International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 3, p. 196-207, 2020

INNSPUB

RESEARCH PAPER OPEN ACCESS

Soil and leaf nutritional profiling of declined citrus orchards of Punjab, Pakistan

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Key words: Citrus, Decline, Variations, Plant Health, Analysis.

http://dx.doi.org/10.12692/ijb/16.3.196-207 Article published on March 18, 2020

Abstract

Citrus tree decline (CTD) is the subject of substantial research worldwide. CTD is a complex problem, involving both biotic and abiotic factors, and causing huge yield losses in citrus. Malnutrition is also playing significant role in citrus decline and its low yield. Objective of this study was to assess nutritional status and other parameters of rhizosphere soil together with nitrogen (N) and phosphorous (P) levels in leaves collected from healthy, partially healthy and declined trees. For samples collection, comprehensive surveys were conducted and soil and leaves samples were collected from declined, partially declined and healthy trees, from four sides and middle of the orchards. Determination of soil pH was done with glass electrode pH meter and Organic matter (OM) was determined by multiplying the organic carbon (OC) with factor 1.724. The total nitrogen (N) in soil samples was measured by Kjeldahl procedure, soil phosphorous (P) was measured using spectrophotometer, while Potash (K) content was measured with flame photometer by using K filters. The N and P level in leaves were determined using standard procedures. The results showed that electrical conductivity (EC) and pH were higher in declined orchards compared to healthy while organic matter, phosphorous, potassium, nitrogen and zinc were low in declined orchards compared to healthy. Significant variation was found in nitrogen and phosphorous levels of leaves collected from healthy and declined citrus trees. The current findings will lead in devising effective management strategies of citrus decline in Punjab.

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Introduction

Malnutrition has significant role in citrus decline. Nutritional imbalance causes chlorosis in citrus trees. Other symptoms are also produced due to nutritional deficiencies including yellowing of leaves and inter-veinal light-green areas around the midrib. Due to these symptoms, citrus tree shows retarded growth and bears narrow leaves with small twigs. Under these conditions, shoots of infected tree show the tendency towards dieback and whole tree gets dried completely in the coming years. Kiely (1957) described the general apparent citrus decline symptoms in Sri Lanka due to sandy soils, and reported that during chlorosis, silvery grey areas appear on the upper side of the leaves which resemble very closely to the marble chlorosis symptoms of manganese (Mn) deficiency. Randhawa *et al.* (1967) further reported that same symptoms were also produced during iron (Fe) and zinc (Zn) deficiencies in northwest orchards of India. In Maharashtra, India, decline of sweet orange has been reported due to micronutrients deficiencies (Srivastava and Singh, 2009). Similar symptoms have been identified in Brazil and Florida where blight affected trees showed symptoms similar to Zinc-deficiency which included wilting of canopy, leaf fall, twigs die-back, irregular flowering, and overall canopy decline. A rating scale has been developed to rationally rate Zn- deficiency in leaves, canopy wilting, dropping of leaves, abnormal lowering, die-back of twigs, broad canopy decline, and development of new internal shoots and reduced fruit size. According to this scale, 0.0–3.0 (0.0 complete healthy, 0.0–1.0 initial decline stage, 1.0–2.0 middle stage, and >3.0 most advanced stage).

Criteria have been designed to differentiate between decline and healthy citrus trees. The declined citrus trees have less uptake of water in the stem followed by occurrence of unstructured plugs in vascular bundles, decline in canopy of tree and vigor, higher level of zinc accumulation in phloem and wood, and less or no flow of water in roots of infected trees (Brlansky *et al.*, 1986). Same criteria are also being followed to differentiate blight infected citrus trees in Florida. Soil fertility has not been found direct limiting factor in decline incidence; though, a substantial relocation of nutrients takes place within the infected/declined trees during secondary transformation. This relocation of nutrients and less water uptake by trunk are the indicators for the identification of citrus decline in different countries. Young *et al.* (1980) reported that Zn accumulation in outer layers of wood while decreased water supply in inner layers of wood take place in declined trees. Wutscher (1981) suggested that anion-cation ratio and soil water dissolvable nutrients are the best methods to describe the decline in citrus. In many studies, an increased level of Zn concentration has been observed in declined trees prior to symptoms appearance. Soil related constraints including waterlogging, irrigation, drainage and increased clay content in soil, constraints related to soil fertility induced by salinity, soil pH and calcareousness, are the chief pedological factors playing role in citrus decline (Sarivastava and Singh, 2009). Under these situations, different modules comprising root-stocks tolerant to these soil stresses, nutrient management according to soil site and integrated soil management systems are the most effective ways to combat citrus decline.

Materials and methods

Estimation of total nitrogen

The present study was conducted in College of Agriculture, University of Sargodha during the year 2018-19. Total nitrogen was estimated using Chapman and Parker (1961) method. The ground leaves were digested in concentrated $H_2SO_4$and copper sulphate, potassium sulphate and ferrous sulphate mixture (10:0.5:10). For this, one 1 gm powder of leaves was taken into Kjeldhal's digestion flask and then added 30 mL concentrated H_2SO_4 and 5 gm digestion mixture. This mixture was first heated gently and then strongly until mixture converted into transparent green liquid. The mixture was then cooled and was shifted to 100 mL volumetric flask. To obtain 100 mL volume in volumetric flask, distilled and sterilized water was added. From that solution, 10 mL was transferred in micro kjeldhal's apparatus to distill it. The micro kjeldhal's apparatus contained

4% boric acid, 40% sodium hydroxide and mixed indicator of methyl red and bromocresol green. Then titration of distillate was done with N/10 sulfuric acid to restore original color of methyl red. The percent nitrogen content was estimated from the amount of acid using following formula:

$$N\ (\%) = \frac{A\ -\ B \times 100\ \times\ 100 \times 0.014}{\text{Volume of digested samples used (ml)}}$$

Where; A = Quantity of N/10 H_2SO_4 used; B = Blank reading, this reading was calculated by estimating the actual percentage of nitrogen in the sample. The digestion, distillation and titration procedures were the same for the blank sample.

Wet digestion for phosphorous (P) estimation

For the estimation of phosphorous, Yoshida *et al.* (1976) method was used. One-gram oven dried ground leaf material was shifted to a 100 mL beaker containing 10 mL tri-acid mixture (HNO_3, $HClO_4$ and H_2SO_4 in ratio of 5:2:1), and then covered the beaker with watch glass and kept it to stand still for almost four hours to subside all the initial reactions. For disappearance of the solid material from the beaker, the beaker was heated slowly and then vigorously on hot plate until a colorless solution was obtained and volume of beaker was maintained to1.5 ml. The beaker was then removed from the hot plate and allowed to cool. Distilled sterilized water was then added to get100 mL volume in volumetric flask. This filtrate was kept in disposable plastic bottles for the estimation of P.

Phosphorous estimation

This was estimated using spectrophotometer (Chapman and Parker, 1961). Samples colour was produced adding following reagents in 100 mL volumetric flask:

1. Digested leaves material = 5 mL
2. Water and H_2SO_4 (6:1) = 5 mL
3. 5% Ammonium molbydate = 5 mL
4. 0.25% Ammonium vandate = 5 mL
5. Distilled water to get the needed volume

Potassium di-hydrogen phosphate was used to obtain standard curves. Spectrophotometer (wavelength = 470 nm) was then used to analyze the samples and colour intensity was recorded and compared with standard curves to determine the P content in parts per million (ppm). The percentage of P was then determined using following formula:

$$P\ (\%) = \frac{\text{ppm on graphs x dilution}}{10^6} \times 100$$

Comparison of soil nutrients of decline, partially decline and healthy citrus Orchards

During survey, soil samples were collected from declined, partially declined and healthy trees, from four sides and middle of the orchards. Samples were collected from 0-30 cm depth around the trees and then mixed to form composite sample (Fenn *et al.*, 2006). The samples were dried under shade and then converted into powder for further procedures.

Determination of soil pH was done with glass electrode pH meter by dipping it in soil suspension to H_2O ratio of 1:2:5 (Jackson 2005).

Organic matter (OM) was determined by multiplying the organic carbon (OC) with factor 1.724 with following formula (Ghosh *et al.*, 1983):

OM content = % (OC) × 1.724

The total nitrogen (N) in soil samples was measured by Kjeldahl procedure in which soil is digested in catalyst mixture and 30% H_2SO_4 (Page *et al.*, 1965). The digest was then distillated with 40% NaOH, after that titrated with H_3BO_3 and 0.01% NH_2SO_4. Soil phosphorous (P) was measured using spectrophotometer at a 660 nm wave length (Olsen *et al.*, 1954), while Potash (K) content was measured with flame photometer by using K filters (Jackson, 2005).

Comparison of nitrogen and phosphorous profilein declined, partially declined and healthy citrus trees

During survey, collection of 50-60 leaves was made from healthy, partially declined and declined citrus trees. The samples were packed in zipper plastic bags and brought to laboratory.

The leaves samples were given two to three washings with distilled sterilized water for removing dust particles. These washed leaves were first shade dried and then oven dried. After drying, the leaves were ground to convert them into powder for the analysis of nutrients before digestion (Razi *et al.*, 2011).

Results

Soil nutritional profiling in relation to citrus decline

pH: Soil pH of citrus growing areas of Punjab was significantly different ($P<0.05$). It was observed that pH was higher in declined orchards as compared to healthy and partially healthy (Table 1).

Table 1. pH of soil samples taken from rhizosphere of healthy, partially declined and declined orchards.

Sr. #	Location	Healthy	Partially Declined	Declined
1.	Kotmomin	7.9b	8.0b	8.4a
2.	Bhalwal	8.2b	8.4ab	8.4a
3.	Sargodha	8.3b	8.4ab	8.5a
4.	Shahpur	7.8a	7.8a	8.3a
5.	Silanwali	7.7b	7.9a	7.9a
6.	Sahiwal	8.2a	8.3a	8.4a
7.	Multan	7.8a	7.8a	8.2a
8.	Faisalabad	7.4b	7.7a	7.9a

Means sharing similar letters are not significantly different at $P>0.05$.

Electrical Conductivity (mS/cm): Soil EC of citrus growing areas of Punjab was significantly different ($P<0.05$). It was observed that it was higher in declined and partially declined orchards as compared to healthy orchards (Table 2).

Table 2. Electrical conductivity of soil samples collected from healthy, partially healthy and declined orchards.

Sr. #	Location	Healthy	Partially healthy	Declined
1.	Kotmomin	1.2a	2.7b	3.0b
2.	Bhalwal	1.2a	2.7a	2.8a
3.	Sargodha	1.3a	1.2a	1.5a
4.	Shahpur	2.2a	1.1a	2.9b
5.	Silanwali	1.2a	1.4a	1.5b
6.	Sahiwal	1.4a	1.2a	1.6b
7.	Multan	1.9a	2.1b	2.8b
8.	Faisalabad	1.9a	2.4ab	2.8b

Means sharing similar letters are not significantly different at $P>0.05$.

Organic Matter (%): Organic matter in citrus growing areas of Punjab was significantly ($P<0.05$) different. In healthy orchards, soil organic matter was higher as compared to partially healthy and declined orchards (Table 3).

Table 3. Organic matter in soil samples taken from healthy, partially declined and declined orchards.

Sr. #	Location	Healthy	Partially healthy	Declined
1.	Kotmomin	1.00a	0.68b	0.56b
2.	Bhalwal	0.84a	0.73b	0.72b
3.	Sargodha	0.86a	0.79ab	0.72b
4.	Shahpur	0.84a	0.72b	0.65c
5.	Silanwali	0.86a	0.79b	0.65c
6.	Sahiwal	0.84a	0.69b	0.67b
7.	Multan	0.93a	0.83b	0.81b
8.	Faisalabad	1.44a	1.00a	0.93b
	Mean	0.93a	0.82b	0.72c

Means sharing similar letters are not significantly different at $P>0.05$.

Phosphorus (mg/kg): Phosphorus in soil of citrus growing areas of Punjab was significantly (P<0.05) different. It was observed that phosphorus was higher in soil of healthy, partially healthy compared to decline orchards (Table 4).

Table 4. Phosphorus in soil samples taken from healthy, partially declined and declined orchards located in different citrus growing areas.

Sr. #	Location	Healthy	Partially healthy	Declined
1.	Kotmomin	8.20a	7.37b	5.60c
2.	Bhalwal	7.25a	7.25a	7.72b
3.	Sargodha	7.00a	6.35b	5.90b
4.	Shahpur	7.50a	7.40a	7.17a
5.	Silanwali	7.80a	7.75a	7.35a
6.	Sahiwal	7.30a	7.20a	6.90b
7.	Multan	8.00a	7.80a	7.75a
8.	Faisalabad	8.25a	7.95b	7.52b
	Mean	7.52a	7.49a	7.26a

Means sharing similar letters are not significantly different at *P>0.05*.

Potassium (mg/kg): In different citrus growing areas of Punjab, potassium in soil was different significantly (P<0.05). It was found that in healthy orchards potassium content was higher as compared to partially declined and declined orchards (Table 5).

Table 5. Potassium in soil samples taken from healthy, partially declined and declined orchards located in different citrus growing areas.

Sr. #	Location	Healthy	Partially healthy	Declined
1.	Kotmomin	246a	182ab	180b
2.	Bhalwal	160a	152a	155a
3.	Sargodha	294a	163b	183b
4.	Shahpur	200a	194a	145b
5.	Silanwali	236a	157b	205a
6.	Sahiwal	201a	172b	105b
7.	Multan	247a	198a	156b
8.	Faisalabad	170a	153a	146a
	Mean	213a	179b	166b

Means sharing similar letters are not significantly different at *P>0.05*.

Zinc (mg/kg): In soil of citrus growing areas of Punjab, zinc was significantly different (P<0.05). In healthy orchards, zinc content was higher as compared to partially declined and declined orchards (Table 6).

Table 6. Zinc in soil samples taken from healthy, partially declined and declined orchards located in different citrus growing areas.

Sr. #	Location	Healthy	Partially healthy	Declined
1.	Kotmomin	2.90a	2.60a	2.10b
2.	Bhalwal	3.57a	2.15a	1.50b
3.	Sargodha	2.10a	1.95a	1.72a
4.	Shahpur	4.25a	3.15b	1.57c
5.	Silanwali	3.30a	2.70b	2.10b
6.	Sahiwal	2.67a	1.82a	1.25b
7.	Multan	2.57a	1.90b	1.25b
8.	Faisalabad	3.05a	2.17b	1.30b
	Mean	1.98a	1.77a	1.41a

Means sharing similar letters are not significantly different at *P>0.05*.

Nitrogen (mg/kg): Nitrogen in soil of citrus growing areas of Punjab was different significantly (P<0.05). Results showed that nitrogen content in healthy orchards were higher as compared to partially declined and declined orchards (Table 7).

Table 7. Nitrogen in soil samples taken from healthy, partially declined and declined orchards located in different citrus growing areas.

Sr. #	Location	Healthy	Partially healthy	Declined
1.	Kotmomin	500a	343b	282b
2.	Bhalwal	397a	415a	357a
3.	Sargodha	432a	397a	362a
4.	Shahpur	423a	362a	337a
5.	Silanwali	432a	397a	327a
6.	Sahiwal	423a	345a	336a
7.	Multan	467a	437a	367a
8.	Faisalabad	720a	502b	451b
	Mean	484a	404a	365a

Means sharing similar letters are not significantly different at *P>0.05*.

Leaf nutritional profiling in relation to citrus decline

Phosphorous (%): Phosphorous in leaves, collected from different citrus growing areas of Punjab, was significantly (P<0.05) different. It was observed that phosphorus content was lower in leaves of healthy trees as compared to leaves collected from partially and declined trees (Table 8).

Table 8. Phosphorous in leaves taken from healthy, partially declined and declined orchards located in different citrus growing areas.

Sr. #	Location	Healthy	Partially healthy	Declined
1.	Kotmomin	0.188a	0.203b	0.215b
2.	Bhalwal	0.164a	0.189a	0.251b
3.	Sargodha	0.162a	0.131a	0.185a
4.	Shahpur	0.152a	0.155a	0.150a
5.	Silanwali	0.112a	0.214b	0.240b
6.	Sahiwal	0.157a	0.150a	0.710b
7.	Multan	0.153a	0.168ab	0.183b
8.	Faisalabad	0.169a	0.189b	0.203b
	Mean	0.156a	0.174a	0.267b

Means sharing similar letters are not significantly different at *P>0.05*.

Nitrogen (%): Nitrogen in leaves, collected from different citrus growing areas of Punjab, was significantly different (P<0.05). It is observed that nitrogen in leaves of healthy trees was higher as compared to leaves collected from partially healthy and declined trees (Table 9).

Table 9. Nitrogen in leaves taken from healthy, partially declined and declined orchards located in different citrus growing areas of Punjab.

Sr. #	Location	Healthy	Partially healthy	Declined
1.	Koatmomin	1.953a	1.785a	1.715a
2.	Bhalwal	2.020a	1.680b	1.736b
3.	Sargodha	1.820a	1.743	1.610b
4.	Shahpur	1.834a	1.589b	1.484b
5.	Silanwali	1.820a	1.673b	1.533b
6.	Sahiwal	1.981a	1.813a	1.750b
7.	Multan	1.806a	1.764a	1.680b
8.	Faisalabad	1.911a	1.764b	1.652b
	Mean	1.893a	1.726b	1.645b

Means sharing similar letters are not significantly different at *P>0.05*.

Discussion

Soil pH directly affects plant health as it is linked with the supply of all plant nutrients. In present research, it was observed that average soil pH of declined orchards (8.3) was higher as compared to partially declined (8.0) and healthy orchards (7.5). These findings are supported by many research workers, who described that above7.5 pH, many nutrients become unavailable to plants (Rajper *et al.,* 2003; Ibrahim *et al.,* 2004; Chaudhry, 2005). So, this is possibility that higher pH might have caused negative effect on citrus tree health. Soil pH from 5.5 to 6.5 is considered best for citrus cultivation and in this respect, pH of declined orchards soil (8.3) is much higher. There are reports that citrus plants can survive in pH ranging from 4.0 to 8.5, but not for longer period of time. Low pH increases the leaching of lime and magnesium while high pH reduces the availability of trace elements (Siddiq *et al.,* 2011). Most of the soil in Pakistan is alkaline in nature having pH 7.5 to 8.5 (Ibrahim *et al.,* 2004). At this pH, availability of Zn, Fe, Mn, Ca, P and K is reduced due to precipitation, immobilization and adsorption to $CaCO_3$ (Siddiq *et al.,* 2011). Hence, our findings revealed that pH is significant factor for citrus decline.

EC refers to the amount of salt present in the soil. EC measurement detects the amount of anions or cations in the solution. Higher value of EC indicates that there is higher amount of anions or cations (salts) in the soil. In present study, soil EC of declined orchards (2.36 mS/cm) was higher as compared to partially declined (1.85 mS/cm) and healthy orchards (1.5 mS/cm). Present results are supported by the findings of many researchers (Ghafoor *et al.,* 2004; Muhammad, 2005; Siddiq *et al.,* 2007), who explained that citrus is highly sensitive to higher EC or soil salinity. If there is higher EC of the soil, plant needs more metabolic energy to absorb water. If a plant does not meet higher energy demands due to higher EC, it remains unable to absorb water, resultantly decrease in water uptake and transpiration is observed, and ultimately plant growth and yield is affected (Ghafoor *et al.,* 2004). Salts in soil come from fertilizers which we apply or come from irrigation water. Some salts in the soil are essential for plant growth while some or not. Those salts which are not required by the plant increase EC and disturb plant growth by disrupting soil-water balance. Keeping in view these considerations, it can be said that high EC negatively affects tree health and leads towards tree death. So our findings revealed that higher EC might be the cause of citrus decline.

Soil organic matter (OM) is important part of soil and plays key role in soil fertility. It maintains soil aggregation, soil water holding capacity, soil aeration and nutrient availability to plants (McCauley *et al.,* 2009). In present study, our results indicate that OM in declined (0.72%) and partially declined (0.82%) was low as compared to healthy citrus orchards (0.93%). Most of the soil in Pakistan is deficient in OM (<1%) (Pervaiz *et al.,* 2002; Ahmad and Khan, 2006; Obaid-ur-Rehman *et al.,* 2010). Higher OM in soil reflects good plant health and high yield. Low OM means less availability of nitrogen, phosphorous and other micronutrients, which ultimately adversely affect plant growth and yield (Obaid-ur-Rehman *et al.,* 2010). Considering previous findings of researchers, OM is vital for citrus, hence, its acute deficiency may be the cause of citrus decline.

Phosphorus is an essential element for plant health. It is one of the major nutrients that predicts plant growth (Ezawa *et al.*, 2002). Phosphorus performs multiple functions in plants. Most of the soils contain about 0.05% phosphorous while plant needs 0.1% (Achal *et al.,* 2007). In present study, it was found that mean phosphorous in healthy (7.52 mg/kg) and partially healthy (7.49 mg/kg) orchards was little higher as compared to declined (7.26 mg/kg) citrus orchards. This little difference might be higher in declined orchards, but as Pakistani soils are already deficient in phosphorus, therefore the difference was not so high (Obaid-ur-Rehman *et al.,* 2010; Sarwar *et al.,* 2018). To overcome phosphorous deficiency in Pakistani soils, phosphatic fertilizers are applied. Still, phosphatic fertilizers remove only 10-25%deficiency as through phosphatic fertilizers only

1.0 mg kg-1 of phosphorus is available to soils. Phosphorus availability to the plants is highly pH dependent, and as in our findings pH of declined orchards was higher, so this may also be the reason of less availability of phosphorous in declined orchards (Aziz *et al.,* 2006). Our findings confirmed that less availability of phosphorous is also the reason of citrus decline.

Potassium (K) is an important plant nutrient and plays vital role in plant health and growth. In present research, mean potassium content in healthy orchards (253 mg/kg) soil was higher as compared to partially healthy (179 mg/kg) and declined orchards (166 mg/kg). This study is in line with findings of Ahmad *et al*., (2003) and Khalid *et al.,* (2012), who reported that potassium content in most of the soils of Pakistan was satisfactory and adequate except in diseased citrus orchards. Ammar *et al*., (2015) reported that soil with <90 mg/kg potassium content is considered poor, 180-200 mg/kg considered satisfactory and with >200 mg/kg are adequate. Hence, the declined orchards have much less K, and this deficiency may be the cause of citrus decline. K helps plants in performing different physiological processes such as water, nutrients and carbohydrates transportation, photosynthesis, utilization of nitrogen, initiation of early growth, and insect pest and disease resistance (Lakudzala, 2013). Hence, its deficiency surely weakens the plant and leads towards tree decline. K also helps plants to regulate the opening and closing of stomata (Egilla *et al.,* 2005) which is important for effective use of water. In addition, a close relationship between higher level of K and plant drought resistance has also been observed (Cakmak, 2005). Thus, its deficiency may decrease the immunity of citrus plants and leads them towards decline.

Nitrogen (N) is most important among all plant nutrients. In our findings, mean nitrogen value in soil samples taken from healthy, partially healthy and declined orchards was 484, 404 and 365 µg/kg respectively. This is consistent with the findings of Leghari *et al*., (2016), who reported less nitrogen in soil samples collected from weak citrus trees. N plays pivotal role in good plant growth (Massignam *et al.,* 2009). Good growth of the plants enables them to fight better against diseases and insect-pests. During survey, it was observed that those orchards showed more decline whose growth was poor. N is often regarded as a component of enzymes, vitamins, chlorophyll molecule, and involved in nucleic acid, amino acid and protein synthesis; thus, its role in plant defense against diseases is very important. There are many proteins which are involved in plant defense against biotic factors. Thus, low nitrogen also deprives the plants of defense proteins and enzymes leading towards the death of plants or trees. Hence, the deficiency of N in declined orchards has leading role in declining the citrus trees. N is important for cell division and growth of young tissues (e.g., buds, flowers, leaves, twigs). N also affects the absorption and distribution of all other nutrients in the plant, and is particularly important to trees during flowering and fruit setting (Neto*et al.,* 2008; Stellacio *et al.,* 2013). Thus, when there will be low N, it will cause irregularity in absorption and distribution of different nutrients in the trees or plants; hence, low N is linked with citrus decline. Further, under limited available N, tree growth becomes slow and defense mechanisms of tree react slowly to the attack of pathogens or abiotic stress which succumb the trees towards death (Zekri *et al.,* 2003). In short, in declined orchards, the deficiency of N confirmed that N has role in citrus decline.

In present study, mean zinc value of soil samples taken from healthy, partially healthy and declined orchards was 1.98, 1.77 and 1.41 mg/kg, respectively. As compared to other micronutrients, zinc deficiency is more prevalent in citrus orchards of Punjab. Even, most of the soil in Pakistan is zinc deficient (Ahmad *et al.,* 2012). Several soil factors and conditions produce Zn deficiency in soil which includes higher pH, sandy texture, high salt concentrations, calcareousness, waterlogging or flooding, low organic matter content, high magnesium and/or bicarbonate concentrations, intensive cultivation and less application of micronutrients (Alloway, 2009). Zn requirement of citrus is high compared to other micronutrients (Yaseen and Ahmad, 2010). Zinc deficiency has been reported in producing several disorders in citrus trees worldwide (Srivastava and Shyam, 2009). Zn deficient citrus trees often produce inter-veinal chlorosis which ultimately leads towards decline (Chang, 2000). In conclusion, Zn deficiency is linked to citrus decline.

Phophorous is important element for plant growth and development. It is required for photosynthesis and breakdown of carbohydrates. Phosphorous helps in transfer of energy, involved in nutrient uptake and translocation, and also plays role in cell division (Zekri and Obreza, 2003). In current findings, leaf phosphorous content mean (0.267%) was higher in leaves collected from declined citrus trees as compared to leaves collected from partially healthy (0.174%) and healthy (0.156%) trees. Zekri and Obreza (2003) reported that optimum range of phosphorus in citrus tree is 0.12-0.16%. Therefore, the phosphorous content in declined tree leaves is quite high and this is possibility that this high level of phosphorus might have caused negative effects on citrus tree health. There are reports that excessive phosphorus in leaves interferes with the availability of other plant nutrients (Grundon, 2006).

Nitrogen is most important mineral in plant nutrition. It is part of amino acid, enzymes and proteins. It is essential for normal cell division, growth and respiration. Optimum range of nitrogen in citrus tree leaves is 2.5-2.7% (Zekri and Obreza, 2003). In present research, mean nitrogen percentage in leaves taken from declined citrus trees (1.645%) was lower as compared to leaves collected from partially healthy (1.726%) and healthy trees (1.893%). These findings are in line with Razi *et al.,* (2011), who reported that citrus trees infected with greening disease had lower content of nitrogen in leaves. Lower nitrogen in leaves may also be due to shortage of nitrogen in declined orchards soil. Further, low nitrogen in soil causes chlorosis and stunted growth in trees, lessen their vigor and reduces canopy size, which in the long run impedes the supply of nitrogen from soil to leaves and eventually tree shows decline symptoms (Ahmed *et al.,* 2006).

Acknowledgement

The authors are thankful to the support provided by Higher Education Commission, Islamabad, Pakistan for funding the project (No. 20-3559/NRPU/R&D/HEC/14/1156) for Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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