

Mineral Characterization, fruit and leaf physiology of *Citrus reticulata* comparative to citrus canker stress in Sargodha, Punjab

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Abstract

Pakistan's economy greatly benefits from citrus production since these fruits are sold and consumed all over the world. Nevertheless, there are difficulties associated with cultivating citrus fruits, such as illnesses brought on by bacteria, viruses, and fungus. The quantity and quality of fruit that is produced might be negatively impacted by these challenges as well as difficulties obtaining the proper nutrients. One tough problem is citrus canker, which is caused by a harmful germ called *Xanthomonas axonopodis*. This germ affects many types of citrus fruits all over the world. This study looked closely at how citrus canker affects the leaves and the quality of the fruit in places like Sargodha, Bhalwal, Kotmomin, and Silanwali, which are big areas for growing citrus in the Sargodha district. What we found was that plants without the disease had more chlorophyll in their leaves compared to the sick plants. Also, the healthy plants had better amounts of important minerals like calcium, magnesium, potassium, and phosphorus in their fruits. But the fruits with the disease had too much sodium, and the iron levels were a bit different. The fruits with the disease also didn't have as much of something that protects them called antioxidants, which made them more likely to get sick. This study helps us understand how citrus canker affects plants and fruit, so we can think of ways to deal with it.

Introduction

Citrus species are fruit trees that have a significant economic impact on the world and have been tamed and grown for many years (Shi et al., 2023), and are distributed worldwide in temperate and tropical regions, mainly on the continents of Southern Africa and Australia (Khalaf et al., 2022). Citrus fruits are grown in Pakistan over an area of 183.8 thousand hectares with a total production of 1,943.7 thousand tonnes and currently 94,806 tonnes of citrus (Kinnow and others) fruits are exported to various countries (Altaf et al., 2008).

Citrus fruit production is limited by a number of variables, including the prevalence of diseases brought on by bacterial, viral, fungal, and nematode pathogens. Citrus nematode, citrus gummosis, citrus canker, citrus greening, and citrus tristeza virus are among the prevalent illnesses that harm citrus fruits in Pakistan. Citrus canker is one of these that is common and has a negative effect on plant growth and fruit quality in all citrus-growing regions of the world (Pruvost et al., 2002). The causative agent of this disease is *Xanthomonas axonopodis* (Sahi et al., 2007). Citrus canker was initially reported in Punjab, Pakistan, but has since become an epidemic throughout the country. Unfortunately, all cultivars, including grapefruit, sweet oranges, lemons, limes, rootstocks, and their hybrids, are susceptible to this disease (Gottwald et al., 2001).

Gram-negative bacterium causing canker possesses a single polar flagellum. It thrives aerobically within the temperature range of 28–30°C and can tolerate temperatures as high as 35–39°C (Shinde et al., 2023). The surge in canker incidences in Brazil and other countries has been linked to insect-induced injuries that create new entry points for the pathogen, leading to merging lesions (Gottwald et al., 2001; Graham et al., 2004). The bacteria can also enter plants through stomata or wounds, including those

caused by leaf miners on citrus leaves. Once inside the plant, they multiply and cause necrotic patches as visible symptoms. These lesion areas serve as a potent source of infection, facilitating further bacterial growth and increasing the risk of disease outbreaks in other parts of the plant or nearby plants (Shahbaz et al., 2022). This emphasizes the importance of managing insect populations and preventing wounds to control the spread of citrus canker effectively.

Citrus canker manifests in various symptoms, such as pustules and necrotic lesions characterized by erupting corky tissues, surrounded by oily or water-soaked margins, and accompanied by a yellow halo. The severity of the disease leads to defoliation, dieback, premature fruit drop, and the development of blemished fruit (Schubert and Sun, 2003). Fruit lesions are particularly economically significant as they render the fruit unsuitable for the fresh market, resulting in substantially reduced prices. Integrated control measures for citrus canker include the production of disease-free stock, application of copper sprays, and efforts to reduce pathogen transmission facilitated by leaf miners or wind (Das, 2003; Graham et al., 2004).

Citrus canker is known for its ease of transmission and its ability to spread rapidly. The primary mode of transmission is through water, especially wind-driven rain, which carries bacterial cells from infected plants to neighbouring healthy ones (Adaskaveg et al., 2023). The bacteria can also be disseminated through irrigation water, contaminated tools, and equipment, and via human activity, such as pruning or handling infected plant material (Machado-Moreira et al., 2019). The global movement of citrus plants and fruit has played a significant role in the spread of citrus canker to new regions, highlighting the importance of strict biosecurity measures and quarantines to prevent its introduction into disease-free areas (Singh et al., 2023).

Citrus canker is a formidable bacterial disease that poses a significant threat to Pakistan's citrus plantations, causing economic losses due to reduced fruit quality and quantity. Despite on-going global efforts to control the disease, there is limited comprehensive literature on its impact on various citrus attributes. This study extensively investigated how citrus canker affects the morpho-physiological, and biochemical attributes of kinnow mandarin (*Citrus reticulata*). The study's findings will offer valuable insights for urgent disease management strategies to safeguard the citrus industry and ensure food security.

MATERIALS AND METHODS

Study area description

Sargodha is known as the "California of Pakistan" for quality citrus production. The city has a harsh climate with scorching summers and freezing winters. The economy of Sargodha is based on agriculture and various industries.

Sampling Tehsils

A systematic approach was used to collect samples in District Sargodha's four Tehsils: Silanwali, Bhalwaal, Kotmomin, and Sargodha. Each Tehsil provided five designated sites (Site A to E) for a total of 20 locations. Within each site, ten Kinnow plants aged 10 to 15 years were selected, half healthy; half infected; marked for subsequent sampling. Each plant yielded samples of 20 leaves and 20 fruits, serving as an individual replication. Harvesting occurred at commercial maturity, enabling a comprehensive assessment of plant attributes.

Total Antioxidant Capacity ($\mu\text{mol} / 100 \text{ g}$) and Crude Fiber Content determination

A 0.1 ml sample solution containing a reducing species (in water, methanol, ethanol, dimethyl sulfoxide, or hexane) was combined with 1 ml reagent solution in an Eppendorf tube (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were sealed and placed in a thermal block for ninety minutes at 95°C. Once the samples had cooled to room temperature, the absorbance of each was measured at 695 nm against a blank. A standard blank solution was prepared as follows: 1 milliliter of reagent solution with an appropriate volume of a solvent that was similar to the one used for the sample. It was then incubated in the same manner as the other samples. For materials with unknown composition, lipid-soluble and water-soluble antioxidant capacities were determined using the α -tocopherol and ascorbic acid equivalents (Prieto, Pineda, & Aguilar, 1999).

The acid-base digestion method was used to calculate the amount of crude fibers (AOAC, 2006). Three grams of the oven-dried sample were taken, the fat was removed from the Soxhlet apparatus, and the sample was digested in separate solutions of 1.25% H₂SO₄ and NaOH. Following filtration, the material underwent three rounds of washing with distilled water. The leftover material was then transferred to a china dish, and the crude fibers were measured after the dish was baked for 24 hours at 105°C. The amount of crude fibers in the sample explained the variation in weights.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of oven dried sample}}{\text{Weight of fresh sample}} \times 100$$

Mineral Assessment (mg/kg)

The fruit samples that had been baked were ground into a fine powder and then subjected to a wet digesting process. After adding 10 ml of HNO₃ to each 0.5 g sample in a digestion flask, the samples were kept there for the entire night. Following that, 5 milliliters of perchloric acid were added to the sample and the digestion process was conducted on a hot plate. Before the sample solution was clear, the process was repeated. Then, up to 100 ml of distilled water was added to form a final solution of 50 ml, which was then put for analysis. Then standard solutions were created, and the digested samples were ready for elemental analysis using those standards. The produced sample solution was fed into the atomic absorption spectrophotometer for elemental analysis. For each metal, a standard curve was created by running samples of known strength. The elemental composition of the samples was calculated using the standard curves created for each metal using the procedure (AOAC, 2006). Standard

approaches were used to estimate Calcium (Ca), Magnesium (Mg), Phosphorus (P), Iron (Fe), Sodium (Na), Potassium (K). The given formula was used to make 1000 ppm concentration stock solutions for each metal.

$$X = \frac{\text{Molecularweightofsalt}}{\text{Molecularweightofmineral}} \times 0.1$$

For the production of the standard curve, X grams of salt were dissolved in 100 ml distilled H₂O to produce a 1000 ppm solution, which was then diluted to a 100-ppm solution. A diluted solution of 1 ppm to 10 ppm was used to calibrate the equipment.

Statistical Analysis

Utilizing SPSS 20, the data was submitted to a Two-way Analysis of Variance (Completely Randomized Design, 2 variables) in order to determine the significance of the differences between means and interactions. For significant factors, Tukey HSD all-pairwise comparisons were used to compare means at the 5% level of significance. Microsoft excel was used to prepare the graphs

Results

Assessment of physiological attributes in diseased and healthy plants

Estimating the impact of Citrus canker on biochemical properties of citrus

Determination of biochemical property in healthy and diseased fruits indicates that CFC was recorded highest at Bhalwal region in healthy fruits while the highest recorded in diseased fruits at Silanwali (Table 1). The results reveal a strong statistical significance in total antioxidant activity concerning the interplay between disease factors and the geographical administrative divisions known as "tehsils." Furthermore, an evident interaction between tehsil-specific attributes and disease conditions has been established, showcasing a substantial impact on total antioxidant activity. For instance, at Bhalwal, TAA in healthy fruits was 1015.0 µmol/100g, while in diseased fruits; it was 526.0 µmol/100g. The difference in TAA values between healthy and diseased fruits was approximately 50 times higher in healthy fruits. Tehsil analysis also revealed notable variations in TAA values at the tehsil level.

Table 1
Determination of biochemical property in healthy and diseased fruits

Parameters	Tehsil	Healthy	Diseased	Mean
TAA ($\mu\text{mol} / 100 \text{ g}$)	Bhalwal	1, 015.0 $\pm$ 35.27a	526.0 $\pm$ 29.56d	770.5 $\pm$ 84.34C
	Kotmomin	1, 054.8 $\pm$ 55.67a	633.5 $\pm$ 40.00c	844.1 $\pm$ 77.30B
	Sargodha	1, 004.8 $\pm$ 23.05a	839.8 $\pm$ 20.61b	922.3 $\pm$ 31.12A
	Silanwali	1, 087.8 $\pm$ 11.74a	435.7 $\pm$ 32.31d	761.7 $\pm$ 109.89C
	Mean	1, 040.6 $\pm$ 17.92A	608.7 $\pm$ 37.45B	
CFC	Bhalwal	6.95 $\pm$ 0.111	5.58 $\pm$ 0.122	6.27 $\pm$ 0.240A
	Kotmomin	6.53 $\pm$ 0.120	5.60 $\pm$ 0.122	6.06 $\pm$ 0.175A
	Sargodha	6.87 $\pm$ 0.214	5.51 $\pm$ 0.220	6.19 $\pm$ 0.269A
	Silanwali	6.71 $\pm$ 0.154	5.74 $\pm$ 0.050	6.23 $\pm$ 0.178A
	Mean	6.76 $\pm$ 0.080A	5.61 $\pm$ 0.068B	
NS = (P > 0.05).				

Determination of minerals in diseased and healthy plants

Findings outlined that calcium and iron levels exhibit noteworthy statistical significance in relation to disease conditions, while potassium also demonstrates a significant association. In the context of interactions between tehsil attributes and disease conditions, calcium, iron, and potassium exhibit a remarkably robust statistical significance ($p < 0.01$). Delving into the interaction dynamics between tehsil factors and disease conditions, calcium retains its significance; iron retains its high level of statistical significance, while potassium's impact becomes statistically non-significant (Table 2).

Table 2
Evaluation of calcium, iron and potassium in healthy/diseased fruits

Parameters	Tehsil	Healthy	Diseased	Mean
Calcium (mg/kg)	Bhalwal	187.76 ± 8.36a	143.96 ± 2.10b	165.86 ± 8.36A
	Kotmomin	135.00 ± 2.20b	105.80 ± 3.51d	120.40 ± 5.24B
	Sargodha	185.36 ± 5.90a	146.03 ± 2.69b	165.70 ± 7.23A
	Silanwali	119.50 ± 2.83c	100.53 ± 1.96d	110.01 ± 3.55C
	Mean	156.91 ± 7.36A	124.08 ± 4.97B	
Iron (mg/kg)	Bhalwal	1.12 ± 0.068ef	0.90 ± 0.076f	1.01 ± 0.060C
	Kotmomin	1.60 ± 0.047d	1.28 ± 0.057e	1.44 ± 0.064B
	Sargodha	2.72 ± 0.093a	1.86 ± 0.095c	2.29 ± 0.157A
	Silanwali	2.57 ± 0.109a	2.21 ± 0.089b	2.39 ± 0.090A
	Mean	2.00 ± 0.158A	1.56 ± 0.122B	
Potassium (mg/kg)	Bhalwal	2, 011.34 ± 57.98	1, 944.86 ± 53.31	1, 978.10 ± 38.75A
	Kotmomin	1, 663.34 ± 55.06	1, 601.33 ± 53.81	1, 632.34 ± 37.74C
	Sargodha	1, 835.34 ± 30.54	1, 730.11 ± 52.69	1, 782.72 ± 33.64B
	Silanwali	1, 362.54 ± 46.22	1, 286.16 ± 67.29	1, 324.35 ± 40.53D
	Mean	1, 718.14 ± 59.27A	1, 640.61 ± 60.69B	
	NS = (P > 0.05).			

Results illustrates that calcium concentration was highest in healthy fruits compared to diseased fruits across all tehsils, with significant differences between the two conditions (Fig. 1A). Tehsil Bhalwal was the site where the highest calcium levels were observed for both healthy and diseased fruits, while tehsil Silanwali was the site with the lowest levels in both cases. Tehsils Kotmomin and Silanwali showed the least fluctuation in calcium concentration among healthy fruits, unlike other tehsils.

Results confirmed Bhalwal as the site where the highest calcium value was recorded for healthy fruits and Silanwali as the site with the lowest, both for healthy and diseased fruits. Tehsil Sargodha had the highest calcium value for diseased fruits. The mean value of calcium in healthy fruits significantly differed from the accumulative mean of diseased fruits, with tehsils Bhalwal and Sargodha having the highest mean values, and tehsil Silanwali having the lowest mean value (Table 2).

Analysis depicted that iron concentration was highest in healthy fruits compared to diseased fruits across all tehsils, showing a substantial difference between the two conditions. Tehsil Sargodha had the maximum iron concentration, whereas tehsil Bhalwal had the lowest iron levels (Fig. 1B). Phosphorus

concentration was higher in healthy fruits compared to diseased fruits in all tehsils, with a slight difference between the two. Tehsil Sargodha displayed the maximum phosphorus value, while tehsil Bhalwal displayed the lowest, showing fluctuations mainly in tehsil Bhalwal (Fig. 1C), while the Sodium concentration was high at Kotmomin Tehsil in healthy fruits (Fig. 1D). Results revealed that tehsil Sargodha recorded the highest iron concentration for healthy fruits, while tehsil Silanwali had the highest for diseased fruits. Conversely, tehsil Bhalwal had the lowest iron concentration for both healthy and diseased fruits. The mean value of iron in healthy fruits significantly differed from the mean value of all tehsils for diseased fruits, with Silanwali having the highest iron concentration, and Bhalwal having the lowest for both healthy and diseased fruits (Table 2).

Graphical data showed significant variations in potassium concentration among different tehsils. Moderate potassium levels were observed in both healthy and diseased fruits in tehsils Sargodha and Kotmomin, while tehsil Bhalwal had the highest levels, and tehsil Silanwali had the lowest levels. Overall, potassium concentration was slightly higher in healthy fruits compared to diseased fruits across all tehsils (Fig. 2A). that magnesium concentration was slightly higher in healthy fruits compared to diseased fruits in all tehsils. Tehsil Silanwali was associated with the minimum magnesium value, while the maximum value was observed at an unspecified location (Fig. 2B).

The diseased to tehsil interaction table for potassium, indicated that the mean value of potassium in healthy fruits differed from the mean value of all tehsils for diseased fruits. Both healthy and diseased fruits showed the highest potassium concentration in tehsil Bhalwal and the lowest in tehsil Silanwali. Tehsil Bhalwal's mean value had a significant difference from the other three tehsils (Table 2).

the results regarding magnesium, sodium, and phosphorus levels, which all display substantial statistical significance in the context of disease conditions. Similarly, the interactions between tehsil-specific attributes and disease factors yield consistently high levels of statistical significance for magnesium, sodium, and phosphorus. However, focusing on the interplay between tehsil characteristics and disease conditions, sodium remains highly significant, whereas phosphorus and magnesium exhibit statistical non-significance (Table 3).

Table 3
Evaluation of magnesium, sodium and phosphorus in healthy/diseased fruits

Parameters	Tehsil	Healthy	Diseased	Mean
Magnesium (mg/kg)	Bhalwal	171.28 ± 16.97	139.10 ± 6.17	155.19 ± 10.06A
	Kotmomin	124.37 ± 4.58	106.12 ± 4.79	115.25 ± 4.36B
	Sargodha	146.89 ± 10.39	115.92 ± 6.39	131.40 ± 7.73B
	Silanwali	131.65 ± 4.56	102.15 ± 3.19	116.90 ± 5.57B
	Mean	143.55 ± 6.33A	115.82 ± 4.09B	
Sodium (mg/kg)	Bhalwal	13.03 ± 0.35g	18.93 ± 0.54e	15.98 ± 1.03C
	Kotmomin	24.42 ± 0.57c	31.17 ± 0.92a	27.80 ± 1.24A
	Sargodha	27.42 ± 0.67b	28.77 ± 0.64b	28.09 ± 0.49A
	Silanwali	16.41 ± 0.76f	21.40 ± 0.67d	18.90 ± 0.96B
	Mean	20.32 ± 1.36B	25.07 ± 1.20A	
Phosphorus (mg/kg)	Bhalwal	261.02 ± 11.31	211.40 ± 04.28	236.21 ± 10.04C
	Kotmomin	328.00 ± 16.50	295.39 ± 18.13	311.70 ± 12.77B
	Sargodha	413.37 ± 08.45	372.49 ± 15.83	392.93 ± 10.86A
	Silanwali	362.52 ± 05.84	305.54 ± 09.38	334.03 ± 10.83B
	Mean	341.23 ± 13.71A	296.20 ± 14.44B	
NS = (P > 0.05).				

Analysis of variance (mean squares) for crude fiber content and TAA shows that TAA and crude fiber contents were highly significant with respect to disease interaction (Table 4). The diseased to tehsil interaction mean for magnesium, it was found that tehsil Bhalwal exhibited the highest magnesium concentration for both healthy and diseased fruits, while tehsil Kotmomin demonstrated the lowest concentration for healthy fruits. Regarding diseased fruits, tehsil Bhalwal also showed the highest magnesium concentration, and tehsil Silanwali had the lowest, similar to healthy fruits. The mean value of magnesium in healthy fruits was found to significantly differ from the mean value of all tehsils for diseased fruits. Among all tehsils, tehsil Bhalwal displayed the highest mean value for both healthy and diseased fruits, whereas tehsil Kotmomin had the lowest. Tehsil Bhalwal's mean value exhibited a significant difference from all other tehsils (Table 5).

Table 4
Analysis of variance (mean squares) table for crude fiber content and TAA

Source of variation	Degrees of freedom	Mean squares	
		Crude fiber content	TAA
Disease	1	13.3749**	1865087.0**
Tehsil	3	0.0763 ^{NS}	56025.0**
Disease*Tehsil	3	0.1416 ^{NS}	102595.0**
Error	32	0.1105	5596
Total	39		
NS = Non-significant (P > 0.05); * = Significant (P < 0.05); ** = Highly significant (P < 0.01)			

Table 5
Analysis of variance (mean squares) table regarding evaluated minerals

Source of variation	Degrees of freedom	Mean squares		
		Calcium (mg/kg)	Iron (mg/kg)	Potassium (mg/kg)
Disease	1	10774.5**	1.94437**	60100.0*
Tehsil	3	8704.7**	4.46710**	760571.0**
Disease*Tehsil	3	306.7*	0.20813**	943
Error	32	90.9	0.03339	14064
Total	39			
Source of variation	Degrees of freedom	Mean squares		
		Magnesium (mg/kg)	Sodium (mg/kg)	Phosphorus (mg/kg)
Disease	1	7684.26**	225.492**	20270.3**
Tehsil	3	3418.10**	382.304**	41993.2**
Disease*Tehsil	3	102.83 ^{NS}	14.084**	279.3 ^{NS}
Error	32	343.72	2.182	744.4
Total	39			
NS = Non-significant (P > 0.05); * = Significant (P < 0.05); ** = Highly significant (P < 0.01)				

Graphical data reveals that sodium concentration was higher in diseased fruits compared to healthy fruits across all tehsils. Tehsil Kotmomin was associated with the maximum sodium value, while tehsil Silanwali was associated with the minimum. Fluctuations were prominent in sodium concentration in diseased fruits of tehsil Silanwali (Fig. 3A). In the context of mineral composition, elements such as calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), and iron (Fe) exhibited elevated concentrations across all healthy samples from the studied sites (Fig. 3B).

Notably, sodium (Na) concentrations were found to be most prominent in the samples labelled as diseased across the investigated sites, presenting a clear distinction. Furthermore, a consistent trend resembling the aforementioned observations was also noted within the studied sites of T3 and T4 (Fig. 4A and 4B). This conformity in results further supports the validity and significance of the identified patterns across multiple locations of district Sargodha. In summary, the results indicate a strong association between the health status of the samples and the recorded parameters, with higher levels of chlorophyll, TAA, CFC, and select mineral compositions being linked to healthy samples. This information provides valuable insights into the potential factors influencing the health of the studied sites within the aforementioned regions (Fig. 5A-5E).

Comparison of sub-sites in each studied tehsil with respect to Chlorophyll a and b, Na, Fe and crude fiber content

Graphical data for the sub-sites at different tehsils show that the highest concentrations of sodium as present at sub-site D at Bhalwal tehsil in diseased fruits while the highest concentration of chlorophyll b was recorded at sub-site A at Bhalwal tehsil in healthy fruits (Fig. 6A). The highest concentration of sodium as present at sub-site C at Sargodha tehsil in healthy fruits while the highest concentration of CFC was recorded at sub-site A at Sargodha tehsil (Fig. 6B). The highest concentration of sodium as present at sub-site D at Kotmomin tehsil in diseased fruits while the highest concentration of CFC was recorded at sub-site A at Kotmomin tehsil (Fig. 6C). The highest concentration of sodium as present at sub-site A at Silanwali tehsil in diseased fruits while the highest concentration of CFC was recorded at sub-site C at Silanwali tehsil (Fig. 6C).

Discussion

Citrus canker poses a significant problem in citrus farming, causing detrimental effects on leaves, fruits, and twigs, leading to overall plant health decline (Stall et al., 1980). Our research aligns with Stall *et al.*'s findings, showing that citrus canker impacts multiple parameters. It is evident from our study that citrus canker reduces tree production and vigor, resulting in lower fruit yields. Fruit from canker-affected trees may experience up to a 30% decrease in fresh market pack out rates, which supports previous studies by McClain (1989) and Amiri-Besheli (2011).

Photosynthetic pigments assessment

Chlorophyll a and b levels in *Citrus reticulata* leaves from different tehsils in Sargodha district were higher in healthy leaves compared to CC-infected leaves, attributed to citrus canker infection. Variations in chlorophyll concentrations among healthy leaves across tehsils may be influenced by farmed regions, growth conditions, soil type, seasonal fluctuations, cultivars, and storage conditions (Imeh & Khokhar, 2002). CC-infected leaves exhibited significantly reduced chlorophyll concentration compared to healthy leaves. Citrus canker-affected trees also showed signs of yellowing and chlorosis (Ito et al., 2002), consistent with our findings. Previous studies also reported a significant decrease in chlorophyll concentration due to citrus canker infection and viral illnesses like Chilli veinal mottle virus (Ali et al., 2020). Reduced chlorophyll content leading to chlorosis is a common sign in various plant diseases.

This study observed a clear difference in chlorophyll concentration between healthy leaves and those infected with citrus canker, possibly influenced by leaf miner attack and CC infections. The relationship between citrus canker occurrence and rainfall was noted, contradicting Derso and Sijam (2007), but supporting with rain splash and wind as important disease diffusion modes (Gottwald et al., 1997). In a study on citrus it was also found a link between short-duration, high-intensity rainstorms and disease increase (Pruvost et al., 1999). Grecco et al., (2014) reported lower chlorophyll a concentration in citrus *reticulata* leaves (0.59 mg/g), differing from our observations for non-diseased leaves, which may be due to genetic variability, leaf age, photoperiod, and soil texture. In our findings, chlorophyll a concentration was lower in diseased leaves compared to healthy ones, possibly due to leaf miner attack and CC infections.

Chlorophyll a content was measured in NML and ML. In Sargodha, TTS, and Vehari, NML showed 1.56, 1.75, and 1.33 mg/g FW, while ML exhibited 1.04, 1.07, and 0.8 mg/g FW, respectively (Nawaz et al., 2021). In Tehsil Silanwali, Kotmomin, Bhalwal, and Sargodha, NML recorded 3.05, 4.11, 4.18, and 4.29 mg/g, and ML showed 2.30, 3.07, 3.56, and 3.09 mg/g, respectively. Our findings aligned with Nawaz *et al.*, indicating lower chlorophyll a concentration in diseased leaves due to leaf miner attack and CC infections. However, the values in our study were higher than Nawaz *et al.*, possibly due to genetic diversity, leaf age, foliar micronutrient administration (Zn, Cu, and B), photoperiod, or soil texture.

The results from Grecco et al. (2014) regarding chlorophyll b concentration (0.65mg/g to 0.75mg/g) differed significantly from our findings in healthy leaves. The variation in chlorophyll b concentration could be attributed to cultivated regions, climatic conditions, soil type, seasonal changes, genetic factors, and the stage of sample collection. Our results were slightly higher than those of Ilyas et al. (2015), possibly due to an increased ratio between chlorophyll a and b with higher light intensity (Matsuda et al., 2007). Shading did not affect the anatomical characteristics of Ponkan tangerine plants (Budiarto et al., 2019), but chlorophyll levels differed in sun and shade leaves. In our study, chlorophyll b concentration was lower in diseased leaves compared to healthy leaves, consistent with Nawaz et al. (2021), indicating variations due to leaf miner attack and CC infections. However, our values were higher than Nawaz *et al.*, possibly due to genetic diversity, leaf age, foliar micronutrient administration (Zn, Cu, and B), photoperiod, or soil texture.

Assessment of antioxidant activity and crude fiber content

In the context of seasonal variations (spring, summer, and autumn flushes), the evaluation of antioxidant activity through DPPH (1, 1-diphenyl-2-picrylhydrazyl) inhibition was conducted. This assessment was performed on two distinct groups: NML (with inhibition percentages of 27.66%, 32.27%, and 45.23% for the respective flushes) and ML (with inhibition percentages of 18.12%, 18.07%, and 32.40% for the corresponding flushes), as detailed in the work by Nawaz *et al.* in 2021. Akin to Nawaz *et al.*'s findings, our investigation on diseased and non-diseased fruits exhibited a consistent pattern, wherein maximal antioxidant activity was observed in healthy fruits, as opposed to their diseased counterparts. This disparity can be attributed to potential infections and disease manifestations. The primary factor influencing the reduced antioxidant activity in diseased fruits appears to be associated with the onset of citrus canker (CC) infection. This phenomenon can be attributed to the degradation of chlorophyll in CC-affected plants, leading to a decline in photosynthetic activity. This deterioration of antioxidant activity could potentially be attributed to the oxidative degradation of vital bioactive components such as vitamin C, carotenoids, and phenolic compounds. This oxidative process might be facilitated by specific enzymatic activation, notably peroxidase and polyphenol oxidase, as discussed by De Ancos *et al.* in 2020.

Notably, the study by Oikeh *et al.* (2013) established that *Citrus sinensis* L.'s flavedo region possessed a notable crude fiber content, measuring at 13.43 ± 0.03 percent. In light of these findings, the observed disparities in crude fiber content between healthy and diseased fruits are comparatively minor. It is plausible that these deviations can be attributed to differences in nutritional composition. Furthermore, our research reveals slight variations in crude fiber content between diseased and non-diseased fruits, with the former exhibiting relatively lower values. These observed distinctions between leaves of diseased and non-diseased plants may be attributed to factors such as leaf miner infestations and CC infections.

Determination of minerals

Citrus reticulata L. elemental profile from four tehsils in District Sargodha revealed the importance of potassium (K) for photosynthesis, protein synthesis, carbohydrate production, fruiting, and overall plant health. A deficiency in K can slow down photosynthesis and lead to disease problems, while an imbalance with nitrogen (N) can cause protein build-up and fruit-related issues. Potassium also plays a vital role in various bodily processes, acting as an electrolyte for cardiac function, digestion, and muscular contraction (Aggett, 2012; Hermansen, 2000).

The study's observations on potassium concentration in *Citrus reticulata* fruits differ from the values reported by Simpkins *et al.* (2000), where our findings were lower for both healthy and diseased fruits. Citrus nutritional content can vary due to factors such as farmed regions, soil type, cultivars, and storage conditions (Ukana *et al.*, 2012). Similar potassium values were observed in our study as reported by Ayub *et al.* (2010). Overall, potassium concentration was higher in healthy fruits compared to diseased fruits, possibly influenced by infection. Potassium plays a crucial role in various cell processes, affecting stomata function, photosynthesis, and plant turgor (Imeh & Khokhar, 2002). Variations in potassium concentration between tehsils could be attributed to soil composition and fertilizer application.

Magnesium (Mg) is essential for chlorophyll and photosynthesis in plants. Detecting magnesium deficiency during early growth stages is challenging. Discrepancies exist between our magnesium concentration test results and previous studies (Dhiman et al., 2013). Simpkins et al. (2000) found 122 mg/kg magnesium, similar to our findings in non-diseased fruit. Overall, healthy fruits exhibited higher magnesium levels than diseased ones, potentially influenced by infections. Variations in magnesium concentration across tehsils may be attributed to differences in soil mineral content (Reference: Dhiman et al., 2013; Simpkins et al., 2000).

The study found the highest phosphorus concentration in citrus pulp, with a small amount also present in fruit skin. Our results for phosphorus levels were higher than those reported by Alina et al. (2009), who found 12mg/100g in oranges, 8mg/100g in grapefruit, and 16.2mg/100g in lemon. However, our findings differed from Simpkins et al. (2000) who reported 181 mg/kg. The variation in phosphorus content in *C.sinensis* L. fruit could be attributed to soil composition and fertilizer application across different tehsils. Calcium levels were also higher in our study compared to Simpkins et al. (2000), with higher concentrations in non-diseased fruits, possibly influenced by soil composition and fertilizer usage. Calcium was found to be highest in healthy fruits compared to diseased ones, and differences in Ca content among tehsils may be due to soil composition and genetic variability. Sodium concentrations showed discrepancies with Simpkins et al. (2000), with lower values in healthy fruits and potential links to soil composition and fertilizer use. Overall, the variations observed may be influenced by infection. Reference: Alina et al., (2009); Simpkins et al., (2000).

Iron is crucial for human health as it forms a part of hemoglobin, facilitating oxygen transport to body tissues. Citrus juices have relatively low iron content, but supplementation in drinks can aid iron deficiency. Our study found iron concentrations slightly higher than Simpkins et al. (2000), who reported 0.98 mg/kg. Variations in iron content may be attributed to soil composition, climate changes, and fertilizer usage. Overall, healthy fruits exhibited lower sodium concentrations than diseased ones, possibly influenced by infection. Differences in sodium content across tehsils may be related to soil mineral concentrations and fertilizer application. (Reference: Simpkins et al., 2000).

Conclusion

Citrus canker continues to be a significant challenge for citrus industries worldwide. Its rapid spread, destructive impact, and economic repercussions make it a constant concern for citrus growers, researchers, and policymakers alike. Implementing stringent biosecurity measures, conducting thorough research, and fostering international collaboration are essential to mitigating the threat of citrus canker and safeguarding the health and prosperity of the global citrus industry.

Recommendations

To manage citrus canker effectively, enforce strict quarantine measures, regularly inspect and monitor orchards, remove infected trees, and promote disease-resistant citrus varieties. Implement proper

sanitation practices and educate farmers and workers about canker management. Utilize copper-bactericides in high-risk areas, limit movement of infected material, and collaborate with agricultural authorities for an efficient management plan, while supporting sustainable research solutions for long-term control.

Declarations

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Funding statement

All authors declare that there was no funding.

Competing Interest

All authors declare that there are no competing interests.

Voucher specimens

Citrus specimens were identified by Dr. Abdul Ghani associate professor of Botany, University of Sargodha, Sargodha, Pakistan and Dr. Mansoor Hameed, Professor at University of Agriculture Faisalabad. Herbarium sheets (number S2023/3421-3467) are deposited at Department of Botany University of Sargodha, Sargodha Pakistan.

Consent for Publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Authors' contributions

Rab Nawaz and Toqeer Abbas conducted the experiments and analyzed the data Muhammad Nadeem and Anis Ali Shah worked on graph and tables and all authors revised the manuscript

Ethics

I Rab Nawaz, Department of Botany, University of Sargodha, Pakistan, declare that most of the sampling sites were my own citrus gardens and some samples were collected from my friends and relatives lands, and the affidavit from my relatives is attached in "Related Files". I also submit that in case of any issue I Rab Nawz will be responsible.

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Figures

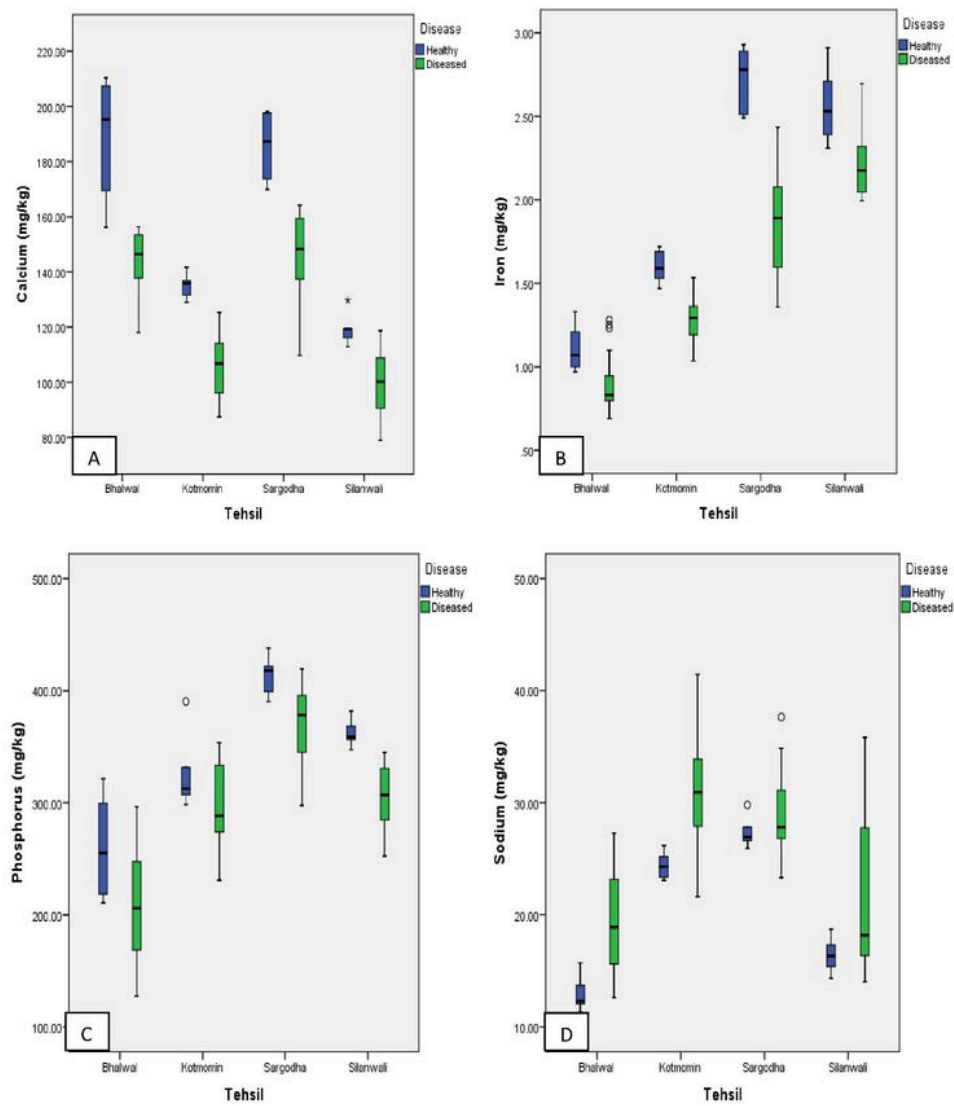


Figure 1: Box plot for Ca, Fe, P and Na conc. in healthy and diseased fruits of studied tehsils

Figure 1

See image above for figure legend.

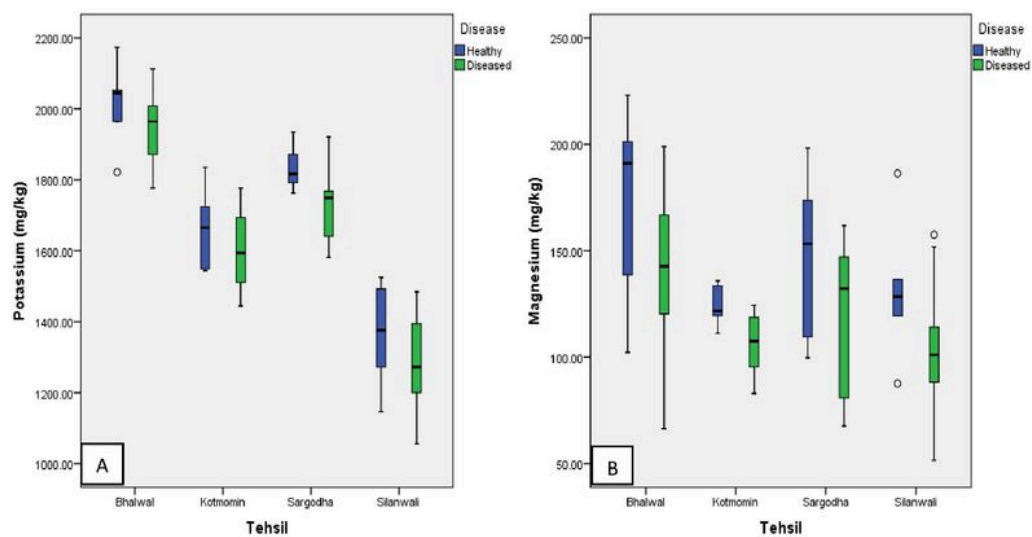


Figure 2: Box Plot for K (Fig. 2A) and Mg (Fig. 2B) conc. in healthy and diseased fruits of studied tehsils.

Figure 2

See image above for figure legend.



HS-healthy samples, DS- disease samples, TAA-Total Amino Acids, CFC-Crude Fiber Contents, K-Potassium, Na-Sodium, P- Phosphorus, Mg-Magnesium, Ca-Calcium) (T₁- Tehsil Bhalwal, T₂- Tehsil Sargodha)

Figure 3: Comparisons of evaluated attributes at various sites of T₁ Bhalwal (Fig. 3A) and T₂ Sargodha (Fig. 3B)

Figure 3

See image above for figure legend.

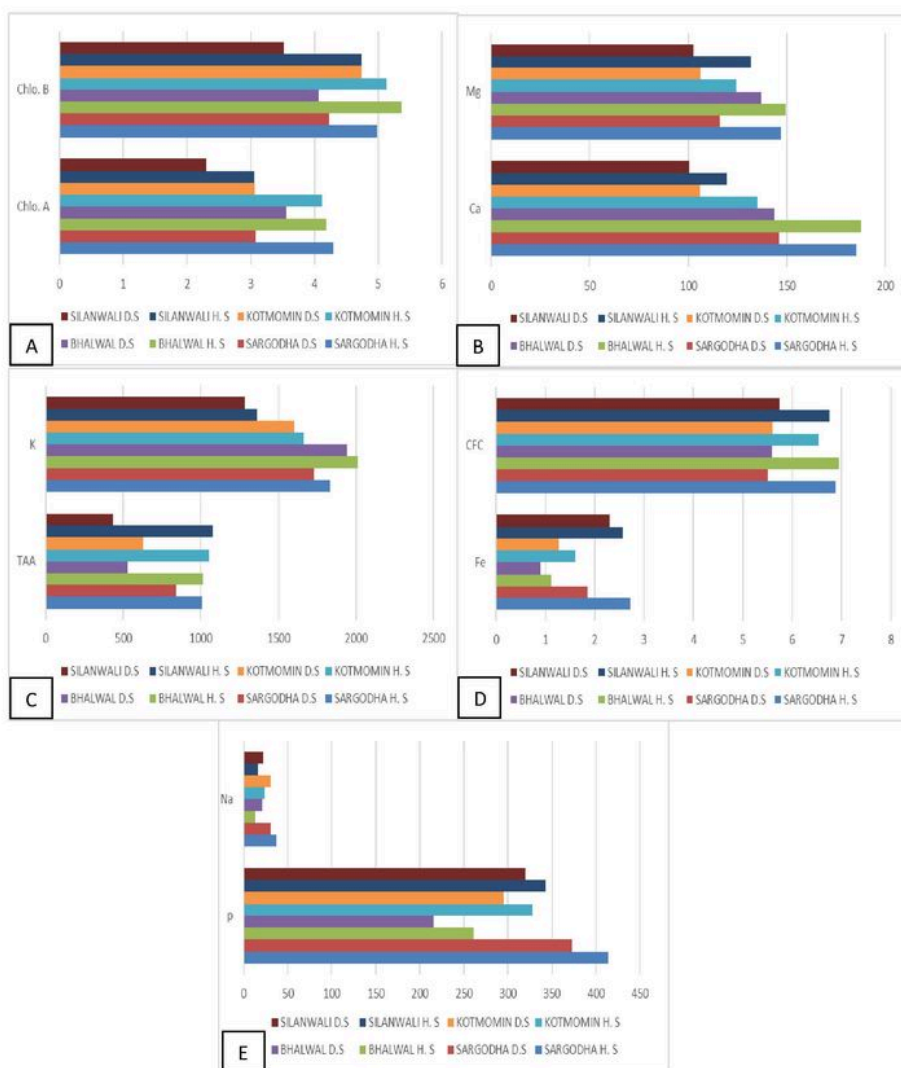


HS-healthy samples, DS- disease samples, TAA-Total Amino Acids, CFC-Crude Fiber Contents, K-Potassium, Na-Sodium, P- Phosphorus, Mg-Magnesium, Ca-Calcium)

Figure 4: Comparisons of evaluated attributes at various sites of T₃ Kotmomin (Fig. 4A) and T₄ Silanwali (Fig. 4B)

Figure 4

See image above for figure legend.

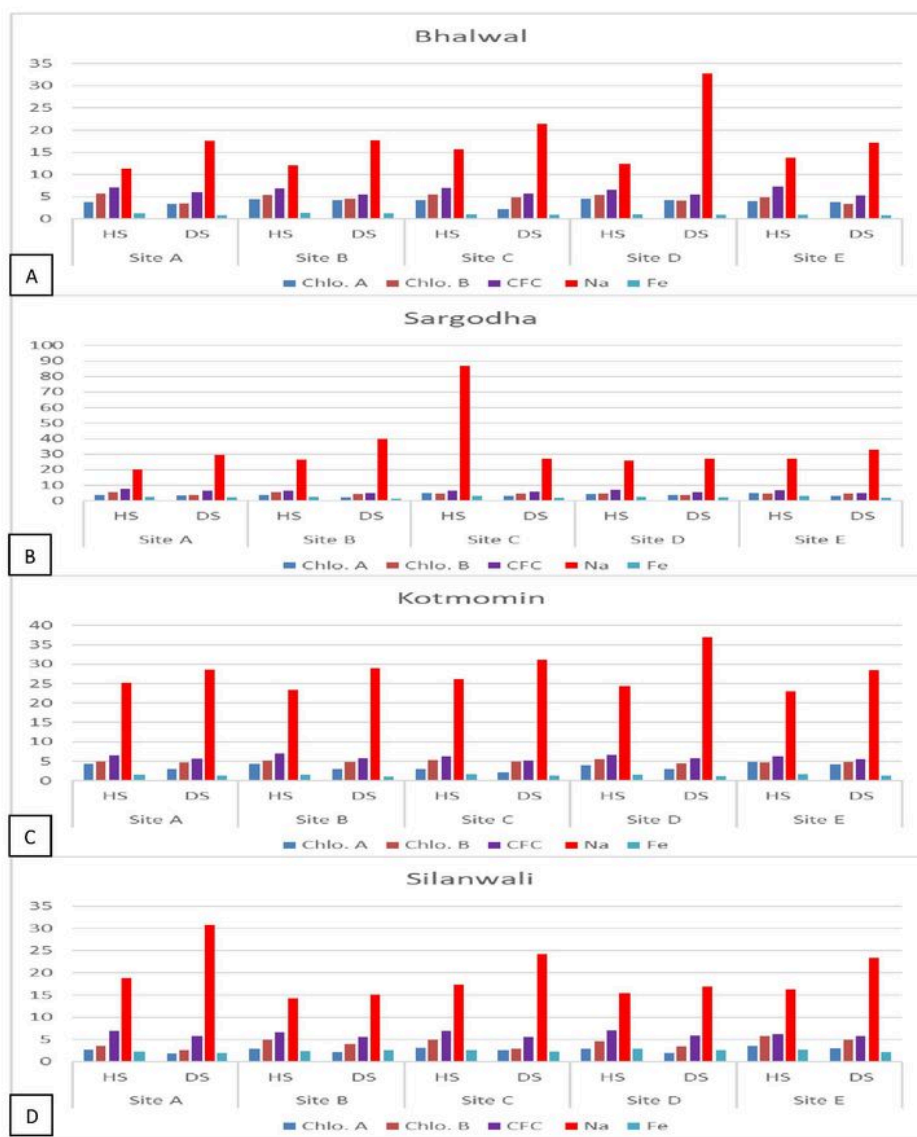


HS-healthy samples, DS- disease samples, TAA-Total Amino Acids, CFC-Crude Fiber Contents, K-Potassium, Na-Sodium, P- Phosphorus, Mg-Magnesium, Ca-Calcium)

Figure 5: Comparisons of tehsils with respect to photosynthetic pigments, minerals, CFC and total antioxidant activity.

Figure 5

See image above for figure legend.



HS-healthy samples, DS- disease samples, TAA-Total Amino Acids, CFC-Crude Fiber Contents, K-Potassium, Na-Sodium, P- Phosphorus, Mg-Magnesium, Ca-Calcium)

Figure 6: Comparison of sites in each studied tehsil with respect to photosynthetic pigments, minerals and crude fiber content

Figure 6

See image above for figure legend.