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QUANTIFICATION OF ALTERATION IN MINERAL CONTENTS OF DIFFERENT CITRUS GENOTYPES WITH VARYING DISEASE RESPONSES INDUCED BY *ELSINOE FAWCETTII*

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ABSTRACT

The objective of the study was to determine the resistance of citrus cultivars to citrus scab (*Elsinoe fawcettii*) and to examine the effects of infection on mineral composition of the leaves. Regardless of the significant economic impact of scab disease, limited comparisons of citrus cultivars to scab disease taken across multiple cultivars and a lack of understanding of nutrient composition in relation to disease severity exists. Citrus (*Citrus* spp.) comprises various species of fruit crops that have world significance and are extensively grown in tropical, subtropical and Mediterranean climates. Its productivity is adversely affected by a fungal disease citrus scab caused by *Elsinoe fawcettii*. This research was conducted in a randomized completely blocked design (RCBD) with three replicates to evaluate the resistance levels of twenty citrus varieties against scab disease and to investigate changes in mineral contents between healthy and diseased leaves. Disease severity was measured on a 0-5 scale and analyzed through ANOVA, means were compared through Tukey HSD comparisons to determine differences at $p \leq 0.05$. Field evaluation of the cultivars over three consecutive growing seasons (2020–2022) revealed statistically significant variation in disease response. Among the twenty citrus cultivars, *Citrus japonica* 'Kumquat', *Citrus reticulata* 'Kinnow' and *Citrus paradisi* 'Duncan' exhibited high levels of resistance with disease severities of 0.71%, 1.75% and 3.92%, respectively. Highly susceptible cultivars showed the greater disease severity, such as *Citrus sinensis* 'Jaffa' (27.48%), *Citrus sinensis* 'Ruby Red' (28.59%), *Citrus paradisi* 'Foster' (30.02%), *Citrus paradisi* 'Shamber' (31.95%) and *Citrus sinensis* 'Valencia Late' (34.00%). Mineral analysis, conducted by using spectrophotometric and flame emission methods, revealed marked reductions in key elements in infected leaves compared to healthy ones. Nitrogen content declined from 10.70% in healthy 'Red Blood' leaves to 2.16% in infected 'Feutral's Early'; phosphorus decreased from 2.84% in healthy 'Red Blood' to 0.09% in infected 'Feutral's Early'; and potassium dropped from 12.70% in healthy 'Red Blood' to 2.86% in infected 'Feutral's Early'. Similar reductions were also observed in calcium, magnesium, sodium, zinc, iron and copper. These findings highlight that mineral depletion increases with disease severity, as it disturbs the nutritional uptake in citrus. The results highlight that integrating resistant cultivars with balanced nutrient management can substantially reduce citrus scab incidence and associated yield losses.

Keywords: Citrus, Cultivars, Citrus Scab, Disease Resistance, *Elsinoe fawcettii*, Minerals Alteration

INTRODUCTION

Citrus is grown in tropical, subtropical and Mediterranean climates where climate has a significant controlling influence on fruit development and quality; it thrives at a temperature around 25–30 °C with avoidance of frost and good peel coloration and taste are preferred in Mediterranean regions like Spain, Italy and North Africa (Caldana *et al*., 2020; Cebadera-Miranda *et al*., 2020; Penjor *et al*., 2013)Similar agro-climatic zones in other regions of Asia, such as China, are also expected to see increased temperatures and a change in precipitation under future climate predictions, posing a risk to the yield and quality of citrus fruits and emphasizing the importance of adaptive management under urgency (Wang *et al*., 2022).

According to FAOSTAT (2023), China, Nigeria, India, Iran, Angola, Mexico, Guinea, Tunisia, Saudi Arabia and Sierra Leone are among the top ten citrus-producing countries worldwide. Global citrus production is reported to be 13,407,216.82 tons from 1,457,547 ha of harvested area. In comparison, Pakistan produces 49,482 tons from approximately 7,000 ha (FAOSTAT, 2023). Nutritionally, citrus depends on balanced macronutrients (N, P and K) and micronutrients (Ca, Mg, Fe, Zn, Cu and Mn) that are the basis of canopy vigor, photosynthesis, peel/juice quality and pest-disease resistance (Zekri and Obreza, 2003). Pathogen pressure tends to interfere with uptake, partitioning and ionic homeostasis and healthy plants tend to exhibit lower disease incidence than those lacking in relatively immobile nutrients. However, nutrient–disease interactions are also influenced by factors such as cultivar/rootstock compatibility, orchard management practices (fertilization and irrigation), soil type and seasonal growth flushes, which affect nutrient uptake and disease expression (Shabbir *et al*., 2022; Tripathi *et al*., 2022).

Among the fungal citrus diseases, *Elsinoe fawcettii* (anamorph *Sphaceloma fawcettii*) ascomycete scab is prevalent in wet production areas, creating corky, wart-like defects in young leaves, shoots and fruit that decrease marketability; disease severity increases during rainy spring flushes with rich susceptible tissues (Pham, 2025). *E. fawcettii* secretes elsinochromes, light-activated perylene quinones which produce reactive oxygen species, induce peroxidation of membranes and lesion formation and serve as major virulence determinants; compatible interactions on leaves of satsuma mandarin replicate normal scab symptomology within days of inoculation. Reactive oxygen species produced by elsinochromes can destroy membrane transporter and root ion channels, impairing the absorption and translocation of important nutrient elements and may explain the observed mineral deficiencies in infected citrus tissues (Chung, 2011; Liao and Chung, 2008a; Shin *et al*., 2021).

Although symptom biology and toxigenesis are well characterized, less is known about how scab perturbs mineral nutrition in citrus tissues. Observations from citrus and general crop systems suggest that disease typically accompanies quantifiable declines in major nutrients (Ca, Mg, K), weakening cell-wall integrity, membrane stability, carbohydrate partitioning and enzyme activity; in citrus, nutrient regimes that highlight Ca, K and Zn can support structural defense and fruit quality under pathogen stress (Bastakoti, 2023; Bhar *et al*., 2023; Huai *et al*., 2022; Negi *et al*., 2023; Zekri and Obreza, 2014). Recent research on *Alternaria citri* (brown spot) further associates' infection with reductions in N, P, K, Ca, Mg and a number of micronutrients in susceptible genotypes, whereas metal homeostasis (Fe/Cu) has interactions with redox processes during host–pathogen interactions. Together, these findings provoke a targeted consideration of whether *E. fawcettii* infection induces similar changes in citrus leaf mineral profiles, with nutritional implications for disease prevention (Iqbal *et al*., 2024; Rai *et al*., 2021).

The objective of this research was to establish how *E. fawcettii* infection changes the macro and micronutrient composition of citrus tissues, by comparing inoculated plants with healthy plants, in order to identify nutrient disturbances, which can then in turn help guide nutritional strategies to mitigate the impact of the disease and improve fruit quality.

MATERIALS AND METHODS

Identification of the pathogen associated with citrus scab: The key citrus-growing areas of the Punjab province (Bhalwal, Kot Momin, Sargodha, Faisalabad, Sahiwal and Toba Tek Singh) were surveyed for diseased leaf sampling. Samples were washed with tap water to remove surface dust and stored at 4 °C to minimize enzymatic and pathogenic activity until processing. Potato dextrose agar (PDA) was prepared using 200 g potato infusion, 20 g dextrose and 15–20 g agar for 1 L, while potato dextrose broth (PDB), lacking agar, was used for inoculum preparation. Media and glassware were sterilized at 121 °C for 20 min (103 kPa) and streptomycin (250 mgL^{-1}) was added to lukewarm PDA media under aseptic conditions. For fungal isolation, 2 mm leaf segments from lesion margins were excised, surface-sterilized with 1% sodium hypochlorite for 60 s, rinsed thrice with sterile distilled water, air dried and incubated on PDA at 25 ± 2 °C for 7 days. Emerging colonies were purified by transferring 5 mm mycelial plugs from actively growing edges to fresh PDA plates until pure cultures were obtained. Morphological characterization was based on macroscopic (colony morphology, pigmentation and growth habit) and microscopic features (septation, conidial shape and sclerotia formation), following the keys of Timmer *et al*. (1996); Liao and Chung (2008b). For short-term storage, pure culture was preserved on PDA slants at 4 °C and in glycerol stocks, kept at –80 °C for long-term maintenance.

Screening of Citrus Germplasm: Twenty citrus varieties (Table 1) were collected from the Ayub Agricultural Research Institute (AARI), Faisalabad and screened for resistance/susceptibility to citrus scab under greenhouse conditions. Plants were grown under advised agronomic practices in a Randomized Completely Blocked Design (RCBD) with three replicates. Each replicate consisted of five uniform three-year-old potted plants per cultivar. Artificial inoculation was done by spraying a conidial suspension (1×10^6 conidia/ml) with the help of a hand atomizer for three consecutive weeks and the control plant were sprayed with the sterile distilled water. Plants were kept under plastic covers to maintain 75–90% relative humidity at 25 °C until symptom expression.

Disease Assessment: Disease severity was assessed using a modified disease rating scale as mentioned in Table 2 (Imran *et al*., 2015). Disease incidence was recorded per plant based on 5 randomly selected young leaves per plant. Weekly data were recorded to calculate disease severity and percent disease index (PDI) using the following formulae (McKinney, 1923; Cooke, 1998):

$$\text{Disease severity (\%)} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves}} \times 100$$

$$\text{PDI (\%)} = \frac{[\text{Sum (Class frequency} \times \text{Score of rating class)}]}{\text{Total number of plants} \times \text{Maximal disease index}} \times 100$$

Varietal resistance/susceptibility was classified as immune, resistant, tolerant, susceptible, or highly susceptible according to the rating scale.

Table 1. Citrus cultivars screened against citrus scab (*E. fawcettii*)

Sr. #	Varieties	Sr. #	Varieties
1	*Citrus aurantium* ‘Bitter orange’	11	*Citrus paradisi* ‘Grapefruit’
2	*Citrus aurantifolia* ‘Key lime’	12	*Citrus sinensis* ‘Jaffa’
3	*Citrus jambhiri* ‘Rough Lemon’	13	*Citrus reticulata* ‘Malta’
4	*Citrus japonica* ‘Kumquat’	14	*Citrus reticulata* ‘Kinnow’
5	*Citrus latifolia* ‘Persian lime’	15	*Citrus sinensis* ‘Sweet orange’
6	*Citrus limonia* ‘Mayer lemon’	16	*Citrus reticulata* ‘Feutral’s Early’
7	*Citrus medica* ‘Citron’	17	*Citrus sinensis* ‘Succari’
8	*Citrus paradisi* ‘Duncan’	18	*Citrus sinensis* ‘Ruby red’
9	*Citrus paradisi* ‘Foster’	19	*Citrus sinensis* ‘Red blood’
10	*Citrus paradisi* ‘Shamber’	20	*Citrus sinensis* ‘Valentia late’

Table 2. Disease rating scale for the assessment of citrus scab disease

Grade	Disease Intensity	Result
0	00.00	Immune (I)
1	0.01-5.00	Highly Resistant (HR)
3	5.01-10.00	Resistant (R)
5	10.01-15.00	Tolerant (T)
7	15.01-25.00	Susceptible (S)
9	>25.00	Highly Susceptible (HS)

(Imran *et al.*, 2015)

Mineral Analysis: Six cultivars (three tolerant: Red Blood, Malta and Succari; and three susceptible: Sweet Orange, Grapefruit and Feutral’s Early) were selected for mineral analysis based on mean PDI scores from Table 3. Plant samples (containing both inoculated and un-inoculated leaves) were collected from the greenhouse and dehydrated in an oven (Heaes D650 Hanau) at 70 degrees Celsius for 48 hours. Samples were ground with a pestle and mortar and 0.5 g of each dried sample was boiled in 10ml of 1.4N HNO_3 on a hotplate (RT2-230V) for 30 min at 100 °C. after cooling, the digest was filtered through Whatman No. 42 filter paper and made up to a final volume of 50 ml with deionized water in a volumetric flask (ASTM-E288). This modification from Bhargava and Raghupathi (1993) was validated by ensuring recovery between 95–102% for certified reference material (CRM: NIST 1573a Tomato Leaves) (Bhargava and Raghupathi, 1993). Diseased samples from both resistive and sensitive response categories were obtained from the area and kept in fridge (Dawlance, 91996 WB) for assessment of various bio-chemical compounds using standard analytical methods under factorial experiment. A reagent blank and a digestion blank were included in each run to account for background contamination. All glassware was acid-washed (10% HNO_3) and rinsed thoroughly with deionized water prior to use.

The phosphorus concentration determination involved wet digestion of a 0.1ml sample, followed by the addition of ammonium heptamolybdate $[(NH4)_6Mo_7O_{24}.H_2O]$ and purified water in a measuring flask (ASTM-E288). Amino naphthol-sulphonic acid, having molecular formula $C_{10}H_9NO_4S$, was added after mixing. Absorbance measurements were taken at 720 nm using a Hitachi U-2001 spectrophotometer, with distilled water as blank. The results were then compared to standard curves (Fiske and Subbarow, 1925; Boltz and Mellon, 1948) using a Hitachi polarized Zeeman atomic absorption spectrometer to determine phosphorus concentration. Although the Fiske and Subbarow (1925) method is classical, it remains widely used for plant phosphorus determination and has been validated against modern spectrophotometric methods (Oliveira *et al.*, 2010).

The measurement of potassium and sodium amounts was performed via flame emission spectrometer (Model: PFP-7, Janway). Standard solutions of sodium chloride and potassium chloride prepared at concentrations of 10, 20, 30 and 40 ppm to create the calibration curves for K and Na, respectively. Fresh standards were prepared immediately before analysis to ensure accuracy. This method allows for quantitative measurement of these essential elements in the sample based on the emission of spectra produced when atomized in the flame (Hald, 1947).

The estimation of copper (Cu), zinc (Zn), magnesium (Mg) and iron (Fe), calcium (Ca) was performed using Hitachi U-2001 spectrophotometer (model 121-003). Standard solutions were prepared using FeSO4, MgSO4, ZnO, CaCl2 and CuSO4 for respective elements. The calibration curve was created using specific concentration ranges for each element: 10.0ppm, 20.0ppm, 40.0ppm, 80.0ppm and 100.0 ppm for Ca, 5.0ppm, 10.0ppm, 15.0ppm and 20.0ppm for Mg, 1.5ppm, 2.0ppm, 2.5ppm, 3.0ppm and 3.5ppm for Cu, 1.0ppm, 2.0ppm, 4.0ppm, 6.0ppm and 10.0ppm for Fe and 0.20ppm, 0.30ppm, 0.50ppm, 1.00ppm and 2.00ppm for Zn. Fresh standards were prepared immediately before analysis to ensure accuracy. An atomic absorption spectrophotometer was then employed to analyze these minerals in citrus cultivars, ensuring accurate quantification of the elemental composition in the samples. Calibration curves were constructed for each element within the respective concentration ranges, yielding $R^2 > 0.995$. Each sample and standard were analyzed in triplicate and analytical blanks were run with each batch. Wavelengths used for AAS were: Fe (248.3 nm), Zn (213.9 nm), Cu (324.8 nm), Mg (285.2 nm) and Ca (422.7 nm) (Oliveira *et al*., 2010; Steponënienë *et al*., 2003).

The determination of total nitrogen in samples was conducted using a Micro Kjeldahl method with a quick fit apparatus (46MC). A known amount of oven-dried sample (using a Heraeus D6450 Hanus Oven) was digested in a Kjeldahl flask with concentrated sulfuric acid and a digestion mixture of K_2SO_4 and $CuSO_4$. The digestion process used a KB8S Kjeldatherm digestion hood. The digested sample was then distilled into a 250mL volumetric flask (ASTM-E288). A 10mL aliquot was further distilled in a VAP20 Gerhardt Micro Kjeldahl distillation apparatus using 40% sodium hydroxide solution, releasing ammonia gas which was trapped in a 2% of boric acid solution with methyl red dye. The mixture was titrated in a standardized (0.1N) sulfuric acid until pink endpoint. Nitrogen percentage was calculated through the following formula (Abrams *et al*., 2014).

$$\mathrm{N}(\%) = \frac{(\mathrm{V}_1 - \mathrm{V}_0) \times \mathrm{N} \times 14.007}{\mathrm{W}} \times 100$$

Where,

V_1 = volume (ml) of H_2SO_4 used for sample titration,
V_0 = volume (ml) of H_2SO_4 used for blank titration,
N = normality of acid (0.1 N),
14.007 = atomic weight of nitrogen,
W = sample weight (g).

Both absolute (X–Y) and percentage ((X–Y)/X×100) nutrient declines were calculated also.

Statistical Analysis: Data were analyzed with ANOVA under RCBD for greenhouse screening trial and CRD for laboratory analysis (Steel *et al*., 1996). Means were separated using Tukey's HSD test ($p \leq 0.05$). To assess relationships between nutrient alterations and disease severity, Pearson's correlation analysis was performed between the percent disease index (PDI) and percentage loss of individual macro and micronutrients across citrus cultivars. The correlations were visualized as heatmaps. Principal component analysis (PCA) was also conducted to determine the contribution of each nutrient variable and to classify healthy and inoculated plants based on their nutrient profiles. Regression analysis between PDI and nutrient losses was applied to quantify the strength of association for each element. Data were standardized before analysis and all statistical computations and graphical visualizations were carried out using R software (v4.3.1).

RESULTS

Response of citrus germplasm against citrus scab: Among the 20 citrus varieties that were screened, three were highly resistant (HR). *C. japonica* 'Kumquat' showed slight symptoms with a severity of 0.71% throughout the trial. It indicated the presence of strong inherent mechanisms of resistance, likely due to thicker cuticle layers and biochemical defense mechanisms. *C. reticulata* 'Kinnow' also exhibited only slight and mild lesions with a severity of 1.75%, proving its strong resistance, for which it is a good commercial variety under scab attack. *C. paradisi* 'Duncan' exhibited only surface spots with no extension beyond that, showing its strong resistance characteristics with a severity of 3.92%. The resistant (R) mechanism was observed in *C. aurantium* 'Bitter orange', *C. jambhiri* 'Rough lemon' and *C. aurantifolia* 'Key lime' with severities of 6.94%, 8.11% and 9.74%. These cultivars showed small lesions but were unable to establish severe infections. Their ability to localize the infection suggests a resistance response and hence they can be utilized as rootstocks or parent material in breeding programs.

Four cultivars were classified as tolerant (T). *C. latifolia* 'Persian lime' with 12.23% showed appreciable but restrained infection, indicating that although the pathogen invaded the tissues, the disease did not advance aggressively. *C. sinensis* 'Red Blood' and *C. reticulata* 'Malta' exhibited intermediate lesion formation rates of 13.526% and 14.037%, indicating partial resistance that enables production under moderate disease attack. *C. sinensis* 'Succari' also falls into this category, displaying visible scab symptoms with a severity of 14.700% but maintaining growth and foliage health, indicating its tolerance potential. The susceptible (S) response was demonstrated by *C. sinensis* 'Sweet orange', *C. paradisi* 'Grapefruit' and *C. reticulata* 'Feutral's Early', *C. medica* 'citron' and *C. limonia* 'Mayer lemon'. These cultivars showed extensive lesions with increased disease severity of 18.60%, 20.23%, 21.91%, 23.73% and 24.55%. Scab lesions caused hardening and distortion of tissues and expressed poor resistance mechanisms. These cultivars can suffer economically if planted in scab-infested regions without protection.

The most affected were the highly susceptible (HS) cultivars such as *C. sinensis* 'Jaffa' with a severity of 27.48%, 'Ruby Red' (28.59%) and 'Valentia Late' (34.00%) and *C. paradisi* 'Foster' (30.02%) and 'Shamber' (31.95%). These developed large necrotic lesions, cracking and leaf distortion, with resulting defoliation. This extensive destruction demonstrated their failure to limit pathogen invasion and spread, making them very vulnerable in endemic areas.

Table 3. Resistance status of citrus cultivars to scab (2020–2022)

Varieties	Mean PDI (%)	Disease Rating	Disease Status
Citrus japonica 'Kumquat'	0.71 ± 0.020 T	1	HR
Citrus reticulata 'Kinnow'	1.75 ± 0.018 S	1	HR
Citrus paradisi 'Duncan'	3.92 ± 0.026 R	1	HR
Citrus aurantium 'Bitter orange'	6.94 ± 0.038 Q	3	R
Citrus jambhiri 'Rough Lemon'	8.11 ± 0.029 P	3	R
Citrus aurantifolia 'Key lime'	9.74 ± 0.026 O	3	R
Citrus latifolia 'Persian lime'	12.23 ± 0.026 N	5	T
Citrus sinensis 'Red blood'	13.53 ± 0.015 M	5	T
Citrus reticulata 'Malta'	14.04 ± 0.015 L	5	T
Citrus sinensis 'Succari'	14.70 ± 0.029 K	5	T
Citrus sinensis 'Sweet orange'	18.60 ± 0.018 J	7	S
Citrus paradisi 'Grapefruit'	20.23 ± 0.020 I	7	S
Citrus reticulata 'Feutral's early'	21.91 ± 0.020 H	7	S
Citrus medica 'Citron'	23.73 ± 0.015 G	7	S
Citrus limonia 'Mayer lemon'	24.55 ± 0.020 F	7	S
Citrus sinensis 'Jaffa'	27.48 ± 0.020 E	9	HS
Citrus sinensis 'Ruby red'	28.59 ± 0.015 D	9	HS
Citrus paradisi 'Foster'	30.02 ± 0.020 C	9	HS
Citrus paradisi 'Shamber'	31.95 ± 0.020 B	9	HS
Citrus sinensis 'Valentia late'	34.00 ± 0.020 A	9	HS
Tukey HSD Value		0.0342	

Means followed by the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$)
Disease ratings correspond to the 1–9 scale defined in Methods; mean values represent three replicates (± SE).

Macro nutrient imbalances induced due to *E. fawcettii*: Each mean represents three biological replicates per cultivar, with three technical replicates per digestion. Nitrogen levels were highest in tolerant cultivars. Red Blood exhibited the maximum nitrogen content in both healthy (10.70%) and inoculated (4.58%) as compared to Malta (10.11% and 4.09%) and Succari (9.37% and 3.79%). In contrast, susceptible varieties such as Sweet Orange showed high nitrogen content of 8.34% in healthy and 2.90% in inoculated as compared to Grapefruit (7.38% and 2.66%), which had significantly lower nitrogen values than Sweet Orange but higher than Feutral's Early. In comparison, Feutral's Early showed the lowest nitrogen percentage of 7.01% in healthy and 2.16% in inoculated. These results indicated that tolerant cultivars retained higher nitrogen content under infection stress and healthy state (Fig. 2A). The concentration of nitrogen lowered significantly for all cultivars. The reductions reported for tolerant cultivars were 6.12% (decline of 57.2%) for Red Blood, 6.02% (decline of 59.6%) for Malta and 5.58% (decline of 59.5%) for Succari, respectively. For susceptible cultivars the higher values reported were a decline of 5.44% (decline of 65.2%) for Sweet Orange, 4.72% (decline of 63.9%) for Grapefruit and 4.85% (decline of 69.2%) for Feutral's Early cultivars. The nitrogen data confirms that the nitrogen retention in tolerant cultivars was higher than in susceptible cultivars, which experienced significant nitrogen depletion.

Phosphorus content also varied considerably among cultivars. Red Blood showed the highest phosphorus level of 2.84% in healthy and 0.80% in inoculated in comparison to Malta (2.58% and 0.71%) and Succari (2.34% and 0.66%). Comparatively, the susceptible cultivars, Sweet Orange, showed the phosphorus percentage of 2.24% in healthy and 0.41% in inoculated, as compared to Grapefruit (1.89% and 0.25%), which had lower phosphorus values, with Feutral's Early recording the lowest level of 1.30% in healthy and 0.09% in inoculated. This reflects a significant reduction in phosphorus availability of susceptible cultivars upon inoculation with the pathogen as compared to healthy plants (Fig. 2B). The tolerance cultivars also had lower reductions in phosphate and relative decreases than the susceptible cultivars (2.04% (71.8%) reduction for Red Blood, 1.87% (72.4%) for Malta and 1.68% (71.8%) for Succari). For susceptible cultivars Sweet Orange showed higher absolute losses of 1.83% (81.7%), Grapefruit 1.64% (86.8%) and Feutral's Early 1.21% (93.1%) loss of phosphate. These observations support a clear relationship with higher retention of phosphate and tolerance.

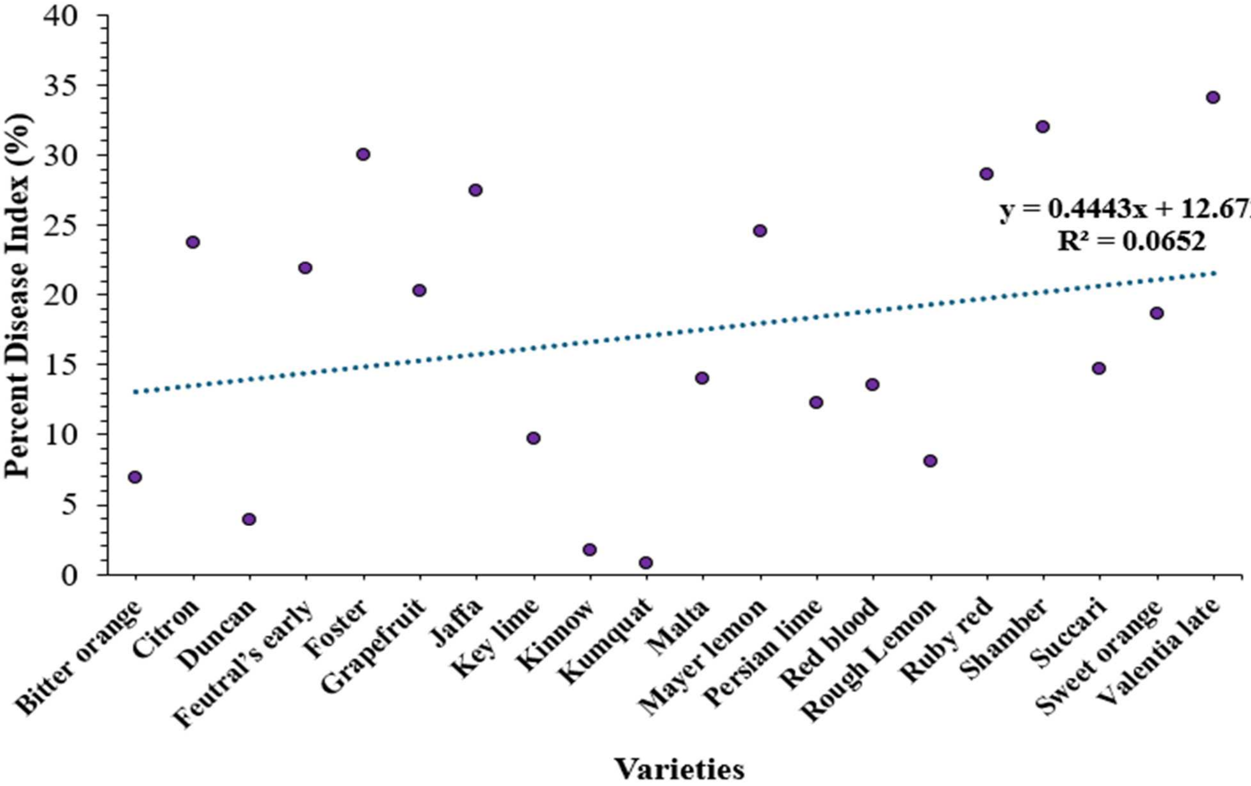


Fig. 1. Graph showing the trendline with the response of citrus against the scab disease

Similarly, tolerant cultivars showed greater potassium content. Red Blood showed the highest values of 12.70 % in healthy and 5.28 % in inoculated, as compared to Malta (12.11 % and 4.79 %) and Succari (11.37 % and 4.49 %). In comparison, Sweet Orange from the susceptible cultivars showed the lower values of 10.34 % in healthy and 3.60 % in inoculated leaves as compared to the tolerant. Feutral's Early, which had the lowest potassium content of 9.01 % in healthy and 2.86 % in inoculated, followed by Grapefruit (9.38 % and 3.36 %). This indicates that the tolerance response is closely linked to potassium retention (Fig. 2C). The absolute and relative declines in potassium content were 7.42 % (58.4 %) in Red Blood, 7.32 % (60.4 %) in Malta and 6.88 % (60.5 %) in Succari, indicating a stronger capacity for potassium retention among tolerant cultivars. In contrast, susceptible cultivars exhibited more pronounced reductions: Sweet Orange (6.74 %, 65.2 %), Grapefruit (6.02 %, 64.2 %) and Feutral's Early (6.15 %, 68.3 %). These results highlight that potassium retention plays a significant role in the tolerance response of citrus varieties against *E. fawcettii* infection.

Micronutrient imbalances induced due to *E. fawcettii*: Sodium concentration was maximum in Red Blood with 2.82 ppm in healthy and 0.90 ppm in inoculated as compared to Malta (2.53 ppm and 0.87 ppm) and Succari (2.34 ppm and 0.72 ppm). Sweet Orange exhibited sodium content of 2.03 ppm in healthy and 0.56 ppm in inoculated leaves, which are higher than Grapefruit (1.79 ppm and 0.43 ppm) and Feutral's Early (1.34 ppm and 0.11 ppm). However, these values were significantly lower than those of tolerant cultivars. Lower sodium in susceptible varieties could be indicative of pathogen-induced damage to ionic uptake (Fig. 2D). Sodium concentrations decreased by 1.92 ppm (68.1%) in Red Blood, 1.66 ppm (65.6%) in Malta and 1.62 ppm (69.2%) in Succari. Sweet Orange decreased by 1.47 ppm (72.4%), Grapefruit by 1.36 ppm (76.0%) and Feutral's

Early decreased by 1.23 ppm (91.8%) for the susceptible cultivars. A greater capacity for sodium to remain in tolerant types suggests that there is less disturbance of ionic equilibrium following infection.

Calcium content was remarkably higher in Red Blood with 2.81 ppm in healthy and 1.92 ppm in inoculated, as compared to Malta (2.70 ppm and 1.80 ppm), which are higher than Succari (2.55 ppm and 1.72 ppm) but lower than Red Blood. The other varieties, such as Sweet Orange, exhibited 2.39 ppm in healthy and 1.48 ppm in inoculated leaves, which are higher than Grapefruit (2.28 ppm and 1.30 ppm) and Feutral's Early (2.13 ppm and 1.03 ppm), which showed very low contents, indicating calcium deficiency in susceptible varieties is lower than in the tolerant ones (Fig. 2E). Calcium decreased by 0.89 ppm (31.7%) in Red Blood, 0.90 ppm (33.3%) in Malta and 0.83 ppm (32.5%) in Succari. The susceptible types, Sweet Orange, Grapefruit and Feutral's Early, exhibited greater absolute losses of calcium at 0.91 ppm (38.1%), 0.98 ppm (42.9%) and 1.10 ppm (51.6%), respectively to establish that the tolerant genotypes had greater calcium homeostasis.

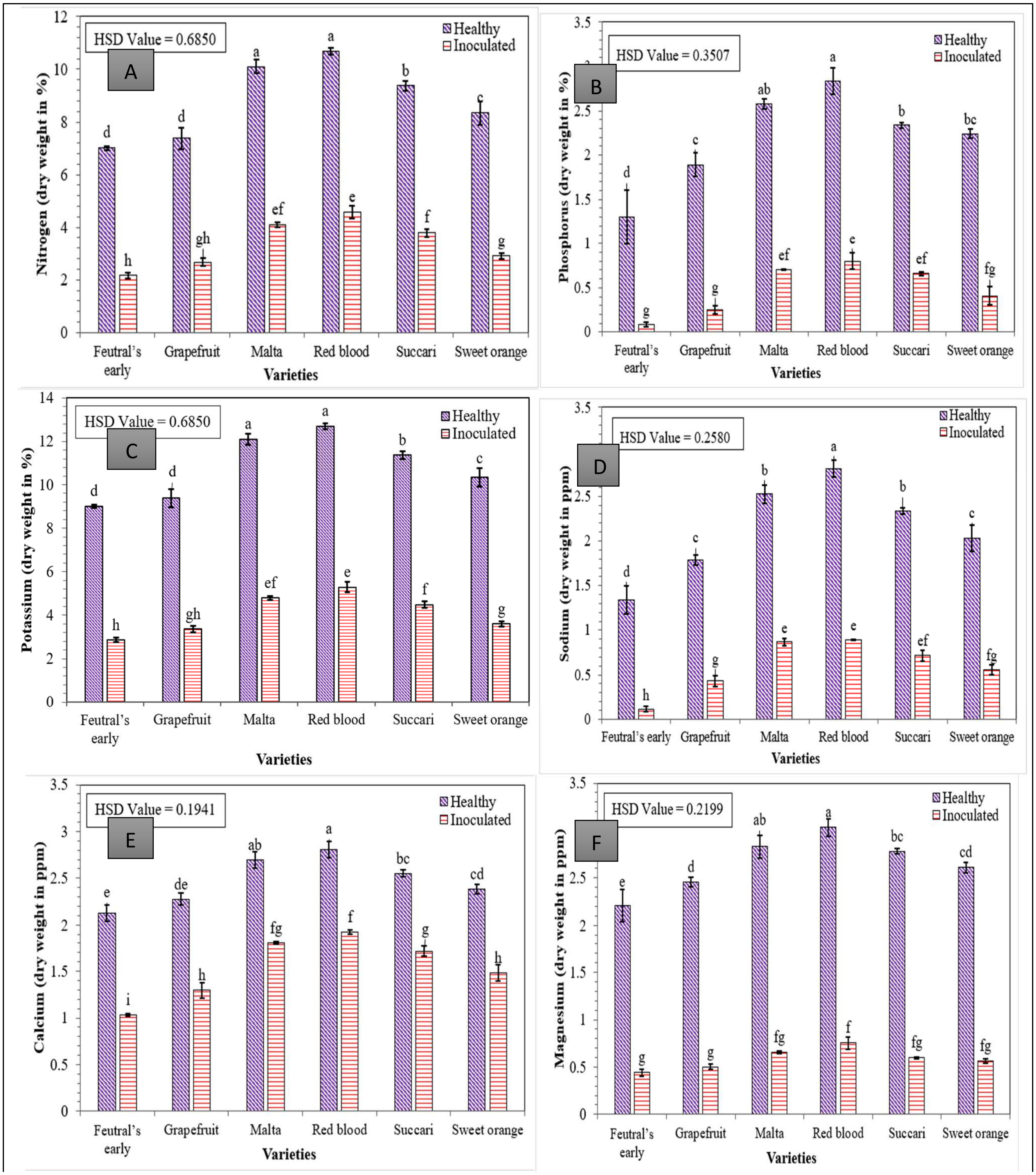
Magnesium was highest in Red Blood, with concentrations of 3.03 ppm in healthy and 0.75 ppm in inoculated samples, compared to Malta (2.83 ppm and 0.65 ppm) and Succari (2.78 ppm and 0.60 ppm). On the other hand, Sweet Orange from the susceptible cultivars showed 2.61 ppm in healthy and 0.56 ppm in inoculated, as compared to Grapefruit (2.46 ppm and 0.50 ppm) and Feutral's Early (2.21 ppm and 0.44 ppm) had lower Mg levels than the tolerant cultivars. Decrease in magnesium in susceptible varieties points towards disturbed chlorophyll biosynthesis during infection (Fig. 2F). Magnesium decreased by 2.28 ppm (75.2%) in Red Blood, 2.18 ppm (77.0%) in Malta and 2.18 ppm (78.4%) in Succari. The susceptible cultivars were Sweet Orange at -2.05 ppm (78.5%), Grapefruit at 1.96 ppm (79.7%) and Feutral's Early at 1.77 ppm (80.1%). The great Mg losses in the susceptible types show the suppression of chlorophyll metabolism and subsequent yield loss in photosynthesis.

Red Blood exhibited the highest zinc content in tolerant cultivars of 2.86 ppm in healthy and 0.57 ppm in inoculated, as compared to Malta with 2.62 ppm in healthy and 0.53 ppm in inoculated, which is higher than Succari (2.32 ppm and 0.50 ppm). Susceptible varieties, such as Sweet Orange, showed the highest zinc content of 2.14 ppm and 0.46 ppm in inoculated samples, compared to Grapefruit (1.50 ppm and 0.38 ppm) and Feutral's Early (1.12 ppm and 0.35 ppm) exhibited significant zinc loss. This micronutrient loss could jeopardize enzyme-mediated defense mechanisms (Fig. 2G). Zinc concentrations declined by 2.29 ppm (80.0%) in Red Blood, 2.09 ppm (79.8%) in Malta and 1.82 ppm (78.4%) in Succari. The susceptible cultivars, Sweet Orange showed a decline of 1.68 ppm (78.5%), Grapefruit at 1.12 ppm (74.7%) and Feutral's Early at 0.77 ppm (68.8%). Collectively, these findings further demonstrate that zinc depletion inhibited enzymatic defenses against infection.

Red Blood had the highest content of iron in both healthy (4.92 ppm) and inoculated (1.94 ppm) samples, compared to Malta (4.78 ppm and 1.77 ppm) and Succari (4.44 ppm and 1.55 ppm). On the other hand, Sweet Orange showed the higher iron content of 4 ppm in healthy and 1.25 ppm in inoculated than Grapefruit (3.42 ppm and 0.88 ppm) and Feutral's Early (3.14 ppm and 0.50 ppm) had lower iron content than the tolerant cultivars, reflecting disruption in defense processes related to lignification. A significant decrease in iron was observed in susceptible varieties (Fig. 2H). Iron concentration decreased by 2.98 ppm (60.6%) in the Red Blood, 3.01 ppm (63.0%) in the Malta and 2.89 ppm (65.1%) in the Succari. In terms of absolute losses, the Sweet Orange, Grapefruit and Feutral's Early had greater reductions, losing 2.75 ppm (68.7%), 2.54 ppm (74.3%) and 2.64 ppm (84.1%), respectively. This shows that iron remained at higher levels in the tolerant varieties, which is important for their function in lignification for defense.

Copper was significantly decreased in infected susceptible cultivars. The highest copper level was reported in tolerant cultivars like Red Blood, with concentrations of 3.05 ppm in healthy and 1.06 ppm in inoculated leaves, compared to Malta (2.89 ppm and 0.49 ppm) and Succari (2.44 ppm and 0.53 ppm). By comparison, Sweet Orange showed the higher copper content of 1.91 ppm in healthy and 0.86 ppm in inoculated leaves than Grapefruit (1.59 ppm and 0.85 ppm) and Feutral's Early (1.28 ppm and 0.88 ppm) had appreciably lower copper levels, indicating Cu depletion in infected leaves (Fig. 2I). Copper concentrations decreased by 1.99 ppm (65.2%) in the Red Blood, 2.40 ppm (83.0%) in the Malta and 1.91 ppm (78.3%) in the Succari varieties. By contrast, the Sweet Orange variety decreased by 1.05 ppm (55.0%), the Grapefruit variety decreased by 0.74 ppm (46.5%) and Feutral's Early reduced by 0.40 ppm (31.3%). The results are consistent with greater copper depletion in tolerant varieties, possibly due to increased use in defense enzyme systems.

Correlations between PDI and Mineral Losses: A strong negative relationship was observed between the Percent Disease Index (PDI) and the loss of macronutrients, including nitrogen (N) (Fig 3A), phosphorus (P) (Fig 3B), potassium (K) (Fig 3C), sodium (Na) (Fig 3D), calcium (Ca) (Fig 3E), magnesium (Mg) (Fig 3F) and iron (Fe) (Fig 3I). This trend indicates that as disease severity increased, the concentration losses of these essential nutrients declined proportionally, suggesting impaired nutrient uptake and translocation under higher disease pressure. In contrast, zinc (Zn) (Fig 3G) and copper (Cu) (Fig 3H) exhibited a positive relationship with PDI, implying that their loss intensified with increasing disease incidence. Among the citrus cultivars, Feutral's early consistently showed the highest nutrient losses, reflecting its greater disease susceptibility, whereas Red Blood maintained comparatively lower losses, indicating stronger tolerance or efficient nutrient retention under disease stress.



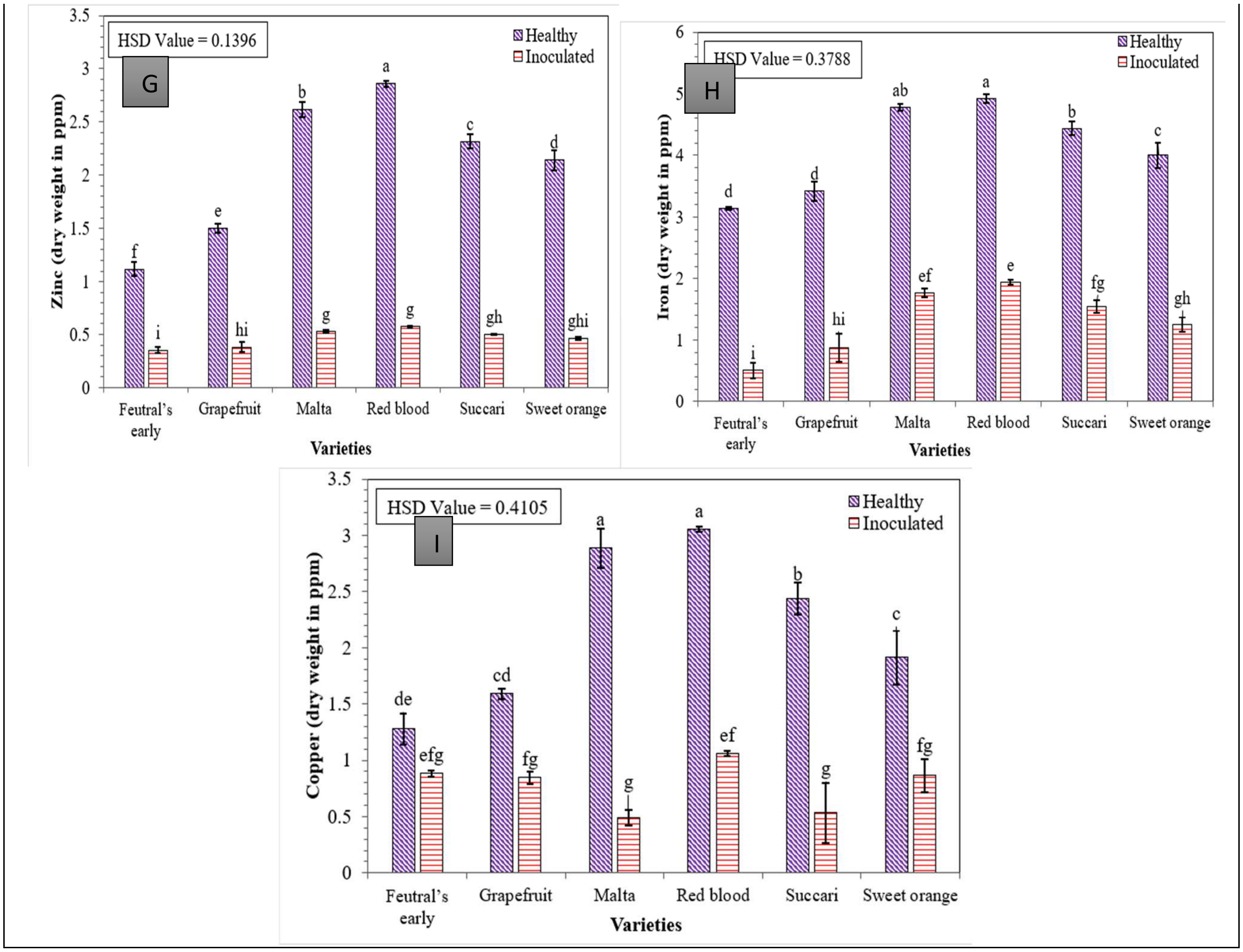
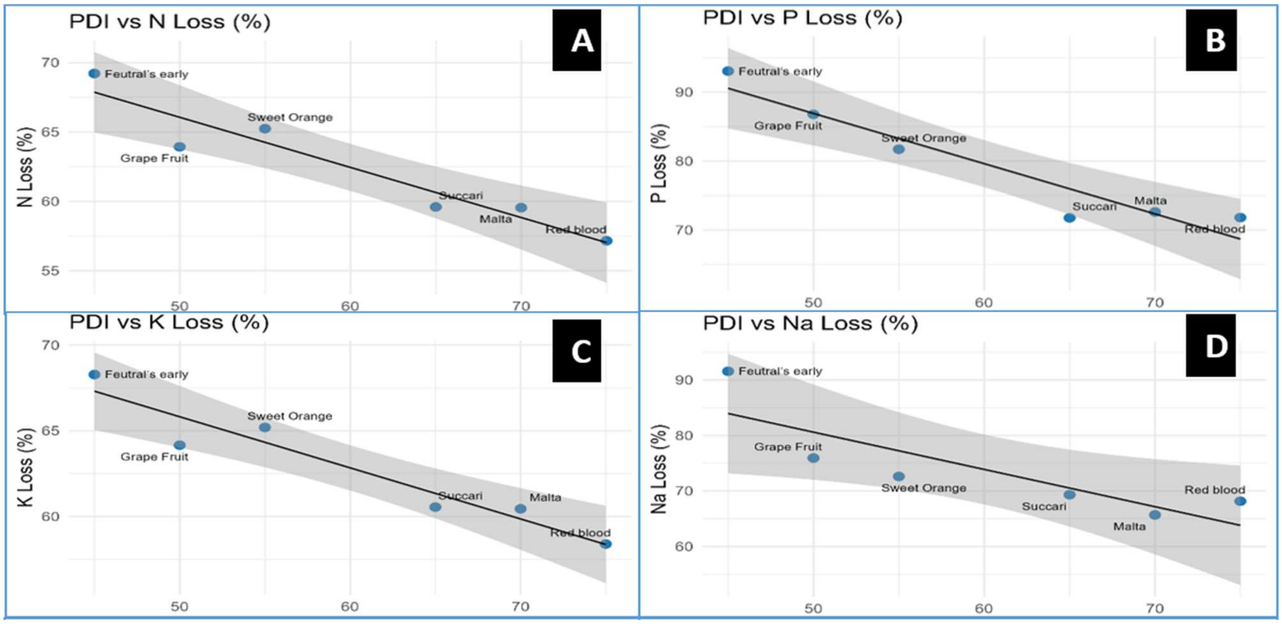


Fig 2. Macronutrient (A–C) and micronutrient (D–I) imbalances in different citrus cultivars in response to *E. fawcettii* infection



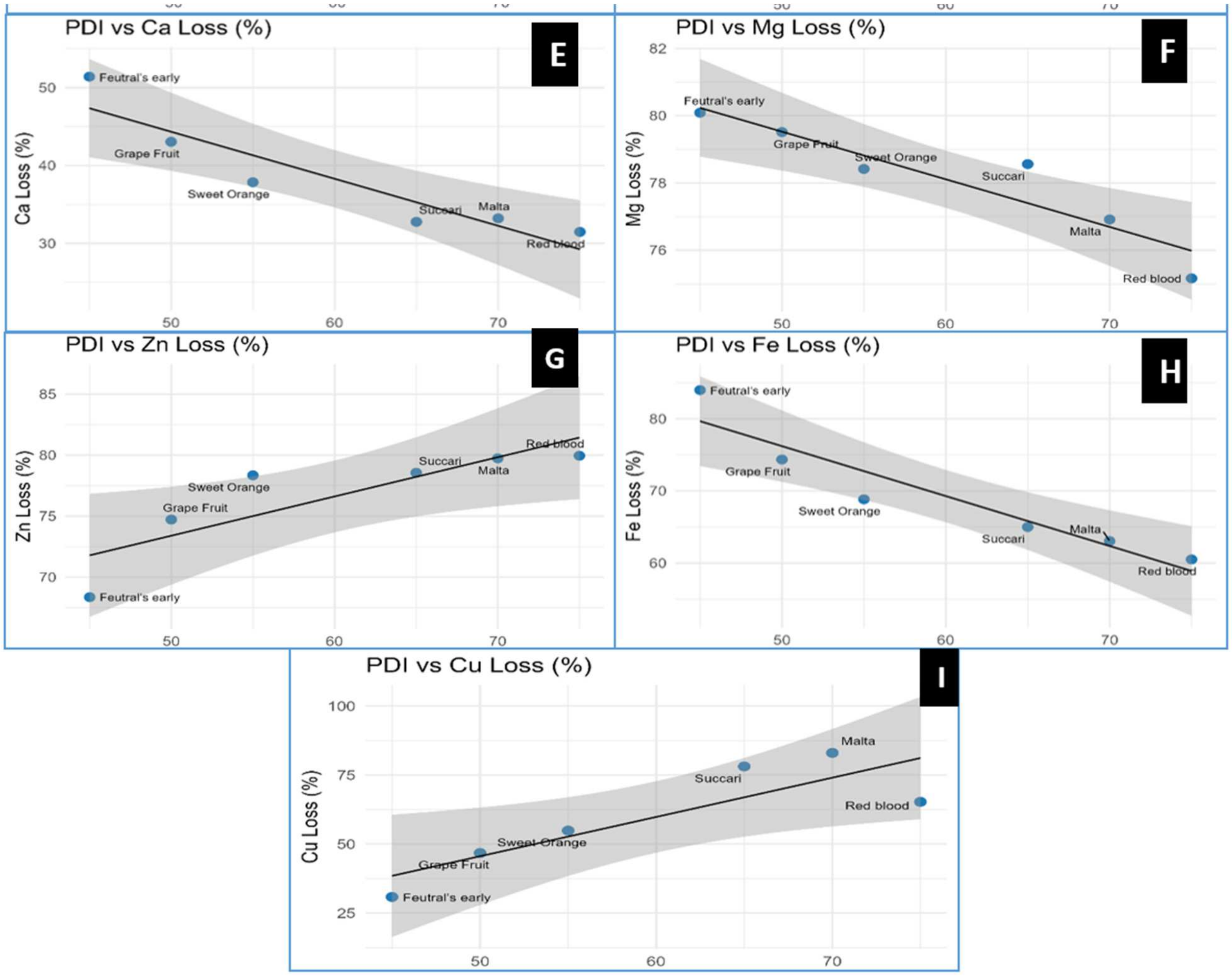


Fig 3. Scatter plots: correlations between percent disease index (PDI) and mineral losses (%). (A) PDI vs N loss. (B) PDI vs P loss. (C) PDI vs K loss. (D) PDI vs Na loss. (E) PDI vs Ca loss. (F) PDI vs Mg loss. (G) PDI vs Zn loss. (H) PDI vs Fe loss. (I) PDI vs Cu loss.

Multivariate analysis of nutrient profiles and disease response: Principal component analysis (PCA) was performed on nutrient profiles for healthy and inoculated citrus varieties in order to demonstrate potential patterns in mineral imbalances in relation to scab tolerance. Fig. 4A illustrates clustering of citrus varieties along Dim 1 (96.9%) and Dim 2 (1.9%), which together explain 98.8% of the total variance. Tolerant varieties (Red Blood, Malta and Succari) are grouped on the positive side of Dim1, reflecting higher overall nutrient retention, particularly of Fe, P and K. In contrast, susceptible varieties (Feutral's Early, Grapefruit and Sweet Orange) cluster on the negative side, showing nutrient depletion following infection. Fig. 4B compares healthy (blue) and inoculated (red) plants. A strong separation along Dim1 demonstrates distinct nutrient profiles between the two groups. Vectors indicate that Cu, Zn, P and N are positively associated with healthy samples, whereas Fe, Ca and K are more closely linked to inoculated ones, suggesting their dynamic response to disease stress.

Heatmap analysis: The heatmap illustrates the scaled nutrient composition across different citrus varieties under two conditions, Healthy and Inoculated. The y-axis represents the citrus varieties, including Red Blood, Malta, Succari, Feutral's Early, Grape Fruit *and* Sweet Orange, each in both Healthy and Inoculated states. The x-axis shows the major nutrients analyzed, such as Zn, Cu, Mg, Ca, P, Na, K and N. Healthy varieties (shown in cyan) exhibit higher nutrient concentrations, particularly for Cu, Mg, Ca, P and Na, as indicated by the warmer (red-orange) colors. In contrast, the Inoculated varieties (shown in pink) demonstrate reduced nutrient levels, especially in Ca, K and N, which appear as cooler (blue) shades, as shown in Fig 5. The clustering clearly separates the Healthy and Inoculated groups, suggesting that disease inoculation significantly affects nutrient balance, leading to nutrient depletion in infected plants compared with healthy ones.

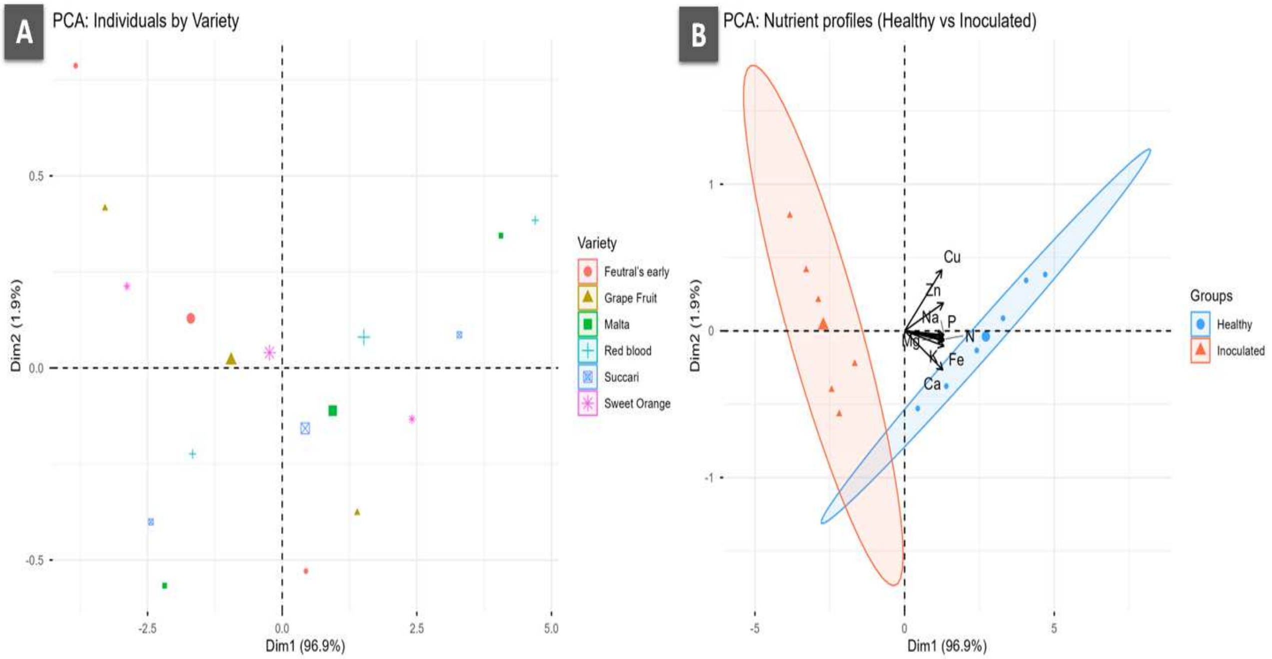


Fig 4. PCA of citrus nutrient composition (Dim1 = 96.9%, Dim2 = 1.9%, explaining 98.8% total variance) in citrus varieties under healthy and inoculated conditions. *(A)* PCA scatter plot showing distinct clustering of citrus varieties (Feutral's Early, Grape Fruit, Malta, Red Blood, Succari and Sweet Orange), reflecting strong varietal differences in nutrient accumulation. *(B)* PCA biplot illustrating clear separation between Healthy (blue) and Inoculated (red) plants, indicating pathogen-induced shifts in nutrient balance.

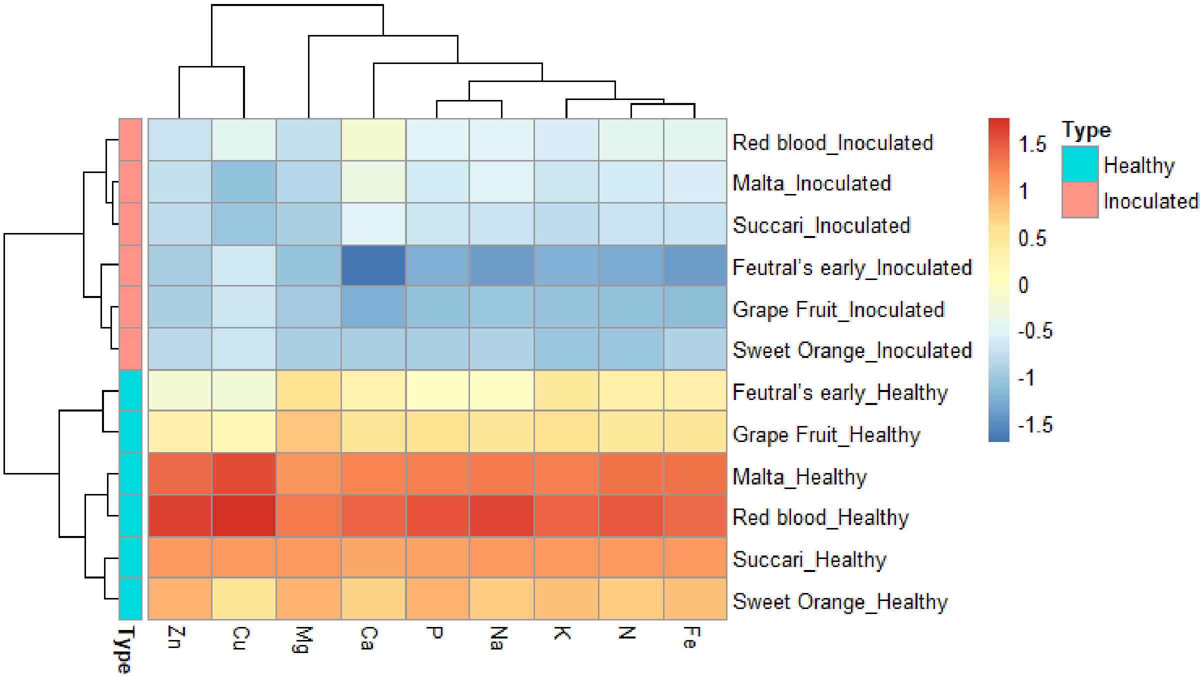


Figure 5. Heatmap showing the scaled nutrient composition in healthy and inoculated citrus varieties. The color gradient from blue to red represents low to high nutrient levels, respectively. Nutrient abbreviations: Zn = Zinc, Cu = Copper, Mg = Magnesium, Ca = Calcium, P = Phosphorus, Na = Sodium, K = Potassium and N = Nitrogen. (Heatmap was generated using the heatmap package in R (v4.5.1, https://cran.r-project.org/bin/windows/base/).

Correlation matrix: A clear association was observed among all nutrient variables of citrus plants under healthy and inoculated conditions. Nutrients such as K, P, Mg and N showed strong positive correlations with each other, indicating their coordinated role in maintaining nutrient balance and physiological stability. In contrast, Fe-Cu and Ca-Na exhibited negative correlations, reflecting possible nutrient antagonism under stress. Overall, healthy plants maintained stronger positive associations among nutrients, whereas inoculated plants showed disrupted correlations, indicating nutrient imbalance and stress-induced metabolic disturbance, as shown in Fig. 6.

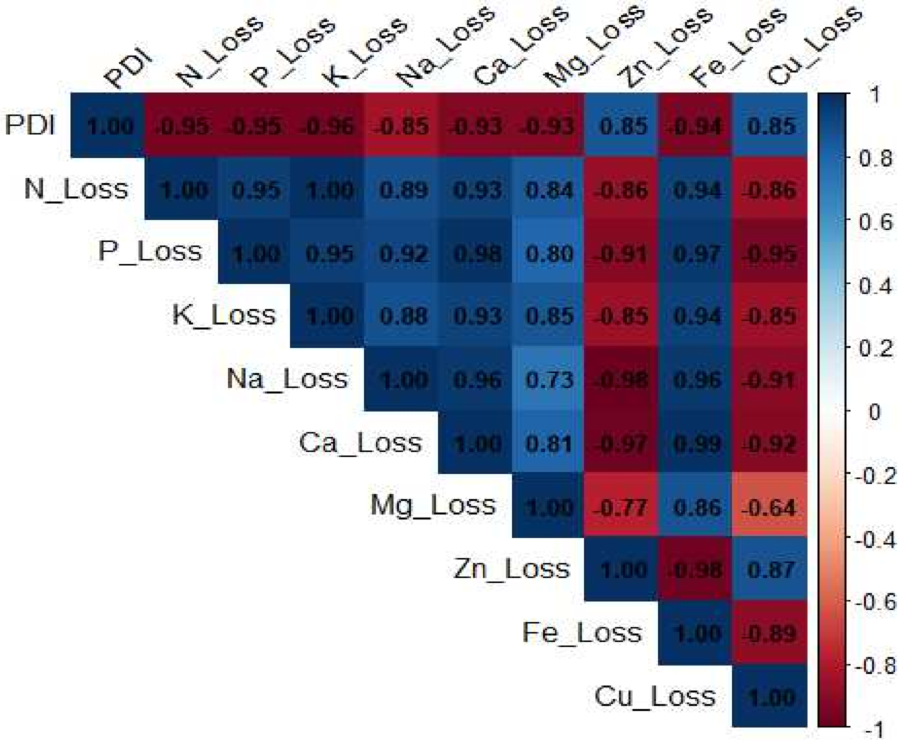


Figure 6. Correlation analysis of the measured nutrient parameters of healthy and inoculated citrus varieties under scab stress. Fe = Iron, Cu = Copper, Zn = Zinc, Mg = Magnesium, Ca = Calcium, Na = Sodium, K = Potassium, P = Phosphorus, PDI = Percent disease index and N = Nitrogen. Strong positive correlations were observed among macronutrients (K, P, Mg, N) whereas antagonistic relationships such as Fe-Cu and Ca-Na indicated nutrient imbalance under infection stress conditions.

DISCUSSION

Different resistance response of citrus varieties to *E. fawcettii* reveals the significant effect of host genetics on resistance to disease. These varieties showed tolerance with milder or fewer symptoms, while some were highly susceptible. This is consistent with previous studies where cultivar-dependent resistance was attributed to parameters such as cuticle thickness, stomatal morphology and biochemical defense mechanisms (Chung, 2011; Satpute and Fadli, 2022). Patterns of susceptibility also vary within Citrus species and hybrids, with specific rootstocks such as rough lemon and some mandarins consistently exhibiting higher resistance in comparative trials (Bowman *et al.*, 2021). In a study conducted on tangerine germplasm, genotypes such as *C. deliciosa*, *C. tangerina*, *C. nobilis* and satsuma hybrids exhibited excellent resistance under natural infection conditions (Palangasinghe *et al.*, 2024; Souza *et al.*, 2011). The high-resistance cultivars identified in this research, including Kumquat, Kinnow and Duncan, represent valuable germplasm for future breeding programs in regions where scab remains a recurring problem.

Mineral nutrition is a primary determinant of plant health and scab disease resistance and the findings of this study clearly establish the contribution of primary macro- and micronutrients towards modulating citrus cultivars' scab disease response (Ortel *et al.*, 2024). Our findings indicate that potassium (K) is an important factor for scab resistance, as resistant cultivars exhibited significantly higher levels of K which promoted stronger defense responses by osmotic regulation, sustaining turgor pressure and stimulating phenolic and phytoalexin production. Adequate K is also associated with stomatal regulation and diminished leaf wetness, which would reduce the probability of fungal infections. Recent studies specific to citrus have shown that K supplementation enhances photosynthetic performance and nutritional status in lemon and acid lime trees, reinforcing its essential role in plant resilience (Papadakis *et al*., 2023; Beheiry *et al*., 2023). In addition, K has been shown to alleviate multiple stress conditions in citrus while managing the rhizosphere microbial community, osmotic substances and enzyme activity levels (Zhang *et al*., 2023). Similar observations have been made in citrus and other crops, where higher K nutrition was linked to inhibited pathogen growth and increased lignification of cell walls (Zekri and Obreza, 2013).

Calcium (Ca) serves a dual function by bonding with pectins to fortify cell wall structure via calcium pectate cross-linking, as well as being a secondary messenger in defense signaling pathways (Wdowiak *et al*., 2024). Resistant cultivars in this study generally had higher Ca levels in their leaves, presumably to allow for a more robust strengthening of cell walls and a quicker defense response upon being challenged by pathogens. Conversely, lower Ca levels would, respectively, weaken the structural integrity and enhance the plant's susceptibility to being infected by fungi (Burrow *et al*., 2017). Magnesium (Mg) is also an important nutritional consideration in citrus diseases, although has not been discussed extensively in related literature. Mg is essential for chlorophyll biosynthesis and energy transfer. Mg concentrations of the susceptible cultivars decreased during the study which may have compromised their photosynthetic capacity and, thus, limited the energy supply necessary to perpetuate the defense response (Guo *et al*., 2024). Other research has documented that Mg deficiency impaired metabolic homeostasis and contributed to foliar disease in other crops (Gransee and Führs, 2013; Huber and Jones, 2013).

Micronutrients were also found to be an important aspect. Zinc (Zn) has a role in the function of numerous metalloenzymes and stabilization of membranes to oxidized stress, while iron (Fe), is necessary for lignin biosynthesis and the production of reactive oxygen species (ROS); both of which are important in limiting the spread of a pathogen (Rao *et al*., 2025). The Zn deficiency and Fe deficiency seen in the susceptible cultivars likely interrupted ROS-mediated signaling and it would have prevented lignin deposition, which was also seen in previous research that tied iron deficiency to increased susceptibility against pathogens (Iqbal *et al*., 2024; Pestana *et al*., 2023). Copper (Cu), in addition to its role in polyphenol oxidase enzyme activity, has some direct antimicrobial protection. The resistant cultivars had some comparatively higher Cu contents, which may have added another protective barrier against pathogen colonization. Cu has a role in enhancing phenolic metabolism and reducing pathogens and that is well documented in citrus pathology (Hippler *et al*., 2017; Zhou *et al*., 2025).

It is crucial to note that while there is a nutrient depletion noted in infected leaves, this shall not be logically attributed to susceptibility, but associated with pathogens activity. Many of the effects of infection are local, involving necrosis, disruption of nutrient transport and degradation of the tissue, which can artificially reduce measured concentration. Differences in soil fertility, fertilizer regime, or vigor may also pre-exist at the initial nutrient status. Baseline soil analyses and leaf nutrient data pre-inoculation were analyzed, however differences in rootstock physiology and leaf development may still have impacted the noted patterns. Recent work by Sharma *et al*. (2024) noted that orchard nutrient management, soil type and genotype had a strong influence on citrus nutrient balance. Again warning, causality and interpretation should be carefully considered. To better separate nutrient cause and effect, future studies would be wise to include nutrient manipulation studies pre-inoculation, isotope tracer work and controlled hydroponics to trace mineral flux during infection, as also suggested by Iqbal *et al*. (2024).

Together, these findings underscore that infection caused by *E. fawcettii* interferes with the nutrient homeostasis of citrus trees, resulting especially in the depletion of certain important minerals in susceptible varieties. Improved mineral nutrition, including K, Ca, Zn, Fe and Cu, promotes physiological vigor of plants and helps defend against citrus scab via a plant's natural defenses. This supports the idea that combining resistance breeding and nutrient management is vital to a successful citrus disease management system.

Conclusion: This study described significant differences in resistance levels for twenty citrus types, ranging from highly resistant (*C. japonica* and *C. reticulata* 'Kinnow') to highly susceptible types (*C. sinensis* 'Jaffa' and 'Valentia Late'). Infection with *E. fawcettii* was associated with measurable declines in both macro and micronutrient concentrations and resistant cultivars tended to have relatively higher concentrations of N, P, K, Ca, Zn, Fe and Cu. Collectively, these findings suggest that nutrient balance may play a role in contributing to, while not solely determining, host resistance to citrus scab development. From a practical standpoint, combining variety selection with efficient nutrient management is likely a sustainable approach to reduce citrus scab losses and sustain higher productivity. Future studies should aim to confirm these associations with field trials, manipulation of nutrients and tracer-based trials and mechanistic studies on lignin deposition, ROS signaling and cuticle characteristics, in conjunction with the use of resistant rootstocks as part of an integrated disease management system.

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

Abrams, D., D. Metcalf and M. Hojjatie (2014). Determination of Kjeldahl nitrogen in fertilizers by AOAC official methods 978.02: effect of copper sulfate as a catalyst. *J. AOAC Int.* 97(3): 764-767. https://doi.org/10.5740/jaoacint.13-299

Bastakoti, S. (2023). Role of zinc in management of plant diseases: A review. *Cogent Food Agric.* 9(1): 2194483. https://doi.org/10.1080/ 23311932. 2023.2194483

Beheiry, H.R., M.S. Hasanin, A. Abdelkhalek and H.A.Z. Hussein (2023). Potassium spraying preharvest and nanocoating postharvest improve the quality and extend the storage period for acid lime (*Citrus aurantifolia* Swingle) fruits. *Plants.* 12(22): 3848. https://doi.org/10.3390/ plants12223848

Bhar, A., A. Chakraborty and A. Roy (2023). The captivating role of calcium in plant-microbe interaction. *Front. Plant Sci.* 14: 1138252. https://doi.org/10.3389/fpls.2023.1138252

Bhargava, B.S. and H. Raghupathi (1993). Analysis of plant materials for macro and micronutrients. In: H.L.S. Tandon (Ed.), *Methods of Analysis of Soils, Plants, Waters and Fertilizers*. FDCO, New Delhi. pp. 49-82.

Boltz, D.F. and M.G. Mellon (1948). Spectrophotometric determination of phosphorus as molybdiphosphoric acid. *Anal. Chem.* 20(7): 749-751. https://doi.org/10.1021/ac60020a021

Bowman, K.D., G. McCollum and U. Albrecht (2021). SuperSour: a new strategy for breeding superior citrus rootstocks. *Front. Plant Sci.* 12: 741009. https://doi.org/10.3389/fpls.2021.741009

Burrow, J.D., T. Vashisth, M. Zekri, S.H. Futch and A. Schumann (2017). Citrus nutrition management practices. *EDIS.* 2017(3). https://doi.org/ 10.32473/edis-hs1292-2017

Caldana, N.F. da S., D.S. Alves and J.A. Martins (2020). Agroclimatic risk zoning of lemon (*Citrus aurantifolia*) in the hydrographic basin of Paraná River III, Brazil. *Geopauta* 4(2): 53–70. https://doi.org/10.22481/rg.v4i2.6457

Cebadera-Miranda, L., P. Morales and M. Cámara (2020). Bioactive compounds in oranges from the Mediterranean climate area. In: V.R. Preedy and R.R. Watson (Eds.), *Mediterr. Diet*. 2nd Ed. Acad. Press, London (UK). pp. 293–309. https://doi.org/10.1016/B978-0-12-818649-7.00027-8

Chung, K.R. (2011). *Elsinoë fawcettii* and *Elsinoë australis*: the fungal pathogens causing citrus scab. *Mol. Plant Pathol.* 12(2): 123-135. https://doi.org/10.1111/j.1364-3703.2010.00663.x

Cooke, B.M. (1998). Disease assessment and yield loss. In: *The Epidemiology of Plant Diseases*. Kluwer Publishers, The Netherlands. pp. 42-71. https://doi.org/10.1007/978-94-017-3302-1_3

FAOSTAT (2023). Food and Agriculture Organization of the United Nations, FAO, Rome. https://www.fao.org/faostat/en/#data/QCL/visualize

Fiske, C.H. and Y. Subbarow (1925). The colorimetric determination of phosphorus. *J. Biol. Chem.* 66(2): 375-400. https://doi.org/10.1016/s0021-9258(18)84756-1

Gransee, A. and H. Führs (2013). Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. *Plant Soil.* 368(1): 5-21. https://doi.org/10.1007/s11104-012-1567-y

Guo, J., X. Li, Y. Wang, W. Hu, L. Zhang, Z. Luo, H. Xu and L.S. Chen (2024). Magnesium deficiency induced leaf chlorosis affects plant growth, mineral concentration and fruit quality in field pomelo trees. *J. Agric. Food Res.* 18: 101338. https://doi.org/10.1016/j.jafr.2024.101338

Hald, P.M. (1947). The flame photometer for the measurement of sodium and potassium in biological materials. *J. Biol. Chem.* 167(2): 499-510.

Hippler, F., R. Boaretto, J. Quaggio and D. Mattos Jr (2017). Copper in citrus production: required but avoided. *Citrus Res. Technol.* 38: ICC067. http://dx.doi.org/10.4322/crt.ICC067

Huai, B., Y. Wu, C. Liang, P. Tu, T. Mei, A. Guan, Q. Yao, J. Li and J. Chen (2022). Effects of calcium on cell wall metabolism enzymes and expression of related genes associated with peel creasing in citrus fruits. *PeerJ.* 10: e14574. https://doi.org/10.7717/peerj.14574

Huber, D.M. and J.B. Jones (2013). The role of magnesium in plant disease. *Plant Soil.* 368(1): 73-85. https://doi.org/10.1007/s11104-012-1476-0

Imran, M., M. Mustafa, M. Azeem, M. Awais and M.A. Khan (2015). Correlation of environmental variables on canker disease development in commercial citrus cultivars of Pakistan. *Int. J. Biosci.* 7(1): 1-13. http://dx.doi.org/10.12692/ijb/7.1.1-13

Iqbal, S., M. Atiq, S.T. Sahi, N. Akbar and N.A. Rajput (2024). Progressive alterations in mineral contents in citrus genotypes toward *Alternaria citri* causing brown spot of citrus. *PLoS ONE.* 19(7): e0306031. https://doi.org/ 10.1371/ journal.pone.0306031

Liao, H.L. and K.R. Chung (2008a). Cellular toxicity of elsinochrome phytotoxins produced by the pathogenic fungus *Elsinoë fawcettii* causing citrus scab. *New Phytol.* 177(1): 239-250. https://doi.org/10.1111/j.1469-8137.2007.02234.x

Liao, H.L. and K.R. Chung (2008b). Genetic dissection defines the roles of elsinochrome phytotoxin for fungal pathogenesis and conidiation of the citrus pathogen *Elsinoë fawcettii*. *Mol. Plant Microbe Interact.* 21(4): 469-479. https://doi.org/10.1094/MPMI-21-4-0469

McKinney, H.H. (1923). Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *J. Agric. Res.* 26(5): 195-218.

Negi, N.P., G. Prakash, P. Narwal, R. Panwar, D. Kumar, B. Chaudhry and A. Rustagi (2023). The calcium connection: exploring the intricacies of calcium signaling in plant-microbe interactions. *Front. Plant Sci.* 14: 1248648. https://doi.org/10.3389/fpls.2023.1248648

Oliveira, S.R., J.A. Gomes Neto, J.A. Nóbrega and B.T. Jones (2010). Determination of macro- and micronutrients in plant leaves by high-resolution continuum source flame atomic absorption spectrometry. *Spectrochim. Acta Part B.* 65(4): 316-320. https://doi.org/10.1016/j.sab.2010.02.003

Ortel, C.C., T.L. Roberts and J.C. Rupe (2024). A review of the interaction between potassium nutrition and plant disease control. *Agrosyst. Geosci. Environ.* 7(2): e20489. https://doi.org/10.1002/agg2.20489

Palangasinghe, P.C., W.K. Liyanage, M.P. Wickramasinghe, H.R. Palangasinghe, H.C. Shih, M.S. Shiao and Y.C. Chiang (2024). Reviews on Asian citrus species: exploring traditional uses, biochemistry, conservation and disease resistance. *Ecol. Genet. Genomics.* 32: 100269. https://doi.org/10.1016/j.egg.2024.100269

Papadakis, I.E., E.V. Ladikou, A. Oikonomou, T. Chatzistathis and G. Chatziperou (2023). Exploring the impact of potassium on growth, photosynthetic performance and nutritional status of lemon trees (cv. Adamopoulou) grafted onto sour orange and Volkamer lemon rootstocks. *Sustainability.* 15(22): 15858. https://doi.org/10.3390/su152215858

Penjor, T., M. Yamamoto, M. Uehara, M. Ide, N. Matsumoto, R. Matsumoto and Y. Nagano (2013). Phylogenetic relationships of citrus and its relatives based on *matK* gene sequences. *PLoS ONE* 8(4): e62574. https://doi.org/10.1371/journal.pone.0062574

Pestana, M., P. García-Caparrós, T. Saavedra, F. Gama, J. Abadía, A. Varennes and P.J. Correia (2023). Nutritional performance of five citrus rootstocks under different Fe levels. *Plants.* 12(18): 3252. https://doi.org/10.3390/plants12183252

Pham, N.Q., B.D. Wingfield, I. Barnes, R. Gazis and M.J. Wingfield (2025). *Elsinoë* species: the rise of scab diseases. *Plant Pathol.* 74(1): 39-58. https://doi.org/10.1111/ppa.14015

Rai, S., P.K. Singh, S. Mankotia, J. Swain and S.B. Satbhai (2021). Iron homeostasis in plants and its crosstalk with copper, zinc and manganese. *Plant Stress.* 1: 100008. https://doi.org/10.1016/j.stress.2021.100008

Rao, M.J., M. Duan, C. Zhou, J. Jiao, P. Cheng, L. Yang, W. Wei, Q. Shen, P. Ji, Y. Yang and O. Conteh (2025). Antioxidant defense system in plants: reactive oxygen species production, signaling and scavenging during abiotic stress-induced oxidative damage. *Horticulturae.* 11(5): 477. https://doi.org/10.3390/horticulturae11050477

Satpute, A.D. and A. Fadli (2022). Diseases of citrus and their control. In: *Citrus Production*. CRC Press. pp. 239-266. https://doi.org/ 10.1201/9781003119852-17

Shabbir, R., T. Javed, S. Hussain, S. Ahmar, M. Naz, H. Zafar, S. Pandey, J. Chauhan, M.H. Siddiqui and C. Pinghua (2022). Calcium homeostasis and potential roles in combatting environmental stresses in plants. *S. Afr. J. Bot.* 148: 683-693. https://doi.org/10.1016/j.sajb.2022.05.038

Sharma, L.D., S.K. Singh, N. Oinam, H. Das, A. Sarkar, L.B. Singh and R. Sadhukhan (2024). Citrus nutrition in India—a snapshot of up-to-date progress. *Ann. Plant Soil Res.* 26(2): 372-386. https://doi.org/10.47815/apsr.2024.10374

Shin, K., D.P. Paudyal, S.C. Lee and J.W. Hyun (2021). Different phytohormonal responses on *Satsuma mandarin* (*Citrus unshiu*) leaves infected with host-compatible or host-incompatible *Elsinoë fawcettii*. *Plant Pathol. J.* 37(3): 268-279. https://doi.org/10.5423/PPJ.OA.12.2020.0224

Souza, M.C.D., E.S. Stuchi and A.D. Goes (2011). Reaction of tangerine genotypes to *Elsinoë fawcettii* under natural infection conditions. *Crop Breed. Appl. Biotechnol.* 11(1): 77-81. https://doi.org/10.1590/S1984-70332011000100011

Steel, R.G.D., J.H. Torrie and D. A. Dickey (1996). *Principles and Procedures of Statistics: A Biometrical Approach.* 3rd Edition. McGraw-Hill Kogakusha, Ltd.

Steponĕnienĕ, L., S. Tautkus and R. Kazlauskas (2003). Determination of zinc in plants and grains by atomic absorption spectrometry. *Chemija.* 14(2): 56-60.

Timmer, L.W., M. Priest, P. Broadbent and M.K. Tan (1996). Morphological and pathological characterization of species of *Elsinoë* causing scab diseases of citrus. *Phytopathol.* 86: 1032-1038. https://doi.org/10.1094/Phyto-86-1032

Tripathi, R., R. Tewari, K.P. Singh, C. Keswani, T. Minkina, A.K. Srivastava, U. De Corato and E. Sansinenea (2022). Plant mineral nutrition and disease resistance: a significant linkage for sustainable crop protection. *Front. Plant Sci.* 13: 883970. https://doi.org/ 10.3389/ fpls.2022.883970

Wang, S., W. Xie and X. Yan (2022). Effects of future climate change on citrus quality and yield in China. *Sustain.* 14(15): 9451. https://doi.org/10.3390/su14159366

Wdowiak, A., A. Podgórska and B. Szal (2024). Calcium in plants: an important element of cell physiology and structure, signaling and stress responses. *Acta Physiol. Plant.* 46(12): 108. https://doi.org/10.1007/s11738-024-03733-w

Zekri, M. and T. Obreza (2013). Potassium (K) for citrus trees. *EDIS.* 2013(7). https://doi.org/10.32473/edis-ss583-2013

Zekri, M. and T. Obreza (2014). Manganese (Mn) and zinc (Zn) for citrus trees. *EDIS* 2014(3). https://doi.org/10.32473/edis-ss616-2014

Zekri, M. and T.A. Obreza (2003). Plant nutrients for citrus trees. *EDIS.* 2003(2): 1-5. https://doi.org/10.32473/edis-ss419-2003

Zhang, M., X. Li, X. Wang, J. Feng and S. Zhu (2023). Potassium fulvic acid alleviates salt stress of citrus by regulating rhizosphere microbial community, osmotic substances and enzyme activities. *Front. Plant Sci.* 14: 1161469. https://doi.org/10.3389/fpls.2023.1161469

Zhou, X., X. Xiong, F. Lu, W. Shi, Y. Zhou, N. Lai, L.S. Chen and Z.R. Huang (2025). Excessive copper induces lignin biosynthesis in the leaves and roots of two citrus species: physiological, metabolomic and anatomical aspects. *Ecotoxicol. Environ. Saf.* 289: 117692. https://doi.org/10.1016/j.ecoenv.2025.117692.