm)			
	0-30	35-60	60-90	Mean
Clay (%)	7.74	5.60	8.07	7.14
Sand (%)	83.12	86.91	82.53	84.18
Silt (%)	9.14	7.49	9.40	8.68
Calcium carbonate				
CEC (meq/100g soil)	6.39	5.35	6.55	6.10
Field capacity (%)	12.12	10.83	12.32	11.76
Wilting point (%)	3.68	2.98	3.79	3.48
Available water content (%)	8.32	7.73	8.41	8.15

Table 4. Soil physicochemical properties of healthy and declined trees of Kinnow mandarin

Soil depths	Physicochemical properties								
	pH			Electrical conductivity (dSm ⁻¹)			Organic carbon (%)		
	Healthy	Declined	Mean	Healthy	Declined	Mean	Healthy	Declined	Mean
0-30	8.31	8.22	8.27	0.046	0.072	0.059	0.42	0.31	0.37
30-60	8.46	8.19	8.33	0.062	0.052	0.057	0.20	0.17	0.19
60-90	8.57	8.49	8.53	0.066	0.064	0.065	0.22	0.14	0.18
Mean	8.45	8.30		0.058	0.063		0.28	0.21	
C.D. at 5%									
Health status	0.10*			N.S.			0.06		
Soil depth	0.13*			N.S.			0.11*		
Status x Depth	N.S			0.016*			N.S.		

*C.D. at 1% level; N.S. = Non-Significant

ii) Soil EC

There was no significant difference in the mean values of soil EC for healthy and declined trees. The differences between means of all the three soil depths were also non-significant. However, the interaction between soil depths and health status of the plants was significant at 0-30 cm depth with mean EC values of 0.046 dSm^{-1} for healthy and 0.072 dSm^{-1} for declined trees.

iii) Organic carbon

The organic carbon content of the soils varied significantly between healthy and declined trees irrespective of soil depth. The soils under the healthy trees contained 33% more organic carbon than the soils under the declined trees. The mean organic carbon content at 0-30 cm soil depth differed significantly relative to higher depths. The differences between the two higher depths were nonsignificant. The interactions of various depths with the health status were also non-significant.

c. Macronutrient status of soil

The data on the available macronutrients at various soil depths is presented in table 5.

i) Soil nitrogen content : There were no significant differences in the available nitrogen contents in soil between the healthy and declined trees as well as among the various soil depths. However, the values for all the trees

Table 5. Macronutrient status of soils of healthy and declined trees of Kinnow mandarin

Soil depth (cm)	Soil macronutrient content (ppm)											
	Nitrogen			Phosphorus			Potassium			Calcium		
	Healthy	Declined	Mean	Healthy	Declined	Mean	Healthy	Declined	Mean	Healthy	Declined	Mean
0 - 30	69.2	86.8	78.0	14.4	20.4	17.4	206.8	132.4	169.6	1120	1120	1120
							120.0			64.4		92.2
30-60	71.4	82.6	77.0	7.4	8.6	8.0	210.0	149.4	179.7	960	976	968
							105.6			100.8		103.2
60-90	77.0	65.8	71.4	5.8	6.4	6.1	232.6	154.2	193.4	1520	1296	1408
							168.0			144.0		156.0
Mean	72.5	78.4		9.2	11.8		216.5	145.3		1200	1130	
							131.2			103.1		

C.D. at 5%

Status N.S.

N.S.

34.7*

N.S.

N.S.

Depth N.S.

6.0*

N.S.

219**

52.59

Status x Depth N.S.

N.S.

N.S.

N.S.

N.S.

*Critical difference at 1%;

N.S. = Non-significant

were below the optimum range.

ii) Soil phosphorus content : There was no significant difference between the mean values of soil phosphorus content in healthy (9.2 ppm) and declined (11.8 ppm) trees. Phosphorus content at 0-30 cm depth was, however, significantly more (17.4 ppm) than at the depths of 30-60 (8.0 ppm) and 60-90 cm (6.1 ppm)

iii) Soil potassium content : The mean available potassium content in the soil of healthy plants was significantly more (216.5 ppm) than that in the soil of declined trees (145.3 ppm). The differences in the available potassium contents at various soil depths as well as their interaction with the health status of the trees were nonsignificant.

iv) Soil calcium content : There was no significant difference in the available calcium content of the soil under healthy and declined trees, however, the calcium content at higher depth of 60-90 cm was more (1408 ppm) relative to 0-30 (1120 ppm) and 30-60 cm (968 ppm) depths. The interaction between soil depth and health status was nonsignificant.

v) Soil magnesium content : The difference between the mean available magnesium contents of healthy (131.2 ppm) and declined (103.1 ppm) tree soils was nonsignificant. However, the mean magnesium level at depth 60-90 cm (156 ppm) was significantly more over 0-30 and 30-60 cm depths.

d. Soil micronutrient status

The data pertaining to the DTPA extractable soil micronutrient contents is presented in table 6.

i) Soil zinc content : The zinc status of soils under healthy and declined trees was at par with each other. There was, however, variation in the zinc status when different depths were compared. At 0-30 cm depth the zinc content was significantly more (0.638 ppm) than at 30-60 cm depth (0.191 ppm) which was also significant over the soil depth 60-90 cm (0.064 ppm). The interaction effect between health status of trees and soil depth was nonsignificant.

ii) Soil iron content: The difference between the means of healthy (1.247 ppm) and declined trees (1.369 ppm) was nonsignificant. The iron content at 0-30 cm soil depth was significantly more (1.564 ppm) than either at 30-60 cm (1.232 ppm) or 60-90 cm (1.128 ppm) soil depths. The interaction between health status and soil depth was nonsignificant.

iii) Soil manganese content : The difference in the soil manganese content in healthy (1.996 ppm) and declined (2.133 ppm) trees was nonsignificant irrespective of soil depth. There was no significant variation in the soil manganese contents at various soil depths. The interaction between soil depth and health status was also nonsignificant.



Table 6. DTPA extractable micronutrient contents in soil profiles of healthy and declined trees of Kinnow mandarin

Soil Depth (cm)	Soil micronutrient contents (ppm)											
	Zinc			Iron			Manganese			Copper		
	Healthy	Declined	Mean	Healthy	Declined	Mean	Healthy	Declined	Mean	Healthy	Declined	Mean
0-30	0.664	0.612	0.638	1.496	1.632	1.564	2.24	2.056	2.148	0.272	0.238	0.255
30-60	0.138	0.244	0.191	1.064	1.400	1.232	2.00	2.116	2.058	0.056	0.078	0.067
60-90	0.056	0.072	0.064	1.180	1.076	1.128	1.75	2.228	1.988	0.048	0.040	0.046
Mean	0.286	0.309		1.247	1.369		2.00	2.133		0.125	0.120	
C.D. at 5%												
Status (S)		N.S.			N.S.			N.S.			N.S.	
Depth (D)		0.25*			0.26			N.S.			N.S.	0.085
S x D		N.S.			N.S.			N.S.			N.S.	

*Critical difference at 5%; N.S. = Non-significant

iv) Soil copper content : The soil copper content did not vary between healthy and declined trees, however, significant variation was observed among the various soil depths. The soil copper content was significantly higher (0.255 ppm) at 0-30 cm soil depth relative to other two depths.

II. Plant nutrient status

a. Leaf nutrient status

The data pertaining to leaf macro and micronutrient contents in healthy and declined tree of Kinnow mandarin are presented in table 7.

i) Leaf nitrogen content : The mean leaf nitrogen content in healthy trees was significantly more (2.20%) than in declined trees (1.81%) although both the values were lower than the optimum value for leaf nitrogen content (2.5%).

ii) Leaf phosphorus content: There was no significant difference between the leaf phosphorus content of healthy (0.17%) and declined (0.18%) trees which were in the optimum range.

iii) Leaf potassium content : The leaf potassium contents in healthy (0.70%) and declined (0.68%) trees were lower than the optimum levels (1.2-1.7%), the difference being nonsignificant.

Table 7. Leaf nutrient status of healthy and declined trees of Kinnow mandarin

Nutrient element	Concentration in leaf		Significance
	Healthy	Declined	
Macronutrients			
N (%)	2.20	1.81	**
P (%)	0.17	0.18	N.S.
K (%)	0.70	0.68	N.S.
Ca (%)	3.62	3.34	*
Mg (%)	0.49	0.35	N.S.
Micronutrients			
Zn (ppm)	25.81	19.17	*
Fe (ppm)	198.20	144.60	**
Mn (ppm)	35.90	45.70	**
Cu (ppm)	13.27	18.44	*

* Significant at 5% level

** Significant at 1% level

N.S.= Non-significant

iv) Leaf calcium content: The leaf calcium content in healthy trees (3.62%) differed significantly relative to declined trees (3.34%) which were in the optimum range.

v) Leaf magnesium content : The leaf magnesium content in healthy (0.49%) and declined (0.35%) trees did not differ significantly and both the values were in optimum range.

vi) Leaf zinc content: The leaf zinc content in healthy trees was significantly more (25.81 ppm) than that in declined trees (19.17 ppm). The zinc content in the leaves of healthy trees was within the optimum range (25-100 ppm) whereas it was low in the declined trees.

vii) Leaf iron content : The leaf iron content in healthy trees (198.2 ppm) was highly significant over that in declined trees (144.6 ppm), both the values, however, were well above the optimum range (60-120 ppm).

viii) Leaf manganese content : The leaf manganese content in declined trees was significantly more (45.70 ppm) than that in healthy trees (35.90 ppm). It was 27 per cent more in declined trees relative to healthy trees.

ix) Leaf copper content : The leaf copper content was also significantly more (18.44 ppm) in declined trees compared to healthy trees (13.27 ppm). It was 38 per cent more in declined trees relative to healthy trees.

III. Bark nutrient contents

i) Bark nitrogen content: The data presented in table 8 indicate that the bark nitrogen content in the healthy (1.32%) and declined (1.32%) trees regardless of the stionic parts was equal. The difference in mean nitrogen content in scion and rootstock bark regardless of health status and time of sampling was, however, found to be significant. The overall mean value for scion bark (1.25% N) was significantly less than that of the roostock back (1.42%).

The mean bark nitrogen content for the two sampling months irrespective of the health status and stionic parts differed significantly. In April, the mean bark N content was 1.29 per cent which was significantly less than that in the month of September (1.38%).

The interaction effects involving health status with stionic part and time of sampling, stionic parts and time of sampling and among all the three factors were nonsignificant.

ii) Bark phosphorus content : The data in table 8 show that the difference of mean bark phosphorus values under healthy (0.0654%) and declined (0.062%) trees was nonsignificant, however, the stionic part mean values for scion (0.069%) and rootstock (0.059%) were significant with each other. The bark samples taken in the month of April contained significantly less (0.058%) phosphorus than those taken in September (0.07%).

Table 8. Spatiotemporal distribution of nitrogen and phosphorus contents in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month	Nitrogen content (%)										Phosphorus content (%)										
	Healthy				Declined				Stionic part x Time means	Healthy				Declined				Stionic part x Time means			
	Sampling	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean		Scion	Root- stock	Scion	Root- stock	Mean	Scion	Root- stock	Mean		Time mean	Scion	Root- stock
April	1.23	1.34	1.29	1.18	1.41	1.30	1.29	1.21	1.38	0.063	0.056	0.060	0.059	0.054	0.056	0.058	0.061	0.055			
Sept.	1.35	1.48	1.35	1.42	1.45	1.35	1.38	1.30	1.47	0.082	0.064	0.073	0.072	0.061	0.067	0.070	0.077	0.063			
Mean	1.29	1.41	1.32	1.22	1.43	1.32	1.25*	1.42*	0.073	0.060	0.065	0.065	0.057	0.062			0.069**	0.059**			
C.D. at 5%																					
Health status (S)										N.S.											
Stionic part (P)										0.09*											
Month of sampling (T)										0.07											
S x P										N.S.											
S x T										N.S.											
P x T										N.S.											
S x P x T										N.S.											

*Critical difference at 1%; N.S. = Non-significant; **Overall stionic part means

The interaction effects between health status and stionic parts with the mean values of 0.073 and 0.06 per cent for scion and roostock respectively of the healthy plants and 0.065 and 0.057 per cent for scion and rootstock respectively of the declined trees were nonsignificant. Similarly, the mean values of the interaction between health status and time of sampling with the means of 0.06 and 0.07 per cent for April and September respectively under healthy trees and 0.056 and 0.067 per cent for April and September, respectively under declined trees were nonsignificant. The interaction between stionic parts and the time of sampling with the mean phosphorus values of scion (0.061%) and rootstock (0.055%) for April and 0.077 per cent of scion and 0.063 per cent of rootstock for September month were nonsignificant with each other. The interaction for the body of the table was also non-significant.

iii) Bark potassium content: Data for mean bark potassium content presented in table 9 indicate that the difference of means for healthy and declined trees was nonsignificant.

The stionic part means for Scion (0.53%) and rootstock (0.36%) differed significantly with each other. Samples collected in the month of September recorded significantly more (0.49%) bark potassium content than those collected in April (0.39%).

The interaction effect for health status and stionic part with mean potassium contents of 0.53 per cent for scion

Table 9. Spatiotemporal distribution of potassium in scion and rootstock bark of healthy and declined trees of Kinnow mandarin.

Month of sampling	Bark potassium content (%)								
	Healthy			Declined				Stionic part x sampling time mean	
	S	R	Mean	S	R	Mean	Time Mean	S	R
April	0.45	0.26	0.35	0.52	0.35	0.43	0.39	0.49	0.30
Sept.	0.60	0.40	0.50	0.54	0.43	0.48	0.49	0.57	0.41
Mean	0.53	0.33	0.43	0.53	0.39	0.46		0.53 ^{**}	0.36 ^{**}

C.D. at 5% level

Health status (S)	= N.S.
Stionic part (P)	= 0.07*
Sampling time (T)	= 0.07*
P x T	= 0.01
S x P	= N.S.
S x T	= N.S.
S x P x T	= N.S.

S = Scion; R = Rootstock

* Critical difference at 1% level

**Overall stionic part means

and 0.33 per cent for rootstock bark in healthy trees and 0.53 per cent for scion and 0.39 per cent for rootstock bark in declined trees was nonsignificant. Similarly, the health status and time of sampling means for April (0.35%) and September (0.50%) in healthy trees and for April (0.43%) and September (0.48%) in declined trees were nonsignificant with each other.

The interaction effects between stionic parts and sampling time were significant with mean values of 0.49 per cent (scion) and 0.30 per cent (roostock) for April and 0.57 per cent (scion) and 0.41 per cent (rootstock) for September.

iv) Bark calcium content: The data pertaining to bark calcium content is presented in table 10. The mean bark calcium contents for healthy (6.98%) and declined (5.42%) trees differed significantly with each other. The means for stionic parts for scion and rootstock were equal (6.20% each). The mean bark calcium values for April (6.09%) and September (6.30%) differed significantly with each other. The interaction effects for health status and stionic part with the mean bark calcium values of 6.98 per cent each for scion and rootstock in healthy trees and 5.42 and 5.41 per cent for scion and roostock, respectively in declined trees were nonsignificant. The health status and time of sampling interaction with mean bark calcium contents of 6.80 and 7.16 per cent for April and September, respectively in healthy

Table 10. Spatiotemporal distribution of calcium and magnesium contents in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month of	Calcium content (%)						Magnesium content (%)											
	Healthy			Declined			Healthy			Declined								
	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Stionic part x Time means	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Stionic part x Time means	Scion	Root- stock		
Sampling	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean	Scion	Root- stock		
April	6.80	6.80	6.80	5.32	5.46	5.39	6.09	6.06	6.13	0.20	0.23	0.216	0.35	0.24	0.295	0.255	0.270	0.235
Sept.	7.16	7.16	7.16	5.52	5.36	5.44	6.30	6.34	6.26	0.23	0.32	0.276	0.26	0.31	0.285	0.281	0.235	0.315
Mean	6.98	6.98	6.98	5.42	5.41	5.42		6.20**	6.20**	0.215	0.275	0.246	0.305	0.275	0.290	0.25**		0.28**
C.D. at 5%																		
Health status (S)																		
Stionic part (P)																		
Month of sampling (T)																		
S x P																		
S x T																		
P x T																		
S x P x T																		

trees were significant with each other. The interaction effects for the body of the table were also significant. The scion and roostock calcium values of 6.8 and 7.16 per cent each for April and September, respectively in healthy plants were significantly more than the corresponding scion and rootstock values for declined trees.

v) Bark magnesium content : It is evident from the data in table 10 that the mean bark magnesium contents of 0.246 per cent in healthy and 0.29 per cent for declined trees were nonsignificant with each other. The mean values of the stionic parts and time of sampling were also nonsignificant.

The interaction effect between the health status and stionic part as well as between the health status and time of sampling were nonsignificant. However, the interaction between rootstock and time of sampling with mean values of 0.235 per cent for April and 0.315 per cent for September was significant.

Figure 1 illustrates the comparative concentrations of the leaf and bark N, P, K, Ca and Mg in the healthy and declined kinnow mandarin trees.

vi) Bark zinc content : The data in table 11 indicate that the difference between the mean bark zinc content between healthy (28.2 ppm) and declined (47.4 ppm) trees was significant. The differences between the zinc content of stionic parts as well as between the means of time of sampling were nonsignificant.

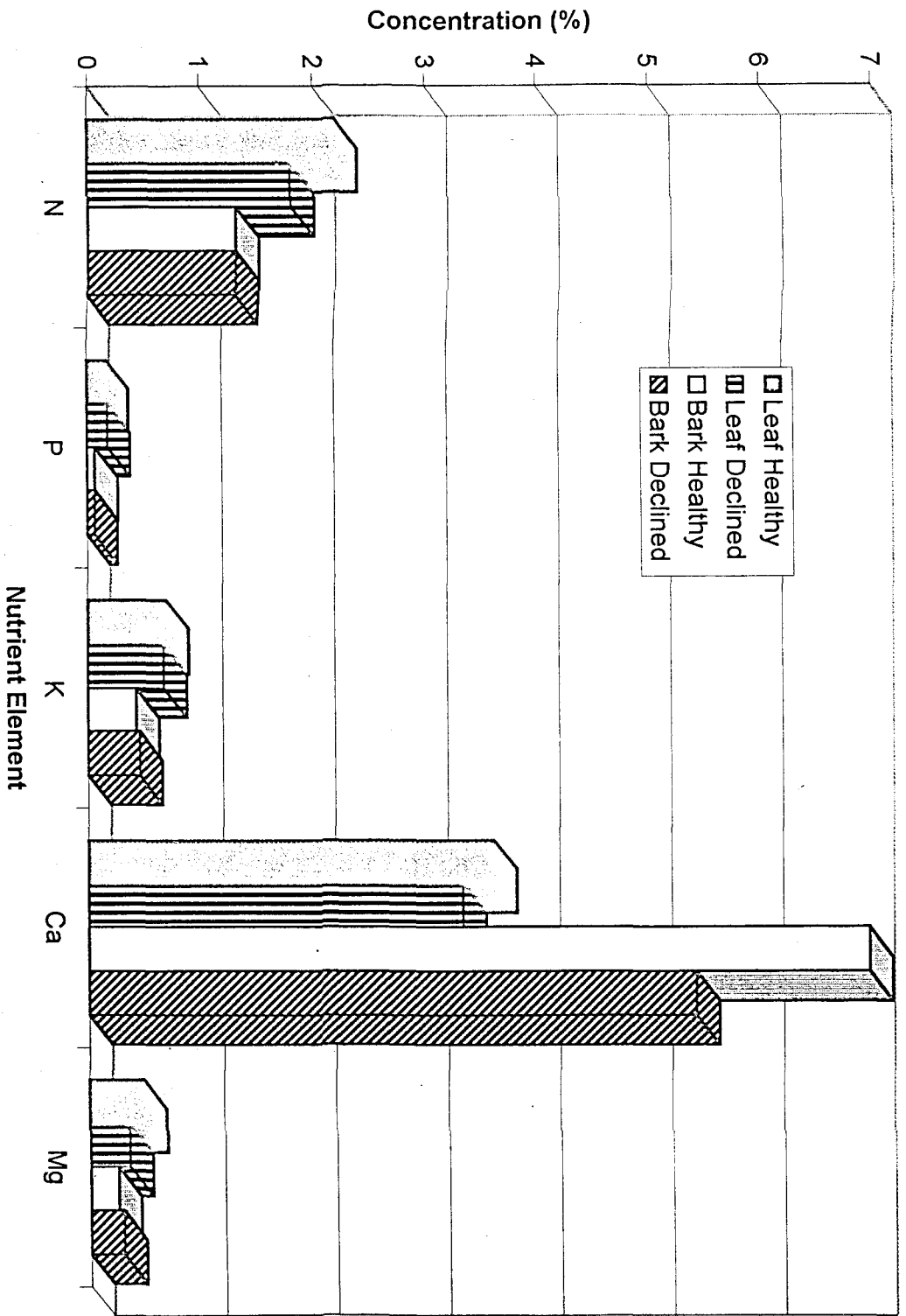


Fig. 1 : Macronutrient contents in leaf and bark of healthy and declined trees of Kinnow mandarin

The interaction effect between health status and stionic part with mean zinc values of 23.6 and 32.8 ppm for scion and roostock respectively in healthy and 48.1 and 46.6 ppm for scion and roostock, respectively in declined trees were nonsignificant. The interaction effects between plant health and time of sampling were significant with 12.3 and 44.1 ppm zinc in April and September, respectively for healthy trees and 58.3 and 36.4 ppm in April and September, respectively for declined trees.

For the month of September, there was significant difference between the zinc content of scion (30 ppm) and rootstock (50.5 ppm). Zinc content of roostock in September was also significantly more than that in April.

Significant differences were also observed in the zinc values in the body of the table. In the month of April, scion bark zinc content (13.2 ppm) under healthy trees was significantly less than the scion zinc content (70.2 ppm) of declined plants. Similar trend was observed in the rootstock bark zinc contents in healthy and declined trees. In the month of September, however, these differences were nonsignificant.

vii) Bark iron content : Data pertaining to bark iron content is presented in table 11. The difference of means of healthy (540 ppm) and declined (645 ppm) trees was significant. the means of two stionic parts, scion (605 ppm) and roostock (580 ppm) did not differ significantly. The

Table 11. Spatiotemporal distribution of zinc and iron contents in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month of	Zinc content (ppm)						Iron content (ppm)											
	Healthy			Declined			Healthy			Declined								
	Sampling	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean	Scion	Root- stock	Scion	Root- stock	Mean	Time mean	Scion	Root- stock		
																	Stionic part x Time means	Stionic part x Time means
April	13.2	11.4	12.3	70.2	46.4	58.3	35.3	41.7	28.9	434	355	394	940	445	693	544	687	400
Sept.	34.0	54.2	44.1	26.0	46.8	36.4	40.3	30.0	50.5	567	803	685	479	715	599	642	523	759
Mean	23.6	32.8	28.2	48.1	46.6	47.4	35.9*	39.7*	501	579	540	710	580	645	605**	580**		
C.D. at 5%																		
Health status (S)																		
Stionic part (P)																		
Month of sampling (T)																		
S x P																		
S x T																		
P x T																		
S x P x T																		

*Critical difference at 1%; N.S. = Non-significant; **Overall stionic part means

mean iron content in the bark collected in the month of September (642 ppm) was significantly more than that collected in the month of April (544 ppm).

The mean iron content in scion bark of healthy trees (501 ppm) was significantly less than that for scion in declined trees (710 ppm). The corresponding rootstock bark contents were at par with each other.

In healthy trees, the mean bark iron content in the month of September (685 ppm) was significantly more than that in the month of April (394 ppm) whereas the difference for the corresponding means in declined trees was non significant. In the month of April, scion tended to accumulate significantly more amount of iron (687 ppm) than that in September (523 ppm) whereas reverse was true for rootstock in the interaction between the stionic part and time of sampling. In other words, scion bark accumulated more iron (687 ppm) than rootstock bark (400 ppm) in April whereas in the month of September rootstock bark accumulated more iron (759 ppm) than scion (523 ppm). The interaction effect for the body of table was nonsignificant.

viii) Bark manganese content : The data in table 12 indicate nonsignificant differences in the means of health status and stionic parts. However, samples collected in April were having significantly more (24.5 ppm) manganese than those collected in September month (18.0 ppm).

The interaction effects of health status and stionic

Table 12. Spatiotemporal distribution of manganese and copper contents in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month of	Manganese content (ppm)										Copper content (ppm)							
	Healthy			Declined			Stionic part x Time means	Healthy			Declined			Stionic part x Time means				
	Scion	Root- stock	Mean	Scion	Root- stock	Mean		Time mean	Scion	Root- stock	Mean	Scion	Root- stock		Mean	Time mean		
Sampling	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean	Scion	Root- stock		
April	31.0	24.0	27.5	19.2	23.6	21.4	24.5	25.1	23.8	46.4	40.6	43.5	83.6	67.0	75.3	59.4	65.0	53.8
Sept.	15.0	23.0	19.0	15.0	18.6	16.8	18.0	15.0	20.8	180.0	133.2	156.6	88.0	68.0	78.0	117.3	126.9	100.6
Mean	23.0	23.5	23.3	17.1	21.1	19.1		20.1**	22.3**	113.2	86.9	100.1	85.8	67.5	76.7		96.0**	77.2**
C.D. at 5%																		
Health status (S)														19.89*				
Stionic part (P)														14.79				
Month of sampling (T)														19.89*				
S x P														N.S.				
S x T														N.S.				
P x T														N.S.				
S x P x T														N.S.				

*Critical difference at 1%; N.S. = Non-significant; **Overall stionic part means

part, health status and time of sampling and the stionic part and time of sampling as well as that for the body of the table were nonsignificant.

ix) Bark copper content : Data on bark copper content presented in table 12 indicate that the mean values in healthy (100.1 ppm) and declined (76.7 ppm) trees differed significantly. There was also significant difference in the mean scion bark (96.0 ppm) and rootstock bark (77.2 ppm) copper content. Bark samples taken in September month exhibited significantly more copper content (117.3 ppm) than those taken in April (59.4 ppm).

The interaction effects for health status and stionic part means were, however, nonsignificant. Bark of healthy plants in April, accumulated significantly less (43.5 ppm) copper than in September (156.6 ppm) whereas there was no significant difference in the temporal distribution of copper in the declined trees. The differences observed in the interaction involving stionic parts and time of sampling as as well in the body of the table were, however, nonsignificant.

x) Bark boron content : Data pertaining to the bark boron content is presented in table 13. The mean boron contents in the bark for healthy (80.6 ppm) and declined (76.0 ppm) trees differed significantly. The differences between stionic parts were, however, nonsignificant. The bark samples collected in September month accumulated

Table 13. Spatiotemporal distribution of boron in scion and rootstock bark of healthy and declined trees of Kinnow mandarin.

Month of sampling	Bark boron content (%)								
	Healthy			Declined				Stionic part x sampling time mean	
	S	R	Mean	S	R	Mean	Time Mean	S	R
April	38.8	79.2	83.0	64.9	70.8	67.9	71.8	75.9	75.0
Sept.	80.8	75.4	78.1	81.2	87.1	84.2	82.6	81.0	81.3
Mean	83.8	77.3	80.6	73.1	78.9	76.0		78.43*	78.13*
C.D. at 5% level									
Health status (S) = 3.67									
Stionic part (P) = N.S.									
Sampling time (T) = 4.95									
P x T = N.S.									
S x P = 7.00									
S x T = 7.00									
S x P x T = N.S.									

S = Scion; R = Rootstock

*Overall stionic part means

significantly more boron than those in April.

Scion bark in healthy trees contained significantly more boron (83.8 ppm) than that in declined trees (73.1 ppm). However, there was no such difference in the rootstock barks of healthy and declined trees. There was no significant difference between the mean bark boron contents of healthy trees in April and September, whereas in declined trees, bark samples collected in September contained significantly more boron (84.2 ppm) than those collected in April (67.9 ppm). There were no significant effects of the interactions between stionic parts and sampling time and within the body of the table.

Figure 2 illustrates the comparative concentrations of various micronutrients in the leaf and bark tissues of healthy and declined trees of Kinnow mandarin.

B. GROWTH, YIELD AND FRUIT QUALITY

I. Vegetative growth

The data in table 14 indicate that there was significant difference in the plant height between healthy and declined trees. Plant height decreased to the extent of 35 per cent in declined relative to healthy trees. The difference in the stock and scion circumference between the healthy and declined trees was, however, nonsignificant. The tree volume in case of healthy trees was 75 per cent higher than in declined trees. Similarly, leaf area was also

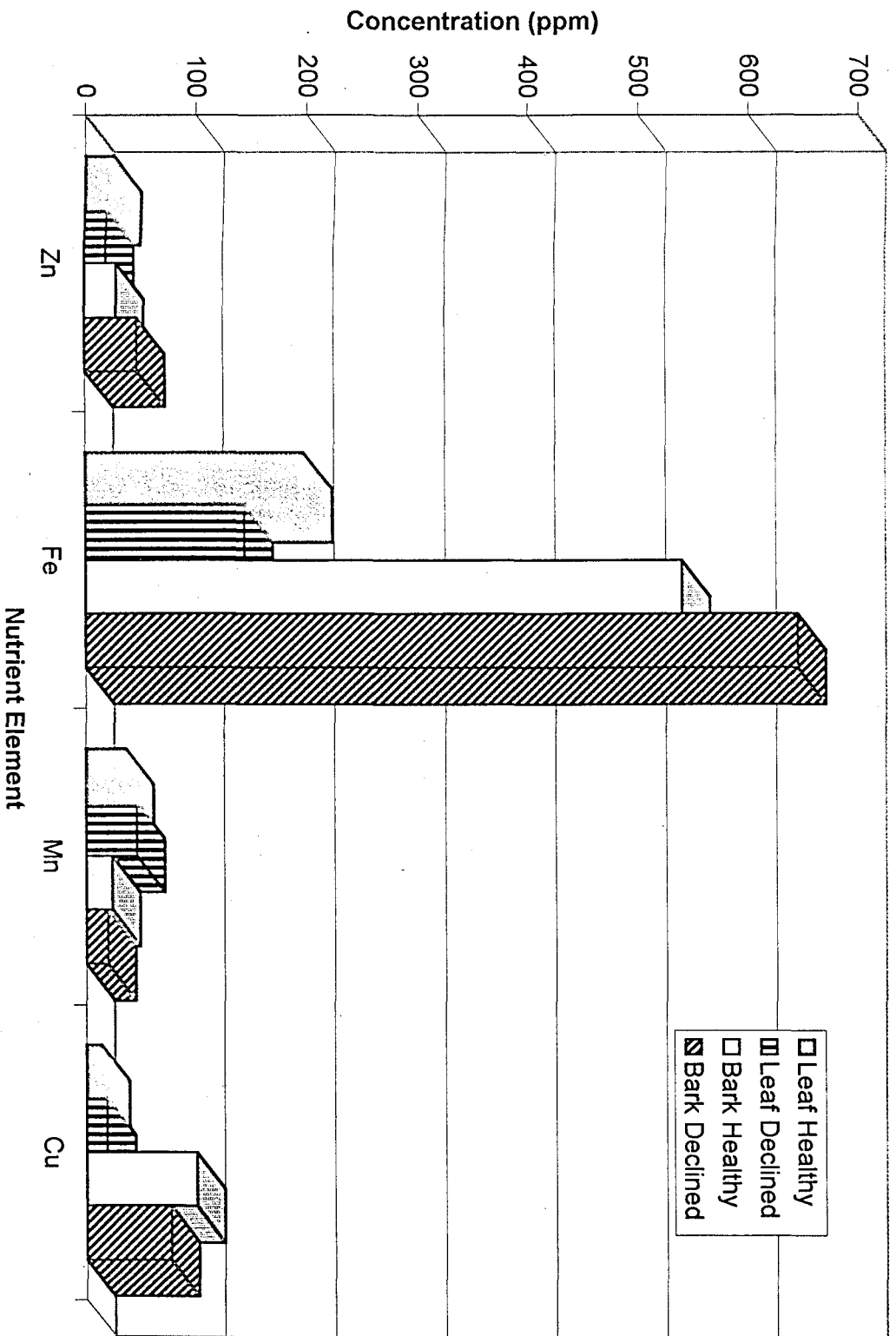


Fig. 2 : Micronutrient contents in leaf and bark of healthy and declined trees of Kinnow mandarin

Table 14. Vegetative growth parameters in healthy and declined trees of Kinnow mandarin

Plant status	Plant height (m)	Stock girth (cm)	Scion girth (cm)	Stock-scion ratio	Tree volume (m ³)	Leaf area (cm ²)
Healthy	5.20	59.5	56.7	1.06	106.34	21.32
Declined	3.53	58.5	55.8	1.05	26.83	13.81
	**	N.S.	N.S.	N.S.	**	**

** Significant at 1% level

N.S. = Non-significant

Table 15. Yield in healthy and declined trees of Kinnow mandarin

Plant status	No. of fruits/tree	Fruit weight (g)	Fruit yield (t/ha)	Tree efficiency (%)
Healthy	832	164.5	37.83	129.07
Declined	77	118.2	2.51	38.18
	**	**	**	**

** Significant at 1% level

Table 16. Fruit physical characteristics in healthy and declined Kinnow trees

Plant status	Fruit length (cm)	Fruit breadth (cm)	Fruit firmness (kg/cm ²)	Rind thickness (mm)
Healthy	5.42	6.72	4.22	2.56
Declined	5.39	6.27	5.69	3.21
	N.S.	N.S.	**	**

** Significant at 1% level

N.S. = Non-significant

significantly higher in healthy trees as compared to declined trees. There was 35 per cent reduction in the leaf area in declined trees as compared to healthy trees.

As regards the visual observations the declined trees wore a deserted look with drastic reduction in canopy, sparse, pale green to chlorotic foliage, abundance of dead wood due to dieback which were in sharp contrast to the profusely growing healthy trees with lush green, abundant foliage and gigantic canopy (plate 1).

II. Yield

The data in table 15 revealed that the average number of fruits/tree and average fruit weight were significantly higher in healthy trees than in the declined trees. Healthy trees recorded more number of fruits (832) compared to the declined trees (77). The average fruit weight was also significantly higher (164.5 g) in healthy trees relative to average weight of fruits (118.2 g) in declined trees. Consequently, the productivity of the Kinnow orchard on a hectare basis was significantly low (2.51 t/ha) in declined trees compared to 37.83 t/ha in healthy trees. The tree efficiency, establishing a relationship between tree volume and fruit yield, was found to be 129.07 per cent in healthy trees which was significantly higher than that in the declined trees (38.18%).



PLATE 1 : A general view showing the status of healthy trees



PLATE 2 : A general view showing the status of declined trees

III. Fruit quality

a) Physical parameters

The data on various physical characteristics of fruit in table 16 show that there was no significant change in the fruit size in respect to length and breadth of the fruit between healthy and declined trees, however, fruit firmness and rind thickness, exhibited a marked difference in the healthy and declined trees. The fruits from the declined trees were relatively more firm than the fruits from the healthy trees. Similarly, the rind thickness was 25% more in fruits of declined trees than that of the healthy ones.

Fruits from the declined trees were smaller in size with rind blemishes, lacking gloss, shine and appeal compared to those from the healthy trees (Plate 3 & 4).

b. Chemical characteristics

The data in table 17 reveals that the juice content significantly varied in fruits from healthy and declined trees. Fruits from the healthy trees contained 47.53 per cent juice as compared to 36.70 per cent in declined trees. Total soluble solids of the fruits on declined trees was higher (11.49%) than of the fruits from the healthy trees (9.51%). The acid content of the fruit juice in terms of citric acid showed significant variation in fruits of healthy and declined trees. Fruits from declined trees showed 35 per cent higher acid content as compared to the



PLATE 3 : Fruits of healthy trees



PLATE 4 : Fruits of declined trees

Table 17. Fruit Chemical characteristics of healthy and declined trees of Kinnow mandarin

Plant status	Juice content (%)	TSS (%)	Acidity (%)	TSS/Acid ratio	Ascorbic acid (mg/100g)	Sugar (%)		
						R	NR	Total
H	47.53	9.51	0.68	13.98	24.05	3.31	3.72	7.03
D	36.70	11.49	1.03	11.16	24.06	4.04	4.40	8.17
	**	**	**	**	N.S.	**	**	**

H = Healthy; D = Declined

R = Reducing; NR = Non-reducing

** Significant at 1 % level

N.S. = Non-significant

Table 18. Moisture and dry matter contents in leaves of healthy and declined trees of Kinnow mandarin

Time of sampling	Moisture content(%)			Dry matter (%)		
	Healthy	Declined	Mean	Healthy	Declined	Mean
March	50.60	38.27	44.45	49.56	61.73	55.65
April	48.45	42.98	45.71	51.55	57.02	54.29
September	44.99	41.90	43.44	55.01	58.10	56.56
Mean	48.02	41.05	44.53	52.04	58.95	55.50

C.D. at 5% level

Health status (S) : 3.08* 3.08*

Sampling time (T) : N.S. N.S.

S x T : 5.35* 5.35*

* Significant at 1 % level

fruits from the healthy trees.

Sugar-acid ratio was higher in the fruits from healthy trees than in the fruits from the declined trees. The reducing, nonreducing and total sugars varied significantly between the fruits from healthy and declined trees. Nonreducing sugar contents were more in the fruits from declined trees.

C. PHYSIOLOGICAL CHARACTERISTICS

I. Leaf moisture content

The data on moisture contents in the leaves of healthy and declined trees presented in table 18 indicate that the mean leaf moisture content for healthy plants was significantly more (48.02%) than that in declined trees (41.05%). The mean values for different months of sampling did not differ significantly. The body of the table showed that the leaf moisture contents in the months of March (50.60%) and April (48.45%) were significantly more in healthy plants than the corresponding values in the declined trees. The September sampling did not show marked variation between healthy and declined trees.

II. Leaf dry matter content

The data pertaining to leaf dry matter content is presented in table 18. The mean leaf dry matter contents for healthy (52.04%) and declined (58.95%) trees differed significantly with each other. No significant differences

were observed among the months of sampling. The interactions of health status and time of sampling showed significant differences between healthy and declined trees in the months of March and April.

III. Bark moisture content

The data pertaining to bark moisture contents presented in table 19 reveals that the mean bark moisture contents in healthy (52.8%) and declined (50.9%) trees differed significantly with each other. The difference of means between bark moisture contents collected in April and September and the difference between the means of scion (51.3%) and rootstock (52.3%) barks was nonsignificant.

Significant difference was observed in the moisture content of the bark collected in April (54.4%) and September (51.2%) in healthy trees.

No significant differences between interaction means of time of sampling and stionic part means as well as the mean values of the interaction of stionic parts with the health status were observed.

IV. Bark dry matter content

The data regarding dry matter content in scion and rootstock barks of healthy and declined trees of Kinnow mandarin is presented in table 19. The overall mean values of dry matter in bark of healthy (47.2%) and declined (49.2%) trees differed significantly. There was no difference in the time of bark sampling means and stionic

Table 19. Spatiotemporal distribution of moisture and dry matter contents in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month	Moisture content (%)								Dry matter (%)					
	Healthy				Declined				Healthy			Declined		
	of				Stionic part x				Time means			Stionic part x		
	Sampling	Scion	Root-stock	Mean	Scion	Root-stock	Mean	Time mean	Scion	Root-stock	Mean	Scion	Root-stock	Mean
April		53.8	55.0	54.4	49.7	51.4	50.5	52.4	51.7	53.2	46.3	45.0	45.6	50.3
												48.7	49.5	47.6
												48.3		46.8
Sept.		51.0	51.3	51.2	50.8	51.7	51.2	51.2	50.9	51.5	49.0	48.7	48.8	49.3
												48.3	48.8	48.8
												49.2		48.5
Mean		52.4	53.2	52.8	50.2	51.5	50.9		51.3*	52.3*	47.6	46.9	47.2	49.8
												48.5	49.2	48.7*
													47.7*	
C.D. at 5%														
Health status (S)														
Stionic part (P)														
Month of sampling (T)														
S x P														
S x T														
P x T														
S x P x T														

*Critical difference at 1%; N.S. = Non-significant; *Overall stionic part means

part means.

The interaction of health status with time of sampling was significant. Dry matter content of healthy plants in September was higher (48.8%) than that in April (45.6%) whereas no difference was observed in the dry matter content of the declined plants. The interaction effects between time of sampling and stionic parts, health status and stionic parts and between all the factors were nonsignificant.

V. Leaf chlorophyll and carotenoid pigments

A perusal of the data in table 20 indicate that chlorophyll-a content in leaves of healthy Kinnow mandarin plants was significantly more ($0.2119 \mu \text{ moles g}^{-1}$) than that in declined plants ($0.1363 \mu \text{ moles g}^{-1}$). Similarly chlorophyll-b was also significantly more in healthy plant leaves ($2.7258 \mu \text{ moles g}^{-1}$) than in declined plant leaves ($1.1411 \mu \text{ moles g}^{-1}$). Consequently, the total chlorophyll content in the leaves of healthy plants was also significantly more ($2.9378 \mu \text{ moles g}^{-1}$) than that in declined plants ($1.1967 \mu \text{ moles g}^{-1}$).

The carotenoid pigments, however, were significantly more in declined tree leaves compared to healthy tree leaves.

VI. Leaf temperature

The data in table 20 indicate that the leaf temperature in the healthy tree leaves was significantly more (35.06°C) than that in declined tree leaves (34.45°C).

Table 20. Physiological characteristics of leaves in healthy and declined trees of Kinnow mandarin

Physiological parameters	Healthy	Declined	Significance
Chlorophyll 'a' (μ moles/g)	0.2119	0.1363	**
Chlorophyll 'b' (μ moles/g)	2.7258	1.1411	**
Total chlorophyll (μ moles/g)	2.9378	1.1967	**
Carotenoids (μ moles/g)	0.0308	0.1839	**
Leaf tempeature ($^{\circ}\text{C}$)	35.06	34.45	**
Stomatal conductance (millimole $\text{m}^{-2}\text{S}^{-1}$)	14.75	19.00	N.S.
Transpiration (millimole $\text{m}^{-2}\text{S}^{-1}$)	0.89	1.09	N.S.
Phytosynthesis ($\mu\text{mole CO}_2\text{m}^{-2}\text{S}^{-1}$)	3.88	2.00	*

* Significant at 5% level

** Singificant 1% level

N.S. = Non-significant

VII. Stomatal conductance and transpiration

The data on stomatal conductance and transpiration is presented in table 20. The values for stomatal conductance for healthy ($14.75 \text{ millimole m}^{-2}\text{s}^{-1}$) and declined ($19.00 \text{ millimole m}^{-2}\text{s}^{-1}$) trees did not differ significantly. The rate of transpiration in healthy trees ($0.89 \text{ millimole m}^{-2}\text{s}^{-1}$) did not differ significantly with that of declined trees ($1.09 \text{ millimole m}^{-2}\text{s}^{-1}$).

VIII. Photosynthesis

The data on the rate of photosynthesis presented in table 20 reveals that the rate of photosynthesis in the leaves of healthy trees was significantly more ($3.88 \mu \text{ mole CO}_2 \text{ m}^{-2}\text{s}^{-1}$) than that in the leaves of declined trees ($2.0 \mu \text{ mole CO}_2 \text{ m}^{-2}\text{s}^{-1}$).

D. BIOCHEMICAL CHARACTERISTICS

I. Peroxidase activity

A perusal of the data presented in table 21 reveals that the mean peroxidase activity in the leaves of healthy tree ($0.83 \text{ O.D. change s}^{-1} \text{ g}^{-1} \text{ fresh leaf tissue}$) was low relative to declined ($1.69 \text{ O.D. change s}^{-1}\text{g}^{-1} \text{ fresh leaf tissue}$) trees. In general the activity of peroxidase enzyme was recorded to be significantly more in the month of April ($1.87 \text{ O.D. change s}^{-1}\text{g}^{-1}$) than in the month of March ($0.65 \text{ O.D. change s}^{-1}\text{g}^{-1}$) irrespective of the health status.

The interaction between health status and the time of

Table 21. Peroxidase activity (O.D. change/s/g fresh weight) in spring flush leaves of healthy and declined trees of Kinnow mandarin

Time of sampling	Peroxidase acitivity (O.D change/s/g fresh wt).		
	Healthy	Declined	Mean
March	0.606	0.694	0.65
April	1.060	2.691	1.87
Mean	0.833	1.69	
C.D. at 1% level			
Status (S)	: 0.56		
Time (T)	: 0.56		
S x T	: 0.79		

Table 22. Seasonal variation in starch content (%) of leaves in healthy and declined trees of Kinnow mandarin

Month of sampling	Starch content in leaves (%)		
	Healthy	Declined	Mean
March	2.48	1.91	2.19
April	3.03	2.61	2.82
September	3.75	3.49	3.62
Mean	3.08	2.67	
C.D. at 1% level			
Health status (S)	: 0.34		
Time of sampling (T)	: 0.42		
S x T	: N.S.		

sampling indicated that the peroxidase activity in the month of March for healthy (0.606 OD change) and declined trees (0.694 OD) was nonsignificant, however, marked variation in April sampling was observed in the healthy and declined trees.

II. Leaf starch content

It is obvious from the data in table 22 that the starch content in healthy tree leaves (3.08%) differed significantly from the declined tree leaves (2.67%). There was significant variation in the starch content of leaves collected in different sampling months irrespective of the health status.

III. Bark starch content

The data pertaining to spatiotemporal distribution of starch content in phloem bark of healthy and declined trees of Kinnow mandarin is presented in table 23.

The difference of means of bark starch content in healthy (2.17%) and declined (1.55%) trees regardless of the spatiotemporal distribution was highly significant. There was also a significant difference in the temporal distribution of bark starch content with the samples collected in April recording 2.07 per cent as against those collected in the month of September (1.65%). The spatial distribution of starch content in stionic parts was also significant where scion and rootstock bark contained 2.39 per cent and 1.33 per cent respectively in both healthy and

Table 23. Spatiotemporal distribution of starch content in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month of sampling	Starch content in bark (%)								
	Healthy			Declined			Time Mean	Stionic part x sampling time mean	
	S	R	Mean	S	R	Mean		S	R
April	2.93	1.71	2.32	2.45	1.17	1.81	2.07	2.69	1.44
Sept.	2.49	1.53	2.01	1.67	0.88	1.28	1.65	2.08	1.21
Mean	2.71	1.62	2.17	2.06	1.03	1.55		2.39*	1.33*
C.D. at 1% level									
Health status (S) = 0.39									
Stionic part (P) = 0.39									
Sampling time (T) = 0.39									
P x T = N.S.									
S x P = N.S.									
S x T = N.S.									
S x P x T = N.S.									

S = Scion; R = Rootstock

*Overall stionic part means

declined trees taken together.

The interaction effects involving health status with stionic parts and time of sampling, stionic part and time of sampling and the body of the table were nonsignificant.

IV. Leaf sugar content

The data on total sugars, reducing sugars and nonreducing sugars in leaves sampled in different months is presented in table 24.

a. Leaf total sugars

The total sugars content of leaves in healthy (4.57%) and declined (3.93%) trees varied significantly. Seasonal variation was also observed to be significant between the mean leaf total sugar content in the samples collected in the months of March (3.98%) and September (4.47%). The March-April and April-September values were at par with each other. There was no effect of interaction values between time of sampling and health status of plants.

b. Leaf reducing sugars

The mean values for leaf reducing sugars in healthy and declined trees as well as for the time of sampling and their interaction values were at par with each other.

c. Leaf nonreducing sugars

The mean values for leaf nonreducing sugars in healthy (2.34%) and declined (1.74%) trees varied significantly. The monthly mean values for all the three sampling months as

Table 24. Seasonal variation in sugar contents (%) in leaves of healthy and declined trees of Kinnow mandarin

Month of sampling	Sugar content in leaves (%)								
	Total sugars			Reducing sugars			Non-reducing sugars		
	Healthy	Declined	Mean	Healthy	Declined	Mean	Healthy	Declined	Mean
March	4.32	3.63	3.98	2.30	2.15	2.23	2.02	1.57	1.80
April	4.68	3.94	4.31	2.02	2.22	2.12	2.67	1.71	2.19
September	4.72	4.23	4.47	2.39	2.31	2.35	2.33	1.91	2.12
Mean	4.57	3.93		2.23	2.23		2.34	1.74	
C.D. at 5%									
Health status (S)	0.38*			N.S.			0.36*		
Month of sampling (T)	0.36			N.S.			N.S.		
S x T	N.S.			N.S.			N.S.		

*C.D. significant at 1% level;

N.S. = Non-significant

well as the interaction between health status and time of sampling were nonsignificant.

V. Bark sugar content

a. Total sugars :

A perusal of the data in table 25 reveals that there was significant difference in the mean bark total sugar content of the healthy (3.71%) and declined (3.11%) trees. The mean values for temporal distribution with 3.36 per cent total sugars in April and 3.46 per cent in September irrespective of health status were nonsignificant. the stionic part means with 3.49 per cent total sugars in scion and 3.33 per cent in rootstock were also nonsignificant.

The interaction effects between health status and stionic parts, health status and time of sampling, stionic parts and time of sampling as well as the starch contents in the body of the table were nonsignificant.

b. Reducing sugars :

The data pertaining to the spatiotemporal distribution of reducing sugars in bark of healthy and declined trees of Kinnow mandarin is presented in table 26.

It is evident from the data that the mean bark reducing sugars content in healthy (1.78%) and declined (1.51%) trees differed significantly. The difference of mean values of bark reducing sugars in scion (1.79%) and rootstock (1.50%) barks was also significant. The difference in monthly means taken together in scion and rootstock barks in healthy and

Table 25. Spatiotemporal distribution of total sugars content in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month of sampling	Total sugar content in bark (%)								
	Healthy			Declined			Time Mean	Stionic part x sampling time mean	
	S	R	Mean	S	R	Mean		S	R
April	3.74	3.62	3.68	3.11	2.96	3.04	3.36	3.43	3.29
Sept.	3.82	3.65	3.74	3.28	3.08	3.18	3.46	3.55	3.37
Mean	3.78	3.64	3.71	3.20	3.02	3.11		3.49*	3.33*
C.D. at 1% level									
Health status (S)			= 0.32						
Stionic part (P)			= N.S.						
Sampling time (T)			= N.S.						
P x T			= N.S.						
S x P			= N.S.						
S x T			= N.S.						
S x P x T			= N.S.						

S = Scion; R = Rootstock

*Overall stionic part means

Table 26. Spatiotemporal distribution of reducing and non-reducing sugar contents in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month of	Reducing sugars (%)										Non-reducing sugars (%)								
	Healthy			Declined			Stionic part x Time means				Healthy			Declined			Stionic part x Time means		
	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean	Scion	Root- stock	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean	Scion	Root- stock	
	Sampling																		
April	1.88	1.64	1.76	1.64	1.22	1.43	1.60	1.76	1.43	1.86	1.98	1.92	1.51	1.74	1.63	1.78	1.69	1.86	
Sept.	1.90	1.68	1.79	1.72	1.46	1.59	1.70	1.81	1.57	1.92	1.97	1.95	1.56	1.62	1.59	1.77	1.74	1.80	
Mean	1.89	1.66	1.78	1.68	1.33	1.51		1.79**	1.50**	1.89	1.98	1.94	1.54	1.68	1.61		1.72**	1.83**	
C.D. at 5%																			
Health status (S)																			
Stionic part (P)																			
Month of sampling (T)																			
S x P																			
S x T																			
P x T																			
S x P x T																			

*Critical difference at 1%; N.S. = Non-significant; **Overall stionic part means

declined trees was, however, nonsignificant.

The interaction effects involving health status of the trees with that of either stionic parts or time of sampling, stionic part and time of sampling and the values of reducing sugar contents in the body of the table were all nonsignificant.

c. Nonreducing sugars :

It is apparent from the data in table 26 that the difference of means in healthy (1.94%) and declined (1.61%) tree phloem bark nonreducing sugar content irrespective of spatiotemporal distribution was significant. The differences between temporal means of barks irrespective of health status and stionic parts and between means of stionic parts regardless of health status and time of sampling were nonsignificant.

The interaction effects involving health status of trees with time of sampling and stionic parts, stionic parts and time of sampling and the nonreducing sugar values within the body of table were nonsignificant.

VI. Leaf protein content

The data regarding seasonal variation in protein content of leaves in healthy and declined trees of Kinnow mandarin is presented in table 27.

a. Soluble proteins

It is apparent from the data that the mean values for

Table 27. Seasonal variation in protein content of leaves in healthy and declined trees of Kinnow mandarin

Month of sampling	Protein content in leaves								
	Soluble			Insoluble			Total		
	Healthy	Declined	Mean	Healthy	Declined	Mean	Healthy	Declined	Mean
March	4.35	2.78	3.56	1.74	0.89	1.31	6.09	3.67	4.88
April	4.52	2.54	3.53	1.22	0.78	1.00	5.74	3.32	4.53
September	7.41	5.23	6.32	1.35	0.95	1.10	8.76	6.18	7.47
Mean	5.43	3.52		1.44	0.87		6.86	4.39	
C.D. at 5%									
Health status (S)		0.48*			0.13			0.53	
Month of sampling (T)		0.59			0.16			0.65	
S x T		N.S.			0.24*			N.S.	

*C.D. significant at 1% level;

N.S. = Non-significant

soluble proteins in healthy (5.43%) and declined (3.52%) trees varied significantly. The monthly mean values for March and April were at par with each other but were significantly lower than that in September. The interaction effects were, however, nonsignificant.

b. Insoluble proteins

Leaf insoluble protein mean values for healthy (1.44%) and declined (0.87%) trees differed significantly. March samples contained more (1.31%) insoluble protein content which was significantly higher over the other two sampling months irrespective of the health status. The interaction of health status and the time of sampling was also significant where healthy trees showed higher insoluble protein content relative to declined trees in all the three months of sampling.

c. Total proteins

It is obvious from the data in table 27 that the mean leaf total soluble protein values for healthy (6.86%) and declined (4.39%) trees varied significantly. Similarly, the time of sampling irrespective of the health status was also significant. Total protein content in the month of March (4.88%) and April (4.53%) were at par with each other, however, they varied significantly when compared to September (7.47%) sampling. There was no significant effect of overall interactions in the body of the table.

VII. Bark protein content

The data pertaining to the spatiotemporal distribution of soluble and insoluble proteins in phloem barks of healthy and declined trees of Kinnow mandarin is presented in table 28.

a. Soluble proteins

It is evident from the data that the difference of means of healthy (3.20%) and declined (2.71%) tree bark soluble proteins was significant. Similarly, temporal mean values for soluble proteins were also significant. Bark samples collected in the month of September contained more (3.19%) soluble protein than those taken in April (2.72%). The rootstock bark in both, healthy and declined trees had relatively more soluble proteins than the corresponding scion bark. The overall stionic part means for scion (2.69%) and rootstock (3.23%) varied significantly.

The interaction effects involving health status of trees with either stionic part or time of sampling, stionic parts and time of sampling and between the values of bark soluble proteins in the body of table were nonsignificant.

b. Insoluble proteins (in 0.3 N NaOH)

The data in table 28 indicate significant difference between the mean values of healthy (1.16%) and declined (0.82%) tree phloem bark insoluble protein contents. The temporal mean insoluble protein values for the two months of sampling (April and September) were, however,

Table 28. Spatiotemporal distribution of soluble and insoluble protein contents in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month of	Soluble proteins (%)										Insoluble proteins (%)								
	Healthy			Declined			Stionic part x Time means				Healthy			Declined			Stionic part x Time means		
	Sampling	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean	Scion	Root- stock	Scion	Root- stock	Mean	Scion	Root- stock	Mean	Time mean	Scion	Root- stock
April	2.71	3.27	2.99	2.12	2.77	2.45	2.72	2.42	3.02	1.10	1.20	1.15	0.76	0.78	0.77	0.96	0.93	0.99	
Sept.	3.15	3.67	3.41	2.75	3.18	2.97	3.19	2.95	3.43	1.14	1.20	1.17	0.80	0.94	0.87	1.02	0.97	1.07	
Mean	2.93	3.47	3.20	2.44	2.98	2.71		2.69**	3.23**	1.12	1.20	1.16	0.78	0.86	0.82	0.95**	1.03**		
C.D. at 5%																			
Health status (S)										0.33									
Stionic part (P)										0.33*									
Month of sampling (T)										0.33*									
S x P										N.S.									
S x T										N.S.									
P x T										N.S.									
S x P x T										N.S.									

*Critical difference at 1%; N.S. = Non-significant; **Overall stionic part means

nonsignificant. Similarly, there was no significant difference in the stionic part means of healthy and declined trees taken together.

The interaction effects involving health status of the trees with either time of sampling and stionic parts, stionic parts with time of sampling and the insoluble protein values in healthy and declined trees in the body of the table were nonsignificant.

c. Total proteins

The data in table 29 reveal that the mean values for bark total proteins in healthy (4.36%) and declined (3.53%) trees differed significantly. The temporal distribution of total protein contents for the two sampling months indicated significant difference with mean values of 3.68 per cent for April and 4.21 per cent for September in healthy and declined trees taken together. The rootstock overall mean protein contents (4.26%) were significantly more than the scion mean (3.64%).

The interaction effects involving health status with either stionic parts or time of sampling, stionic part and time of sampling and the total protein values in the body of the table were nonsignificant.

VIII. Leaf total phenols

The data in table 30 reveal that the mean leaf total phenol content in healthy tree (2.54%) was significantly lower than in the declined tree leaves (3.0%). There were no

Table 29. Spatiotemporal distribution of total protein contents in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month of sampling	Total proteins in bark (%)								
	Healthy			Declined				Stionic part x sampling time mean	
	S	R	Mean	S	R	Mean	Time Mean	S	R
April	3.81	4.47	4.14	2.88	3.55	3.22	3.68	3.35	4.01
Sept.	4.29	4.87	4.58	3.55	4.12	3.84	4.21	3.92	4.50
Mean	4.05	4.67	4.36	3.22	3.84	3.53		3.64*	4.26*
C.D. at 5% level									
Health status (S)			= 0.43						
Stionic part (P)			= 0.43						
Sampling time (T)			= 0.43						
P x T			= N.S.						
S x P			= N.S.						
S x T			= N.S.						
S x P x T			= N.S.						

S = Scion; R = Rootstock

*Overall stionic part means

Table 30. Seasonal variation in total phenols content in leaves of healthy and declined trees of Kinnow mandarin

Month of sampling	Total phenols content (%)		
	Healthy	Declined	Mean
March	2.55	2.87	2.76
April	2.27	3.22	2.75
September	2.69	2.91	2.80
Mean	2.54	3.00	

C.D. at 5% level

Health status (S) : 0.28*

Time of sampling (T) : N.S

S x T : 0.38*

N.S. = Non-significant

* C.D. at 1% level

significant differences in the temporal mean values of total phenols among all the three months of sampling. The leaf phenol contents in healthy plants were significantly low (2.27%) than those in declined trees (3.22%) in the month of April.

IX. Bark total phenols

The data presented in table 31 indicate that the total phenol contents in healthy (1.41%) and declined (1.20%) trees differed significantly. The difference of means between stionic parts with scion mean of 1.39 per cent and rootstock mean of 1.22 per cent was nonsignificant. Similarly, there was no difference in the time of sampling irrespective of the health status.

The interaction effect involving health status and stionic parts in healthy trees with scion mean total phenol content of 1.60 per cent and rootstock mean total phenol content of 1.22 per cent were significant whereas in declined trees these values did not differ significantly. The other interaction effects involving health status of plants with time of sampling, stionic parts with time of sampling and total phenols values in the body of the table were nonsignificant.

Figures 3 & 4 illustrate the comparative concentrations of leaf and bark biochemical constituents in healthy and declined trees of Kinnow mandarin.

Table 31. Spatiotemporal distribution of total phenols in scion and rootstock bark of healthy and declined trees of Kinnow mandarin

Month of sampling	Total phenols (%) in bark								
	Healthy			Declined				Stionic part x sampling time mean	
	S	R	Mean	S	R	Mean	Time Mean	S	R
April	1.52	1.16	1.34	1.20	1.32	1.26	1.30	1.36	1.24
Sept.	1.68	1.28	1.48	1.16	1.12	1.14	1.31	1.42	1.20
Mean	1.60	1.22	1.41	1.18	1.22	1.20		1.39*	1.22*

C.D. at 5% level

Health status (S)	= 0.20
Stionic part (P)	= N.S.
Sampling time (T)	= N.S.
P x T	= N.S.
S x P	= 0.29
S x T	= N.S.
S x P x T	= N.S.

S = Scion; R = Rootstock

*Overall stionic part means

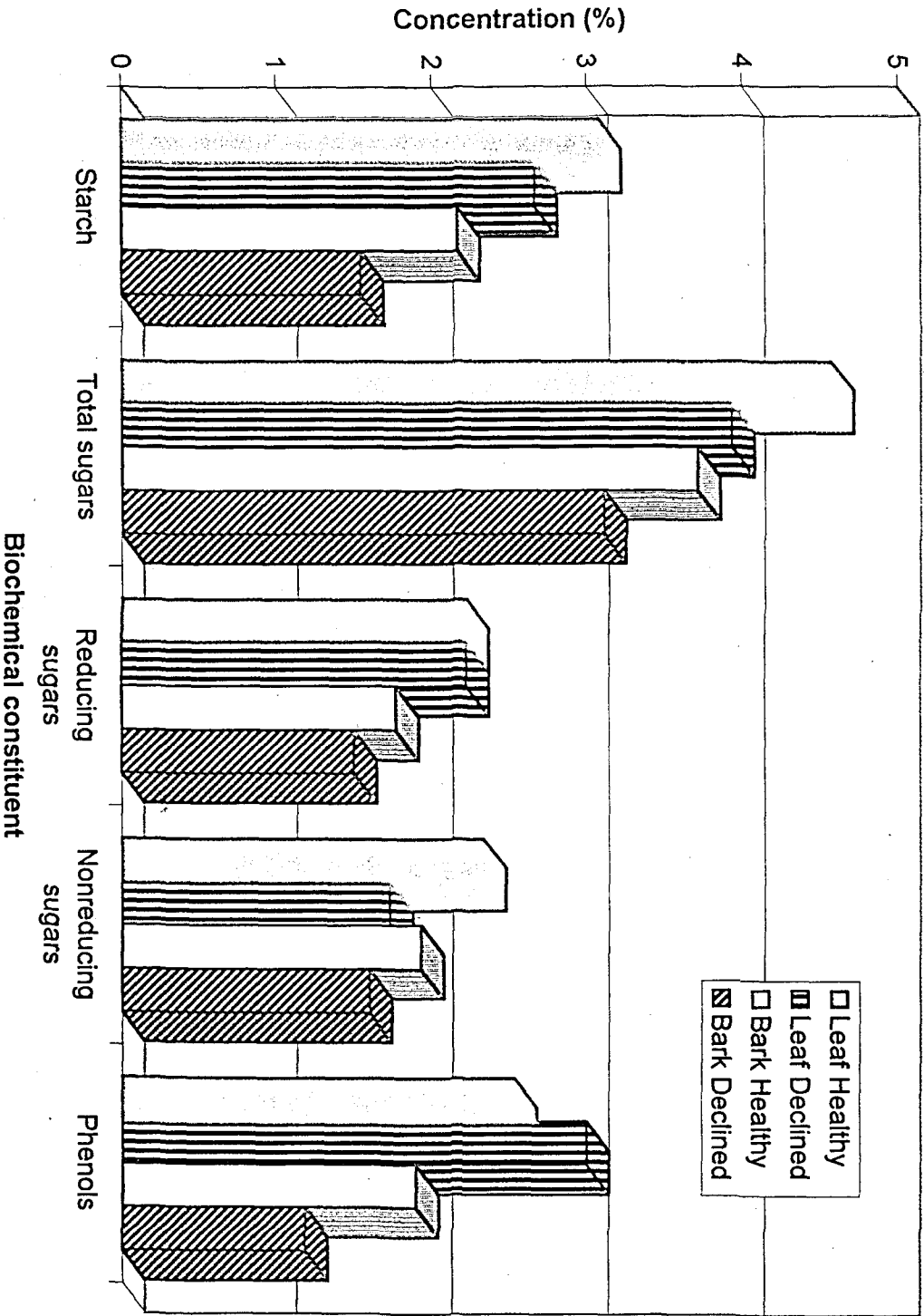


Fig. 3 : Concentration of biochemical constituents in leaf and bark of healthy and declined trees of Kinnow mandarin

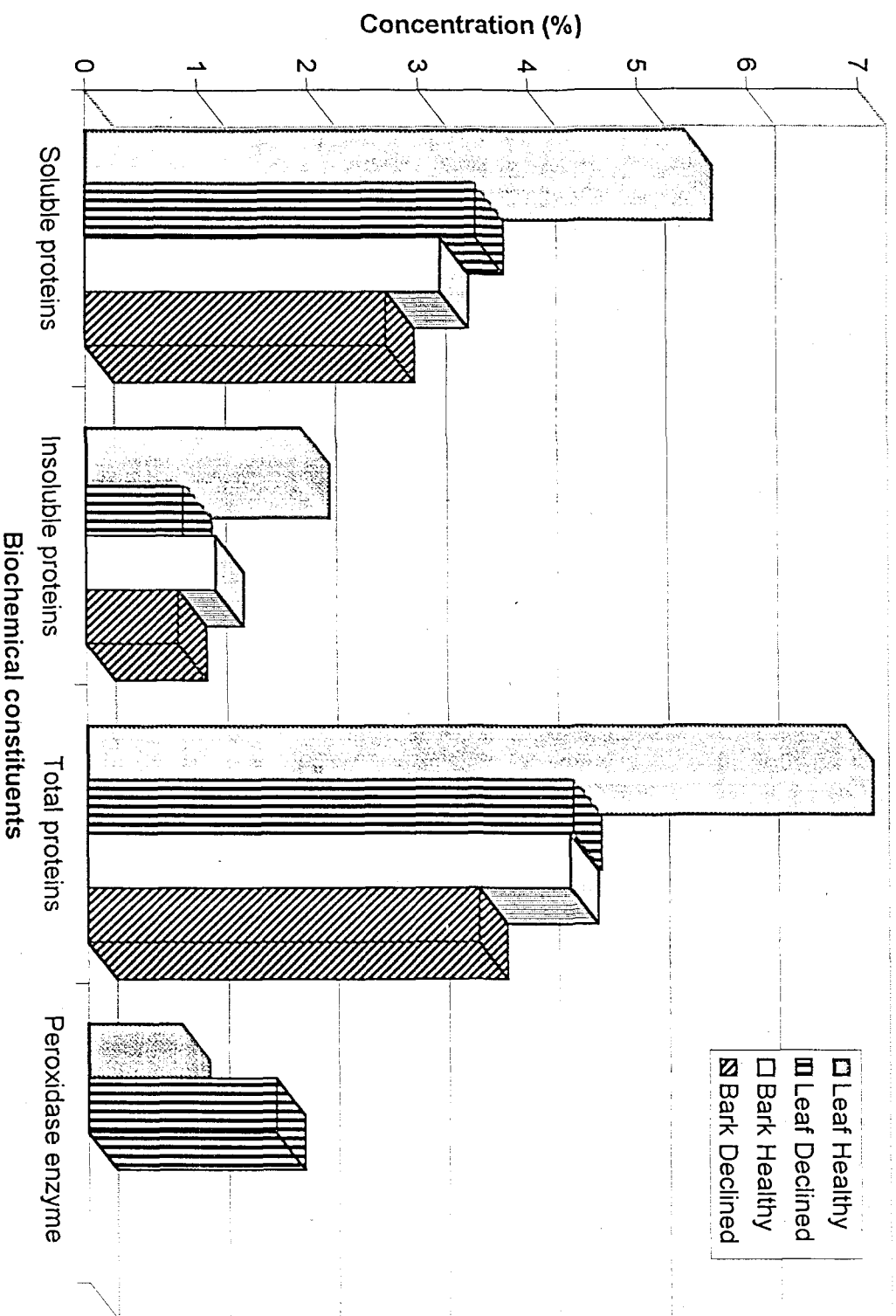


Fig. 4 : Concentration (%) of biochemical constituents in leaf and bark of healthy and declined trees of Kinnow mandarin. The peroxidase enzyme activity is indicated in O.D. change/s/g fresh weight

The present investigations were carried out with the objectives of studying the soil, nutritional, biochemical and physiological factors affecting the health of Kinnow mandarin. The results obtained during the investigation are discussed below:

A. SOIL AND NUTRITIONAL ASPECTS

I. Soil characteristics:

a. Physicochemical properties

i) Soil pH: The pH is one of the most important chemical characteristics of the soil which affects the physical environment and availability of nutrients. At higher pH (> 9.0) values of soil, the nutrient availability is less. However, in the present study (Table 4), the soils under healthy trees showed slightly higher (8.45) pH than declined trees (8.30). Wander (1952) reported that citrus

could do fairly well in soils having pH 6.5 to 9.0.

Low soil pH in declined trees may be due to the accumulation of sulphur ions in the soils of declined trees as a result of the application of sulphur containing fertilizers which might have been unused. Lande et al. (1977) reported the accumulation of sulphur ions in the soil lowers its pH. As for the availability of nutrients, the presence of high organic carbon in healthy compared to declined tree soils might have counteracted the possible ill effects of slightly high pH of healthy tree soil. Raina (1988) reported the availability of Zn to the citrus plant was significantly and positively correlated with organic carbon, the relationship being of the lower order with clay and pH. Thus, as far as soil pH was concerned, the pH of both the soils under healthy and declined trees seemed to be ideal in the present study.

ii) Electrical conductivity (EC) : It was observed in the present study that there was no significant difference in the mean values of soil EC between healthy and declined trees(Table 4), however, at a depth of 0-30 cm i.e. the surface soil which harbours most of the feeder roots, the EC value was significantly lower (0.046 dSm^{-1}) for healthy than the declined trees (0.072 dSm^{-1}). These EC values are much lower because of sandy texture of soil than the values of upto 0.5 dSm^{-1} considered safe for citrus by Kanwar et al. (1965).

iii) Organic carbon: It was evident from the data that the soil under canopy of healthy trees contained significantly more organic carbon than that under the declined trees (Table 4). The organic carbon was also significantly more in healthy tree soil as compared to declined tree soil at 0-30 cm depth. The organic carbon in soil at root zone was in deficient range as per the standards given by Malewar and Jadhav (1979). Low organic carbon in declined tree soil might be the result of sparse foliage in the trees resulting in little leaf fall and humus formation relative to healthy tree soil. These observation are in conformity with those of Richards (1968); Lande et al. (1977); Melewar and Randhawa (1978); Awad et al. (1985) and Raina (1988).

b. Macronutrient status of soil

i) Soil Nitrogen: It was evident from the data in table 5 that the available nitrogen content in both the healthy and declined tree soils was lower than the optimum (2.5-2.7) as per the standards given by Subbiah and Asija (1956). Though the difference in the soil N content was nonsignificant between the healthy and declined trees, the lower level of soil nitrogen content in the healthy trees might be due to its better uptake due to higher organic carbon content in healthy tree soils. This was consistent with earlier reports that the available nitrogen content in the soil depends upon its organic carbon content as well as

the pH of the soil (Nijjar and Singh, 1971; Nilangekar and Patil 1981a and Raina, 1988).

ii) Soil phosphorus: The study revealed that there was no variation in the phosphorus content of both healthy and declined tree soils (Table 5). It was, however, higher in upper layer (0-30 cm) but abrupt reduction was observed in deeper layers (30-60 and 60-90 cm). The available phosphorus content in soil irrespective of the health status was lower than the critical limit reported by Olsen *et al.* (1954). There was a significant negative relationship between available phosphorus and the calcium content and pH of the soil (Lynd and Turk, 1948; Harding and Chapman, 1950) whereas it was positively correlated with organic carbon content in soil (Buckman and Brady, 1983).

iii) Soil potassium: It was observed in this study that the available potassium content in the soil below the canopy of healthy trees was significantly more than that under the canopy of declined trees (Table 5). However, the levels in both healthy and declined trees, were in the optimum range according to the levels as reported by Jackson (1973).

Higher soil potassium under the healthy trees could be partly due to the release of potassium from decaying of dropped leaves from the greater canopy and leaf area of healthy trees compared to declined ones and partly due to retention of K by organic matter as organic matter was

higher in healthy trees soil compared to declined tree soil. Rickard and Keough (1968) also reported that higher level of potassium content of the soil below the plants was due to release of potassium from fallen leaves of deep rooted plants in arid climate.

iv) **Soil calcium:** The available calcium concentration in the soils under the canopy of healthy and declined trees did not differ, however, the calcium content at lower depth of 60-90 cm was more than upper depths (Table 5) probably due to accumulation of calcium of lower depth.

The higher calcium concentrations at lower depth could also be associated with high soil pH and EC (Lynd and Turk, 1948; Pearson *et al.*, 1957 and Lee, 1978).

v) **Soil magnesium :** There was no significant difference between the available magnesium contents of healthy and declined tree soils (Table 5). Nilangekar and Patil (1981b) also reported a marginal difference in water soluble magnesium of healthy and chlorotic orchard profiles. Similar to calcium more accumulation of magnesium was observed at lower depth.

c) Micronutrient status of soil

i) **Soil zinc :** The DTPA extractable zinc status of the soils under healthy and declined trees was at par with each other (Table 6). However, at 0-30 cm depth, the zinc content was significantly more than at 30-60 and 60-90 cm

depths. Similar variation in DTPA extractable Zn was observed by Yadav et al. (1997). The DTPA extractable zinc contents under both the types of trees were below the optimum standard as reported by Lindsay and Norvell (1978). Low to deficient ranges of DTPA extractable Zn levels in citrus soils were earlier reported by Ercivan and Karaca (1979); Ouyang et al. (1984); Sharma et al. (1986) and Raina (1988). Linear decrease in the zinc content at various soil depths might be due to parallel variation in organic carbon at various soil depths. Moreover, the low levels of Cu at higher depths did not affect the zinc uptake by the roots from higher depths. Higher level of Cu has been reported to depress the availability of zinc to the trees (Smith, 1956).

ii) Iron : The present studies revealed that there was no significant difference in the DTPA extractable iron contents in the soils of healthy and declined trees (Table 6). The iron content at 0-30 cm soil depth was significantly more (1.56 ppm) than either at 30-60 or 60-90 cm soil depths. However, all the levels of soil iron were below the critical levels as reported by Lindsay and Norvell (1978). The iron contents in the soils of declined trees were slightly more at 0-30 and 30-60 cm depths, which may be due to less uptake by the declined trees as reflected in leaf analysis. The iron contents in soils generally decreased with depth in healthy and declined trees. Chadda (1966) and

Chavan et al. (1980) also observed vertical variation in soil iron content.

iii) Soil manganese : It was observed in the present study that there were no significant differences in DTPA extractable Mn contents in soils of healthy and declined trees as well as among the different depths (Table 6). However, all the values were well above the critical limits reported by Lindsay and Norvell (1978).

iv) Soil copper: There was no significant difference in the copper contents of soils under healthy and declined trees (Table 6). At 0-30 cm soil depth, the Cu content was significantly higher than at lower depths. In the upper layer (0-30 cm) it was, however, in the optimum range in both, healthy and declined trees as per the standards of Lindsay and Norvell (1978).

d. Leaf macronutrient status

i) Leaf nitrogen : The present study revealed that the leaf N content (Table 7) in healthy trees was significantly more (2.20%) than that in declined trees (1.81%). The nitrogen content in the leaves of healthy trees was in the low range and in the leaves of declined trees, it was in the deficient range as per the Florida citrus leaf nutrient standards (Koo et al., 1984). Deficiency of nitrogen in declined trees of citrus has been widely reported (Mann et al., 1978; Ahlawat et al., 1982; Yamdagni

et al., 1983; Awasthi et al., 1984; Coelho et al., 1984; Dhillon and Dhat, 1988; Kohli et al., 1997). However, Dey and Singha (1998) observed no differences in leaf nitrogen contents of healthy and declined trees of mandarin in Assam although many of the declined trees had lower leaf nitrogen than those on healthy trees, the levels being sufficient for both the types of trees.

ii) Leaf phosphorus : There was no significant difference between the leaf phosphorus content of healthy (0.17%) and declined (0.18%) trees which were in the high range (Table 7). These observations were in conformity with those of Randhawa et al. (1967). Mann et al. (1979); Wutscher et al. (1983); Ahlawat et al. (1982); and Yamdagni et al. (1983). However, Sekhon et al. (1977) observed low leaf phosphorus content in declined citrus trees. Thus, neither the soil nor the leaf phosphorus content seem to be associated with decline in Kinnow mandarin.

iii) Leaf potassium : It was evident from the leaf analysis data in table 7 that the leaf potassium contents for healthy and declined trees did not differ significantly and both the values were around the deficient range as per the foliar nutrient standards (Koo et al., 1984). These results conform to the findings of Coelho et al. (1984) in Sao Paulo, Brazil; Awasthi et al. (1984) and Sharma and Mahajan (1990) in Himachal Pradesh and Dhillon and Dhatt (1988) in Punjab also reported low potassium in leaves of

declined trees compared to healthy trees whereas high to excess leaf potassium were found to be associated with decline in citrus (Mann *et al.*, 1979; Ahlawat *et al.*, 1982; Reddy and Sharma, 1982 and Yamdagni *et al.*, 1983).

In the present study, the levels of potassium in the soil under healthy trees were significantly more than those under the soils of declined trees. The leaf K levels were, however, at par with each other. This could be due to the balance of other soil nutrients (Rajput and Sriharibabu 1985). Dey and Singha, (1998) also reported that the uptake of potassium in citrus was not related to its absolute concentrations in soils but to its relative concentration and forms of potassium in the soil.

iv) Leaf calcium : The leaf calcium contents in this study were significantly more in healthy trees than in declined trees which were in the optimum range (Table 7). Calcium deficiency was reported in declined citrus trees by Kanwar *et al.* (1963); Sekhon *et al.* (1977); Reddy and Sharma (1982) and Dey and Singha (1998).

Calcium is required for better structural build up of the plant parts. The higher levels of Ca in healthy and lower levels in declined trees indicate involvement of calcium, directly or indirectly in citrus decline.

v) Leaf magnesium : The leaf magnesium contents in healthy and declined trees did not differ significantly and were in optimum range (Table 7). The sufficient levels of

leaf Mg in the healthy and declined trees could be due to the higher levels of exchangeable Mg in the soils of both, healthy and declined trees. Nilangekar and Patil (1981a); Sharma et al. (1986) and Nunez and Valdez (1994) also reported optimum to high levels of magnesium in declined citrus trees.

e. Leaf micronutrient contents

i) Leaf zinc : It was evident from the data in table 7 that the leaf zinc content in healthy trees was significantly more (25.81 ppm) than in the declined (19.17 ppm) trees. Thus, the leaf zinc content of healthy trees was just about optimum range whereas that in declined trees was at critical level. Low to deficient levels of foliar zinc contents have widely been reported in declined citrus trees (Prasad et al., 1981; Ahlawat et al., 1982; Reddy and Sharma, 1982; Chauhan et al., 1984; Sharma et al., 1986; Sharma and Mahajan, 1990 and Yadav et al., 1997).

ii) Leaf iron: The study revealed that the leaf iron content was significantly higher in healthy than in declined tree leaves (table 7). Both the values were in higher range inspite of the low levels of soil iron content according to the leaf nutrient standards of Koo et al. (1984). The increased uptake of iron by Kinnow mandarin might be due to the presence of sufficient available iron. The present observations are contrary to the findings of

Prasad *et al.* (1981) who observed high foliar levels of iron in declining mandarin orchards of northeastern India.

iii) Leaf manganese : It was evident from the leaf analysis data in table 7 that the leaf manganese content in declined trees was significantly more (45.70 ppm) than that in healthy trees (35.90 ppm). The levels in both the trees were in optimum range. Kanwar and Randhawa (1960), Randhawa *et al.* (1966), Coelho *et al.* (1984) and Sharma *et al.* (1986) also observed higher leaf manganese levels in declined than in healthy citrus trees. However, Kovanci *et al.* (1978); Mann *et al.* (1979) and Yamdagni *et al.* (1983) observed no marked differences in manganese content in chlorotic and healthy citrus trees, whereas low to deficient foliar levels were observed in healthy and declined citrus trees (Reddy and Sharma, 1982; Wuwel *et al.*, 1998). The higher level of leaf Mn as observed in the present study in the declined trees might be due to slightly lower pH in the soil of declined trees.

iv) Leaf copper : The leaf copper content in declined trees was in higher range and significantly more than in healthy trees (Table 7). The healthy trees were also having copper in optimum range. Higher levels of leaf copper in declined tree leaves were also reported by Prasad *et al.* (1981), Yamdagni *et al.* (1983) and Coelho *et al.* (1984) whereas Wuwel *et al.* (1998) reported lower copper contents in leaves of declined trees.

f. Bark macronutrient contents

i) **Bark nitrogen** : It was evident from the present study that there was no difference between nitrogen contents of healthy and declined tree barks irrespective of the rootstock and scion (Table 8). The results obtained in present investigation are not consistent with the findings of Wutscher and Hardesty (1979) and Williams and Albrigo (1984) who observed higher nitrogen levels in the bark phloem of blight affected citrus trees. Hence, there does not seem to be any association of phloem bark nitrogen levels in healthy and declined trees in the present study.

Chauhan (1968) reported that due to the impediment in translocation through phloem there was accumulation of starch and total nitrogen above the union in declining than in healthy trees of sweet orange. On the contrary, the present study revealed that there was increase in nitrogen content of the rootstock bark than the scion bark regardless of the health status of the trees. Hence the possibility of impediment between the phloem through the stock-scion union was ruled out in this study.

The seasonal variation observed in phloem bark nitrogen contents may be due to less uptake by leaves in the beginning (in early April) and higher uptake in September.

ii) **Bark phosphorus** : It was revealed that there was no difference in phosphorus content of phloem bark

between healthy and declined trees (Table 8). Albrigo (1984) did not observe any significant difference in the blight affected and healthy citrus trees in Florida.

The higher phosphorus content in scion bark may be due to increased phosphorus requirement due to active growth in April and September.

The higher bark phosphorus content in April could be due to its translocation through the phloem in the form of metabolites like ATP to the activating root system in the spring.

iii) **Bark potassium** : The results indicated that there was no difference in the bark potassium contents in healthy and declined trees (Table 9). Similar observations were reported by Williams and Albrigo (1984) who did not observe a consistent increase in bark potassium content in declined citrus trees. There was, however, difference in the spatial distribution of potassium which was higher in the scion and lower in the rootstock bark which could be due to more metabolic activities in the scion portion than in the rootstock portion. Wutscher and Hordesty (1979) and Wutscher *et al.* (1981) also observed higher potassium levels in the wood of the branches and the trunk of declined trees.

iv) **Bark calcium** : It was evident from the data that bark calcium in healthy trees was more than that in declined trees (Table 10). This might be due to the more uptake of calcium by the healthy plants to meet the

requirements of large canopy since calcium is a structural component of cell wall. The corresponding leaf calcium contents were also higher in healthy plants. Bark samples collected in April contained less calcium than those collected in September. Wutscher and Hardesty (1979), however, did not observe such variation in blight affected citrus trees.

v) Bark magnesium : It was evident from the data that the bark magnesium contents in healthy and declined trees were nonsignificant (Table 10). This observation was in conformity with that of Wutscher and Hardesty (1979) who also observed no marked difference in bark magnesium in blight affected and healthy citrus trees in Florida. However, Wutscher *et al.* (1981) reported usually higher levels of magnesium in trunk wood of declined than in healthy trees.

The rootstock bark magnesium content differed significantly with the time of sampling. September sampling contained more magnesium than that in April. Accumulation of magnesium in September might be the result of higher accumulation and least utilization because of lowering of temperature in September.

g. Bark micronutrient status

i) Bark zinc : It was evident from the data that the declined tree bark accumulated more zinc than healthy

trees (Table 11). The elevated zinc levels in the phloem bark of the declined Kinnow trees in the present study were in conformity with the earlier findings of Wutscher and Hardesty (1978), Graca et al. (1979), Albrigo and Young (1980) and Williams and Albrigo (1984).

In the month of April the healthy tree bark contained less zinc compared to that in September whereas inverse was true for declined trees. The seasonal cycles have also been reported for Zn levels in the outer trunk wood of both blighted and healthy trees (Wutscher, 1981) who observed increased levels in winter and reduced levels in the summer.

The reduced levels of zinc in the April sampling might be due to its increased utilization for auxin biosynthesis during the increased meristematic activity.

ii) **Bark iron** : The data revealed that the bark of declined tree contained significantly more iron than that of the healthy trees (Table 11). There was a marked difference in the scion bark of healthy and declined trees. The scion bark of declined trees contained 710 ppm as compared to 501 ppm in healthy trees.

As far as the temporal distribution of bark iron contents is concerned, the healthy tree bark accumulated significantly more iron in September than in April whereas no such consistent seasonal variation existed in declined trees. The decrease in bark iron in healthy trees in April could be due to increased metabolic activities in the

meristematic regions during the flush period.

When the stionic parts were compared with time of sampling, the scions tended to accumulate more iron in April than in September. No such spatiotemporal variations in phloem bark iron contents with respect to healthy and declined citrus trees were reported earlier. The accumulation of iron in phloem bark of declined trees irrespective of stionic parts could be due to its nonutilization and excessive uptake of iron by the sparse foliage. Wutscher and Hardesty (1979) could not find consistent pattern of bark iron distribution in blight affected citrus trees.

iii) **Bark manganese** : The bark manganese contents in present investigation did not vary in healthy and declined trees (Table 12). Wutscher and Hardesty (1979) also did not observe any significant difference between healthy and declined citrus tree barks in Florida, however, Williams and Albrigo (1984) reported significantly higher manganese levels in declined trees.

The temporal distribution in bark manganese was found to be significant. The samples collected in April had significantly higher manganese than those collected in September. This marginal seasonal variation in present study could be due to differential metabolic activities in the trees.

iv) **Bark copper:** It was revealed that the bark copper content in healthy trees was significantly more than that in declined trees (Table 12). The scion barks accumulated more copper than rootstock barks. Samples collected in September showed significantly higher copper content than those collected in April. Williams and Albrigo (1984) observed significantly higher copper levels in tree trunk phloem of declined trees at some sampling dates.

v) **Bark boron :** The bark boron contents in the healthy trees were marginally higher than those in the declined trees (Table 13). Williams and Albrigo (1984) also reported significantly lower bark boron levels in declined trees whereas Wutscher and Hardesty (1978) reported no significant difference in healthy and declined tree bark boron contents. The boron deficiency in declined trees could be one of the factors for citrus decline. Epstein (1972) also reported that boron deficiency leads to phenolic acid production. Wutscher *et al.* (1977) also observed accumulation of phenolics in blight affected citrus trees.

It can be concluded from the above discussion that the bark zinc and iron levels increased whereas bark copper and boron levels decreased in declined trees. No specific difference was observed in the bark manganese levels. Since zinc redistribution occurs early in the citrus blight syndrome in Florida (Wutscher *et al.*, 1982 and Young *et al.*, 1980) the metabolic systems that involve zinc could be a

significant factor contributing to citrus decline. In the present study, bark zinc and iron levels increased markedly in declined trees. Therefore, study of redistribution of these two elements in the development of decline symptoms could hold a key in diagnosing the onset of decline in citrus.

The investigations also indicate that neither the bark manganese nor the bark boron differences from healthy tree levels appear large enough to suggest toxicity of manganese or deficiency of boron, however, boron deficiency can lead to phenolic acid production (Epstein, 1972) and blight affected citrus trees accumulate phenolics (Wutscher et al., 1977).

B. GROWTH, YIELD AND FRUIT QUALITY

I. Vegetative growth

The results indicated that there was significant difference in the plant height between healthy and declined trees (Table 14). The tree volume was 75 per cent higher in healthy than in declined trees. The normal tree growth is an important feature of a good healthy plant which varies with the nature of abnormalities in the plant. Shoot dieback and stunted growth of the plants leading to reduced tree volume as the time progresses is commonly observed in citrus decline (Chadha, 1970; Azeri, 1981; Randhawa and Srivastava, 1986).

The stock-scion circumference and stock-scion ratio in healthy and declined trees showed no differences indicating that there was a smooth union between rootstock and scion. The stock-scion incompatibilities were reported to be the cause of decline in citrus (Chauhan, 1968; Schneider et al., 1978; Azeri, 1982; Bridge and Youtsey, 1996). However, in the present study no such abnormality was apparent.

It was also indicated in the present studies that the leaf area in the declined trees reduced significantly. Reduction in leaf area has a direct bearing on the productivity of the tree. Reduction in leaf area in declining citrus trees was also reported by Kanwar and Randhawa (1960). Blight affected citrus trees had fewer and smaller leaves, less leaf area/tree than the healthy trees (Wutscher et al., 1978; Syvertsen et al., 1980) whereas soil salinisation also affected the leaf area (Patil and Bhambota, 1978). The reduction in leaf area in the present studies could be due to physiological and nutritional disorders.

The declined trees as observed in the present study wore a deserted look with drastic reduction in canopy, sparse, pale green to chlorotic foliage, abundance of dead wood due to dieback which were in sharp contrast to the profusely growing healthy trees with lush green, abundant foliage and gigantic canopy (Plate 1 & 2). Similar observations were reported by Rajput and Sriharibabu (1985), Randhawa and Srivastava (1986) and Diware and Kolte (1990).

II. Yield

The fruit size and yield are directly related to the health and vigour of the plant. The results on the various yield parameters in the present study revealed that the number of fruits/tree, fruit weight and yield/tree was markedly reduced in declined trees (Table 15). The tree efficiency relating to tree volume and fruit yield was found to be significantly lower, in declined trees. Yadav *et al.* (1997) also reported reduction in yield with reduction in tree canopy in Nagpur mandarin. Deterioration of cropping and fruit quality in declined citrus trees has also been reported (Chadha, 1970, Diware and Kolte, 1990, Nunez *et al.*, 1994; Shyam Singh, 1997).

Randhawa (1960) also reported that citrus fruits from declined trees were small and malformed whereas Burnett *et al.* (1982) observed that blight affected citrus trees produced fruits normal in appearance but reduced in size and sometimes irregular in shape.

III. Fruit quality

a. Physical parameters

The studies revealed that there was significant deterioration of fruit quality in terms of increased firmness and rind thickness in declined trees (Table 16). Chadha *et al.* (1970) also observed an increase in roughness and luster in the fruits of declined trees deformed or

irregular shaped fruits in declined trees also reported (Randhawa, 1960 and Burnett et al. 1982).

b. Chemical characteristics

The data on fruit chemical characteristics revealed that there was significant reduction in juice content in declined trees (Table 17). It was consistent with the earlier report by Chadha et al. (1970). There was increase in total soluble solids and sugars in declined tree fruits which could be due to their less juice content. The acidity was also more in declined trees. However, the TSS/Acid ratio was about 14 in the healthy trees which is desirable compared to about 11 as observed in declined trees. Since number of fruits in the healthy trees was more, the overall brix yield per tree thus was more in these trees.

C. PHYSIOLOGICAL CHARACTERISTICS

I. Leaf bark moisture and dry matter contents

It was evident from the data in table 18 that the leaf moisture content was more in the healthy plants as compared to that in declined plants. Consequently, the dry matter content in leaves was more in declined plants. Similar pattern of moisture and dry matter was observed in case of bark samples (Table 19). The fresh and dry weight of plants depend upon the availability of moisture in the soil as well as on the absorption capacity of the root system. The

reduced moisture contents in leaf and bark tissues of the declined trees in the present study could be due to the reduced uptake of water by weakened activity of root system. Pearson *et al.* (1967) reported that the fluctuating soil water table was associated with reduced fresh weight of the grapefruit trees. The increase in dry matter of the plant tissues in declined trees was due to less moisture content. Considering the large foliar area and canopy size in healthy trees, the total biomass dry matter in healthy tree was higher. Wutscher *et al.* (1980), Brlansky *et al.* (1984, 1985) reported that water status in blight affected citrus trees was reduced as the water uptake was affected.

II. Leaf chlorophyll and carotenoid pigments

It was evident from the data in table 20 on the chlorophyll pigments that the chlorophyll 'a' & 'b' pigments were higher in healthy trees as compared to declined tree leaves. The better availability of N and Fe in healthy trees could have increased the chlorophyll pigments. Devi *et al.* (1994) reported the association of the micronutrients like Zn, Fe and Mn with leaf chlorosis in Sathgudi orange in Tamil Nadu and they were able to correct the symptoms of chlorosis through the sprays of micronutrient salts. Similarly, Rao *et al.* (1977) reported less chlorophyll pigments in declined citrus plants.

The carotenoid pigments were observed to be more in declined tree leaves relative to healthy trees. Since the

chlorophyll pigments were degraded in the declined tree leaves, the metabolic pathway could have been shifted to carotenoid synthesis resulting in increase in carotenoid pigments.

III. Leaf temperature, stomatal conductance and transpiration

It was evident from the data that the leaf temperature was more in healthy trees than that in declined trees. There was no difference in the leaf stomatal conductance and the rate of transpiration in healthy and declined tree leaves. The decrease in the leaf temperature in declined trees relative to healthy trees might be due to unusually higher stomatal conductance and rate of transpiration in declined tree leaves. These results were not consistent with those of Syvertsen *et al.* (1980) who observed lower stomatal conductances and transpiration rates in the blight affected sweet orange trees than the healthy trees.

The higher rate of photosynthesis observed in healthy trees in the present study was obviously due to the abundant chlorophyll content coupled with better status of leaf nitrogen, zinc and iron in these trees. The observations were in conformity with those of Devi *et al.* (1998) who reported the increased chlorophyll contents and photosynthetic activity in sweet orange trees treated with combination of Zn, Fe and Mn mineral elements.

D. BIOCHEMICAL CHARACTERISTICS

I. Peroxidase enzyme activity

The results of the present study revealed that the peroxidase enzyme activity in the leaves of healthy trees was lower than that in the declined trees, the difference being more pronounced in the month of April (Table 21). The increased enzyme activity in the declined trees might be due to the fact that the plants infected with pathogens show higher peroxidase activity. The increase in peroxidase enzyme activity is amply demonstrated in declining citrus trees (Bor-Joseph and Loebenstein, 1973; Mantoo, 1975 and Nieves *et al.*, 1994).

Bar-Akiva (1968) reported that the enzymatic activity was positively related to the level of the corresponding metal in the substrate and to the growth response of the trees. There was increase in iron content in the bark of declined trees and decrease in leaf iron content.

II. Biochemical constituents in leaf and bark tissues

a) Starch: It was evident that the starch content in the leaves and bark of healthy trees was more than that in the declined trees (Table 22 & 23). There were also temporal variations in the leaf and bark starch contents. These observations are consistent with the earlier reports (Fawcett, 1946; Schneider, 1954; Mosse, 1962; Chauhan, 1968). However, Mendal and Cohen (1967) did not find any

difference in starch levels of the two components of incompatible combinations of Shamouti orange on Sweet lime. Jones and Steinacker (1951) also reported seasonal variation of starch contents in leaves of Valencia orange and Eureka lemon.

b) Sugars: The total and non-reducing sugars in leaves of healthy trees were more than that in the declined trees. However, reducing sugars did not vary significantly in healthy and declined trees (Table 24).

The bark total, reducing and non-reducing sugars were also observed to be more in healthy trees compared to those in declined trees (Table 25 & 26). There was, however, no temporal variation observed.

The decrease in sugar contents in leaves of declined trees of sweet orange (Mantoo, 1975) and acid lime (Singh and Verma, 1977) was reported. Chauhan (1968) also observed marked spatial redistribution of reducing sugars in scion and rootstocks barks, the scion bark accumulating more reducing sugars than the rootstock bark in declining sweet orange trees. In the present study accumulation of reducing sugars in scion bark was nonsignificant.

c) Proteins : It was evident from the results that the soluble, insoluble and total proteins in leaf and bark samples were significantly more in healthy as compared to declined tree leaves (Table 27, 28 & 29). The bark samples collected in the month of April contained less soluble and

total proteins than those collected in the month of September. Nieves et al. (1994) found low proteins in vegetative and reproductive organs (leaves, buds, ovaries etc.) in the poor yielding Washington Navel oranges relative to high yielding Valencia Late oranges.

Although most of the reports on protein fractions are concentrated on viral infections owing to the fact that virus is formed from ammonia nitrogen and non-nitrogenous carbon sources, Commoner and Dietz (1952) stated that the bulk of the nitrogen incorporated into virus protein was drawn from the leaf's pool of soluble N. However, in the present study the leaf nitrogen content in healthy trees was more than that in declined trees whereas there was no difference in the bark nitrogen contents in the healthy and declined trees. Monselise and Goren (1965) reported that total nitrogen and protein nitrogen contents of leaves and bark were found to be essentially identical for all classes of psorosis infected Valencia orange trees as compared to control. Since no pathological association with citrus decline was studied in the present investigation, it can only be said that increase in the soluble, insoluble and total protein content in the leaf and bark samples is associated with the decline in citrus.

d) Phenols : It was revealed that the total phenol content was higher in leaves of declined trees than in the healthy trees, whereas, converse was true for the bark

phenol contents in healthy and declined trees (Table 30 & 31). The interaction effect revealed that the scion bark phenol content in healthy trees was more than the rootstock bark whereas no such spatial distribution was observed in case of declined trees. Errea (1998) reported that phenol synthesis can be triggered by various stress situations such as wounding, infections and plant defence mechanisms.

The leaves of psorosis infected Valencia trees were reported to bear high levels of caffeic acid, quercetin, eriodictyol and scopoletin (Monselise and Goren (1965) which is consistent with the findings of this study, however, Mantoo (1975) observed that only the phenolic compound coumarin was absent in the leaves of healthy trees and was present in declined trees.

High levels of water soluble phenolics in the wood were also observed in the declining citrus trees in South Africa (Graca et al., 1979). Similarly, Deen Mohammad (1996) also reported that the growth of the various stionic combinations was found to be inversely related with the total amount of phenolic compounds in the bark. A fluorescent phenolic compound gentisoyl- β -D-glucose was found to be present in the bark and fruit albedo of greening affected citrus trees (Schwarz, 1968).

Hence, it appears that the specific phenolic compounds rather than the total phenol content in a plant tissue are associated with decline in citrus. Young et. al. (1966) reported that many phenolic compounds even in the trace

forms are phytotoxic in nature and their glucosidisation greatly reduces their phytotoxicity. Even deficiency of trace element boron induces phenolic acid formation (Epstein, 1972).

Thus, it can be concluded from the present study that the low leaf N, Ca, Zn and Fe levels were associated with declined Kinnow mandarin trees whereas the high leaf Mn and Cu contents were observed in declined trees compared to healthy trees.

The leaf and bark calcium contents in healthy trees were more than that in declined trees.

One of the most significant findings of this study was that there was a marked inverse relationship in the leaf and bark Zn, Fe and Cu contents in the healthy and declined trees. The leaf Zn and Fe contents in declined trees were low relative to healthy trees whereas the corresponding bark Zn and Fe contents were higher in declined trees. Similarly, leaf copper content was more in declined than in healthy trees and the corresponding bark Cu contents was higher in healthy trees relative to declined trees. Interestingly, there were no differences in the soil contents of these micronutrients in healthy and declined trees. This indicates that the micronutrient deficiencies in declined trees is a problem of disturbed translocation and redistribution of the elements within the plant rather than their uptake from the soil. Further studies with histological and radioisotopic aids could elucidate these findings and establish their

association in citrus decline.

Since the declined trees had sparse foliage, the synthesis of carbohydrates and proteins was poor as a result there might be very little downward translocation of carbohydrates and proteins in the bark of declined trees resulting in lower leaf and bark carbohydrates and protein contents in declined trees. Out of the sugar fractions in leaf and bark tissues, low nonreducing sugars in leaves and low reducing sugars in bark were associated with declined trees. The leaf phenols content in declined trees was higher than that in healthy trees and bark phenols were higher in healthy trees. It appears that the specific phenolic compounds rather than the total phenols content in a plant tissue might be associated with citrus decline.

CHAPTER IV SUMMARY AND CONCLUSIONS

The investigation entitled "Studies on biochemical and physiological aspects of citrus decline" were carried out at the Department of Horticulture, CCS Haryana Agricultural University, Hisar during 1998-99 with the objective to study the soil, nutritional, biochemical and physiological factors affecting the decline of Kinnow mandarin. The salient findings of the study are summarised below.

The pH and EC of the soils under the healthy and declined trees were found to be ideal. Soils under the healthy trees contained significantly high organic carbon (0.28%) than those under declined trees (0.21%).

Available nitrogen and phosphorus contents in healthy and declined tree soils were lower than optimum. The available soil potassium content under healthy tree soil was more than that in declined tree soil. The DTPA extractable soil Zn and Fe contents under healthy and declined tree soils were below the optimum levels whereas Mn and Cu levels

were in optimum range in both, healthy and declined trees.

Leaf nitrogen content in healthy trees was low (2.20%) but significantly more than that in declined trees (1.81%) which was in deficient range. Leaf phosphorus level in healthy (0.17%) and declined (0.18%) trees was in the high range whereas potassium content in both the trees was lower than the optimum range (1.2-1.7%). The leaf calcium content in healthy trees was more than that in declined trees.

Leaf zinc content in declined trees was below the optimum range (25-100%) whereas iron content in healthy trees was significantly higher than in the declined trees. The leaf manganese and copper contents were significantly more in declined trees compared to healthy trees.

Bark nitrogen content in healthy and declined trees was equal however the scion bark contained less nitrogen compared to rootstock bark in healthy and declined trees. Bark phosphorus, potassium and magnesium contents did not vary significantly in healthy and declined trees, however, scion bark accumulated more phosphorus and potassium than the rootstock bark.

The healthy tree bark contained significantly high amount of calcium than the declined tree bark whereas the redistribution in scion and rootstock bark was equal.

Bark zinc content was significantly more in declined than in healthy trees. Bark iron content in declined trees was also significantly more than that in healthy trees. There was no difference in bark manganese content in healthy

and declined trees. Bark copper and boron contents in healthy trees significantly more than those in declined trees.

Healthy plants exhibited significantly more plant height and tree volume than declined trees. Leaf area was significantly higher in healthy trees compared to declined trees. Declined trees exhibited deserted look with drastic reduction in canopy, sparse, pale green to chlorotic foliage, abundance of dead wood due to dieback in contrast to the profusely growing healthy trees with lush green, abundant foliage and gigantic canopy.

The number of fruits/tree and fruit weight were significantly higher in healthy trees. The yield per hectare was significantly more in healthy trees compared to that in declined trees. The fruit firmness and rind thickness were too much to be acceptable in declined tree fruits. The fruit juice content was more in healthy trees than in declined trees. The TSS and acidity were more in declined tree fruits but TSS/Acid ratio was optimum in healthy tree fruits.

The leaf and bark moisture content in healthy trees was more than in declined trees. The percentage leaf and bark dry matter content was significantly more in declined than in healthy trees. The chlorophyll pigments, chlolorophyll 'a', 'b' and total chlorophyll were more in healthy tree leaves than in declined tree leaves. The carotenoids were, however, more in declined tree leaves. The leaf temperature was slightly more in healthy tree leaves than in declined

tree leaves. There was no difference in stomatal conductance and rate of transpiration in healthy and declined tree leaves. The rate of photosynthesis was more in healthy than in declined tree leaves.

The peroxidase enzyme activity was more in declined trees than in healthy trees. The starch contents in the leaves and bark of healthy trees were more than that in declined trees. The total and nonreducing sugars were more in the leaves and bark of healthy trees than in declined trees. Reducing sugars were more only in the bark of declined trees. The leaf and bark soluble, insoluble and total proteins were more in healthy than in declined trees. The total phenols were more in leaves of declined trees than that of healthy trees whereas higher in the bark of healthy trees compared to that of declined trees.

Thus, it can be concluded from the present study that low organic carbon, leaf nitrogen content and leaf and bark calcium contents were associated with declined trees. An inverse relationship with respect to the leaf and phloem bark Zn, Fe and Cu status in healthy and declined trees was observed indicating that it was a problem of transport and redistribution within the plant rather than the uptake of these elements.

Lower contents of leaf and bark starch, total sugars and protein contents were associated with declined trees. Similarly, low nonreducing sugar content in leaves and low reducing sugars in bark of declined trees were related with decline in citrus. The leaf and bark total phenol contents in healthy and declined trees indicated that the specific phenolic compounds rather than total phenolic content might be associated with the decline in citrus.

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ABSTRACT

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The studies on the horticultural, biochemical and physiological aspects of citrus decline were carried out on fifteen year old Kinnow mandarin trees budded on rough lemon planted at a distance of 6 x 6 m in a sandy loam soil at Chaudhary Charan Singh Haryana Agricultural University, Hisar during the year 1998-99. There were no differences between the soil pH and electrical conductivity of healthy and declined trees. The soil under healthy trees had significantly higher organic carbon (0.28%) than that under declined trees (0.21%). The leaf nitrogen content in healthy trees was higher (2.2%) than in declined trees (1.81%). The leaf and bark calcium contents were more in healthy trees. An inverse relationship with respect to the leaf and phloem bark Zn, Fe and Cu status in healthy and declined trees was observed

indicating that it was a problem of transport and redistribution within the plant rather than the uptake of these elements.

Growth, yield and fruit quality was better in healthy trees as compared to declined trees. The yield on a hectare basis in declined trees was drastically reduced (2.51 t/ha) as compared to that in healthy trees (37.83 t/ha). The leaf chlorophyll and the rate of photosynthesis were more in healthy trees. The peroxidase enzyme activity was more in declined trees than in healthy trees. There was significant reduction in the leaf and bark starch, sugars and protein contents in declined trees. Low nonreducing sugars in leaves and low reducing sugars in bark were associated with declined trees. The leaf total phenols content in declined trees was more than that in healthy trees.

