Journal of Tropical Resources and Sustainable Science
Website : http://journal.umk.edu.my/index.php/jtrss/
eISSN : 2462-2389
Vol: 14, Issue 2, 2026, 403-409
DOI: https://doi.org/10.47253/jtrss.v14i2.1774

RESEARCH ARTICLE

A case study on the macronutrient differences between healthy and insidious fruit rot–infected Harumanis mango leaves in West Malaysia

Khairun Nisa Kamarudin[1*], Nur Firdaus Abdul Rashid[1], Mohd Saiful Akbar Mohamad Sahal[1], Nurhaliza Mohamad Shahidin[1], Normardhiyah Roslan[1] and Khairun Naim Maulana[2]

[1]Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Perlis Branch, Arau Campus, Perlis, Malaysia
[2]Bank Pertanian Malaysia Berhad (Agrobank), Leboh Pasar Besar, Kuala Lumpur, Malaysia

ARTICLE HISTORY
Received : 30 October 2025
Accepted : 5 February 2026
Online : 30 June 2026

KEYWORDS
Nutrient imbalance,
Internal breakdown disorder,
K/Ca ratio,
Mangifera indica,
N/Ca ratio

☒* **CORRESPONDING AUTHOR**
Khairun Nisa Kamarudin
Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Perlis Branch, Arau Campus, Perlis, Malaysia
Email: irunkha@hotmail.com

ABSTRACT

Insidious fruit rot (IFR) is a major problem in mango cultivation. It can reduce fruit quality without any visible symptoms except when the fruit is cut in half. This problem is an increasing concern among Harumanis growers, one of the premium mango varieties in Malaysia, due to its high cost and maintenance requirements. A previous study found a highly significant difference in soil nutrient content between Harumanis orchards with and without IFR. However, no information on plant nutrient content was provided. The objectives of this study were to measure and compare the macronutrient contents between mango trees with and without IFR incidence and to evaluate the nutrient relationships at each study site. A total of 22 leaf samples were collected from 10 and 12 mango trees with and without IFR incidence, respectively. Approximately 20 leaves were collected from each mango tree, specifically from the third or fourth leaf of the current flush, and processed before analysis. Total nitrogen (N) was determined by combustion, while phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were determined by dry ashing. The N/Ca and K/Ca ratios were manually calculated. The results demonstrated significantly higher N, P, K, Mg, N/Ca ratio, and K/Ca ratio in trees with IFR incidence at 0.57%, 0.05%, 0.15%, 0.27%, 1.50, and 0.37, respectively, while Ca was significantly lower at 2.05%. A highly negative correlation was observed between Ca and the N/Ca ratio. These findings indicate that nutrient imbalance in plants was associated with this problem.

1. INTRODUCTION

Mango (*Mangifera indica*) is among the top six fruit crops in the world, together with guava and mangosteen (FOA, 2023). It is mainly grown in tropical and subtropical countries such as India, Indonesia, China (mainland), Mexico, Brazil and Thailand, which are among the top mango-producing countries (FAO, 2023; MDOA, 2019). In Malaysia, Harumanis (MA128) is one of the most popular commercial mangoes, grown mainly in West Malaysia, specifically in Perlis and Kedah (MDOA, 2019). Recently, this mango variety has been grown in the eastern part of Peninsular Malaysia, mainly in Terengganu (Saiin, 2023; NST, 2020). Other commercial mangoes, such as Chokanan (MA224), Golek (MA162), Maha 65 (MA165), and Malele (MA204), can be found in many areas of Malaysia (MDOA, 2023; MDOA, 2019). Among the commercial mango varieties in Malaysia, Harumanis is the most sensitive to climate variability, as it requires a significantly dry period to initiate flowering (Talib et al., 2020). This variety also requires intensive, high-maintenance (Kamarudin et al., 2023). Despite the high operational costs, this mango variety can yield higher profits than other varieties (MDOA, 2024). Malaysian mangoes are mainly grown by smallholder farmers (MDOA, 2019), whereas large areas of mango cultivation are managed by companies and agencies such as the Muda Agricultural Development Authority (MADA) and the Department of Agriculture (DOA). This mango variety is well-suited for cultivation in Perlis state due to the weather conditions, which include two months of drought (MDOA, 2019; Shahidin et al., 2018). Previously, many paddy fields were converted into Harumanis farms (Kamarudin et al., 2023). However, many of these mango farms have been abandoned or changed recently due to low fruit quality caused by physiological problems that take a long time to resolve.

Insidious fruit rot (IFR) is a common internal disorder in mango fruits (Ullah et al., 2024). In Malaysia, the earliest incidence of IFR was reported in the Harumanis cultivar by Lim and Khoo (1985), followed by Tarmizi et al. (1993). According to Tarmizi et al. (1993), this disorder is similar to soft nose due to the dissolution of the flesh tissue, but it occurs in the sinus region. The quality of the affected fruit is poor, potentially reducing its marketability (Ullah et al., 2024). The tissues of the affected fruit turn yellowish-brown with a yeasty odour and become soft and watery (Oldoni et al., 2022; Shivashankar, 2014), and these symptoms can only be seen after the fruit has been cut in half (Oldoni et al., 2022; Tarmizi et al., 1993). This problem occurs due to physiological disturbances that lead to cell collapse, resulting from metabolic imbalance caused by harvesting factors (Ullah et al., 2024). The major cause of this physiological disorder has not yet been determined (Shivashankar, 2014), but poor soil and nutrient management are likely causes (Kamarudin et al., 2023; Shivashankar, 2014). The incidence of IFR in Harumanis is associated with an imbalance in the nitrogen-to-calcium ratio, particularly during the fruiting stage in both fruits and leaves (Ullah et al., 2024). Although some studies have been conducted on soil-leaf relationships, there is still a relative lack of information to understand this issue, and it remains unresolved.

Leaf nutrient analysis can be used to assess plant nutritional status (Prado, 2021) as leaf composition can reflect the amount of nutrients taken up (primarily from the soil) and assimilated by plants. This analysis has been widely used for nutrient evaluation across a range of plant species, including mangoes (Ram et al., 2020), as well as in fruit disorder studies because leaves reflect the tree's nutritional status during critical stages of fruit development. The results of soil analysis from Harumanis orchards with IFR incidence reported by Kamarudin et al. (2023) showed significantly higher electrical conductivity, total carbon (C), total nitrogen (N), C/N ratio, and exchangeable magnesium (Mg), while significantly lower exchangeable calcium (Ca), potassium (K), and sodium (Na), compared to Harumanis orchards without IFR incidence. Significantly higher and lower levels of N and Ca were found in the soil with IFR incidence, respectively, which may have influenced the nutrient content in Harumanis trees. Additionally, synergistic effects between exchangeable Mg and/or available phosphorus (P) may increase the availability of one of them, and antagonistic effects between exchangeable Mg and available P, and exchangeable Ca may cause precipitation of less soluble calcium phosphates in the vicinity of nutrient-absorbing roots (Jakobsen, 1993). This condition may lead to undesirable changes in nutrient absorption, resulting in nutrient imbalances in the leaves and influencing the IFR problem in Harumanis mango fruits. Therefore, the purposes of this study were to measure and compare the mango plant macronutrient contents between trees with and without IFR incidences in Harumanis mango trees and to evaluate the relationship between nutrients at each site.

2. MATERIALS AND METHODS

2.1. Study location

Sampling was conducted within Perlis state. The climate in Perlis is tropical monsoon (*Am*) according to the Köppen-Geiger classification system. The average annual temperature and precipitation in this state over the past 30 years (1990–2021) were 27.4°C and 1,893.7 mm, respectively (MMD, 2022).

Specifically, leaf samples were collected from two Harumanis orchards to compare their nutrient contents. One of the Harumanis orchards had trees with IFR incidence (6° 23' N, 100° 17' E) located at the Perlis-Kedah border, while the other had trees without IFR incidence (6° 27' N, 100° 15' E) located in Arau, Perlis (Figure 1).

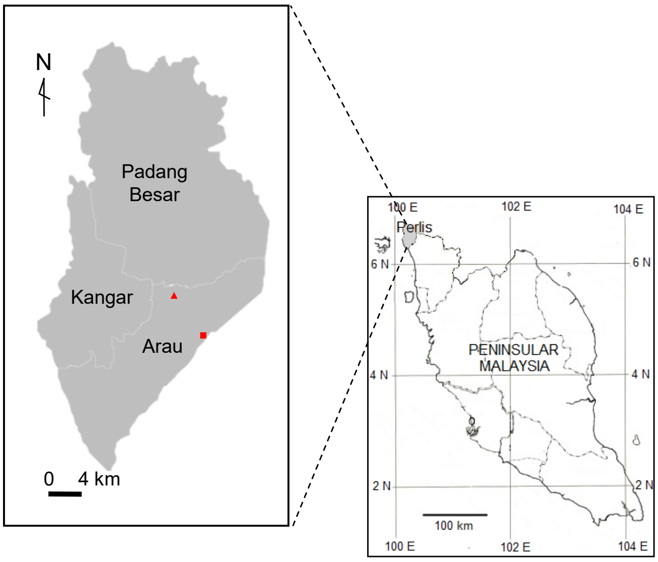

Figure 1: Location of sampling points for mango trees with (squared) and without (triangle) insidious fruit rot incidences

The Harumanis trees with IFR incidence were nine years old during the study. The trees were planted in a square system within 0.25 ha, with a standard planting of 9 m × 9 m, and some were planted near the fence without a specific distance, for a total of 70 trees. Previously, this area was a paddy field, which was later converted into a mango orchard. These trees had been affected by IFR since 2019. During the study, this orchard was already being conserved by adding dolomite, and the fruits still had black spots when cut in half, indicating that the IFR stage was at stage 1 and/or 2 (Sabdin et al., 2022). For cultural management practices, NPK 12:12:17 fertiliser was applied during the vegetative stage, and foliar fertilisers were applied during the flowering and fruiting stages. Pests and diseases were controlled using chemical applications (i.e., pesticides and insecticides), and weeds were controlled using both chemical (i.e., herbicides) and mechanical (i.e., mowers) applications. Other cultural practices, such as the application of paclobutrazol and pruning, were carried out according to standard mango cultivation practices (Kamarudin et al., 2023; MDOA, 2019).

Harumanis trees without IFR incidents were planted in a standard square system with a 9 m × 9 m spacing. Most of the planted trees were 12 years old, and a few were 38 years old (Kamarudin et al., 2024). Previously, this area was cultivated with fruits and rubber trees before being fully planted with mango trees. These mango trees experienced an IFR incident in 2007 and were rehabilitated for three years before fully recovering. Here, NPK 12:12:17 controlled-release fertiliser was applied during the vegetative stage at least once every three months, whereas foliar fertilisers were sprayed during the flowering and fruiting stages at least once every two weeks. Other cultural practices were almost identical to those mentioned above for the Harumanis orchard with IFR incidence.

Harumanis orchard with IFR incidence was located on sandy clay loam soil (clay = 31.1%; silt = 20.4%; coarse sand = 25.0%; fine sand = 23.5%), which has moderate water-holding capacity and relatively higher clay content, while Harumanis orchard without IFR incidence was situated on fine sandy loam soil (clay = 14.8%; silt = 17.5%; coarse sand = 10.2%; fine sand = 57.5%), which is lighter, well-drained, and typically has lower moisture and nutrient holding capacity. The basic soil properties of both orchards were summarised in Table 1, and the soil details at both study sites were discussed in Kamarudin et al. (2023).

Table 1: Basic soil properties at the study sites (Kamarudin et al., 2023).

Property	Unit	Mango Orchard with IFR incidence	Mango Orchard without IFR incidence
pH	–	6.93 ± 0.82	6.48 ± 0.84
Electrical conductivity	mS cm^{-1}	0.09 ± 0.05	0.05 ± 0.02
Total carbon	g kg^{-1}	16.67 ± 6.49	4.71 ± 2.23
Total nitrogen	g kg^{-1}	2.56 ± 0.58	1.54 ± 0.63
C/N ratio	–	6.52 ± 2.07	3.02 ± 0.74
Available phosphorus	mg kg^{-1}	80.92 ± 32.61	116.40 ± 67.47
Exchangeable calcium	cmol kg^{-1}	4.01 ± 2.76	6.63 ± 2.23
Exchangeable magnesium	cmol kg^{-1}	4.61 ± 1.95	0.81 ± 0.17
Exchangeable potassium	cmol kg^{-1}	2.06 ± 1.41	3.40 ± 1.14
Exchangeable sodium	cmol kg^{-1}	3.51 ± 2.42	5.80 ± 1.95
Exchangeable acidity	cmol kg^{-1}	0.57 ± 0.48	1.03 ± 0.94
Effective cation exchange capacity	cmol kg^{-1}	14.76 ± 6.03	17.66 ± 4.88
Base saturation	%	96.23 ± 1.57	93.04 ± 7.98
Soil texture	–	Sandy clay loam	Fine sandy loam

2.2. Leaf sampling

Leaf sampling was conducted in April 2021 during the fruiting stage. To avoid younger or older leaves, third- or fourth-leaf samples were collected from the current flushes. Leaf samples were collected from all cardinal directions, namely north, south, east and west, to avoid any bias. In total, 20 leaves were collected from each mango tree and combined into a single sample for subsequent analysis. The leaf samples were kept in sealed plastic bags and brought to the laboratory. In total, 10 samples were collected from 10 Harumanis trees with IFR incidences, and 12 samples were collected from 12 Harumanis trees without IFR incidences. These Harumanis trees were selected randomly.

2.3. Laboratory analysis

The leaf samples were cleaned and washed with running tap water, then rinsed with distilled water at least 3 times. The rinsed leaf samples were dried in an oven at 80°C until constant weight was attained, then ground to a fine powder using a grinder before analysis. Total N was determined using the combustion method with a CHNS Elemental Analyser (Perkin Elmer 2400 Series II, Waltham, Massachusetts). The extraction of P, K, Ca, and Mg was determined using the dry ashing method. The ground leaf samples were burned in a muffle furnace at 450°C for at least 3 h and a maximum of 5 h. Subsequently, the ash was dissolved in hydrochloric acid. Element concentrations were determined using an inductively coupled plasma-optical emission spectrometer (PerkinElmer Optima, Waltham, Massachusetts, USA). The N/Ca and K/Ca ratios were calculated by dividing N by Ca and K by Ca.

2.4. Statistical analysis

Descriptive statistics, such as minimum, mean, median, standard deviation (SD), median absolute deviation (MAD), maximum, and coefficient of variation (CV), were calculated. The CV was ranked as low (CV < 10%), moderate (CV = 10%–90%), or high (CV > 90%) (Kamarudin et al., 2019; Kamarudin et al., 2023). Meanwhile, Shapiro-Wilk and Levene tests were used to assess the normality and homogeneity of variance of the dataset, respectively. The differences in measured plant parameters between the two groups were tested using Welch's t-test due to unequal variances and sample sizes. The relationships among the variables in each group were computed using Pearson's correlations. All statistical analyses were performed using R software version 4.2.0 (R Core Team, R Foundation for Statistical Computing).

3. RESULT

The descriptive statistics of the leaf samples with and without IFR incidence are presented in Table 2. Generally, the leaf N content showed that 100.0% of samples with IFR incidence were higher than the optimum level, while 91.7% of the leaf samples without IFR incidence were within the optimum range, and 8.3% were above the optimum level. The leaf P content showed that 90.0% of the leaf samples with IFR incidence were within the optimum level, and 10.0% were above the optimum level. A similar pattern was observed in leaf samples without IFR incidence: 91.7% were within the optimum level, and 8.3% were below it. The leaf K content showed that 90.0% of the leaf samples with IFR incidence were within the optimum range, and 10.0% were below the optimum level. Meanwhile, 33.3% of the leaf samples without IFR incidence were within the optimum range, and 66.7% were below it.

Table 2: The descriptive statistics of leaf macronutrient content with and without the insidious fruit rot (IFR) incidences.

Variable	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	N/Ca ratio	K/Ca ratio
Harumanis leaves with IFR incidence (n = 10)							
Mean	1.83	0.14	0.44	0.99	0.42	1.94	0.47
SD	0.20	0.03	0.11	0.22	0.12	0.51	0.17
Median	1.81	0.15	0.44	0.99	0.37	1.97	0.49
MAD	0.19	0.02	0.08	0.17	0.04	0.43	0.10
Minimum	1.56	0.10	0.23	0.65	0.29	1.18	0.17
Maximum	2.17	0.19	0.60	1.38	0.65	2.79	0.77
CV	10.84	17.71	25.01	22.11	29.45	26.08	8.70
Harumanis leaves without IFR incidence (n = 12)							
Mean	1.26	0.09	0.29	3.05	0.15	0.44	0.10
SD	0.17	0.01	0.06	0.87	0.03	0.10	0.03
Median	1.19	0.09	0.29	2.81	0.15	0.47	0.10
MAD	0.12	0.01	0.07	0.79	0.04	0.11	0.03
Minimum	1.09	0.07	0.22	2.13	0.09	0.25	0.07
Maximum	1.59	0.11	0.42	4.68	0.20	0.55	0.14
CV	13.15	13.12	19.69	28.52	21.09	23.91	25.60
Optimum range	1.0 – 1.5%	0.08 –0.18%	0.3 – 0.8%	2.0– 3.5%	0.15 – 0.40%	< 0.5	< 0.2

Abbreviation: SD = standard deviation, MAD = median absolute deviation, CV = coefficient of variation

The leaf Ca content showed that 100.0% of the leaf samples with IFR incidence were lower than the optimum level, whereas 75.0% of the leaf samples without IFR incidence were within the optimum level, and 25.0% were higher than the optimum level. The leaf Mg content showed that 70.0% of the leaf samples with IFR incidence were within the optimum level, and 30.0% were lower than the optimum level, while 58.3% of the leaf samples without IFR incidence were within the optimum level, and 41.7% were lower than the optimum level. The N/Ca ratio showed that 100.0% of the leaf samples with IFR incidence had a higher N/Ca ratio than the threshold value of <0.5, while 58.3% of the leaf samples without IFR incidence had a lower N/Ca ratio than the threshold value, and 41.7% had a higher N/Ca ratio than the threshold value. The K/Ca ratio showed that 90.0% of the leaf samples with IFR incidence were higher than the threshold value of <0.2, and 10.0% were lower than the threshold value, while 100.0% of the leaf samples without IFR incidence were lower than the threshold value. Meanwhile, all measured parameters in both groups showed moderate CVs, except for the K/Ca ratio in leaf samples with IFR incidence, which showed a low CV. Moderate and low CV indicate moderate and low variations, respectively.

The comparison showed significant differences ($p < 0.05$) in all measured parameters between leaf samples with and without IFR incidence (Figure 2). The leaf samples with IFR incidence showed significantly higher N, P, K, and Mg contents than the leaf samples without IFR incidence. The N, P, K, and Mg contents in the leaf samples with IFR incidence were higher at 0.57%, 0.05%, 0.15%, and 0.27%, respectively, than those in the samples without IFR incidence. Meanwhile, the Ca content was lower in leaf samples with IFR incidence, at 2.05% lower than in those without IFR incidence. The significantly higher N/Ca and K/Ca ratios were observed in the samples with IFR incidence at 1.50 and 0.37, respectively, compared with the ratios in the sample without IFR incidence.

Table 3 shows the Pearson correlation coefficients for the measured parameters of leaf samples with and without IFR. A few significant correlations were observed among the measured parameters of leaf samples with IFR incidence compared to those without IFR incidence. The leaf samples with IFR incidence showed a strong positive correlation (R = 0.70–0.90) between the N/Ca and K/Ca ratios. A highly negative (R = -(0.70–0.90)) correlation was found between Ca and the N/Ca ratio and between Ca and the K/Ca ratio, which was also observed in the leaf samples without IFR incidence. A moderate negative (R = -(0.50–0.70)) correlation was found between Ca and Mg in leaf samples with IFR incidence, but the opposite situation was found in leaf samples without IFR incidence, which showed a moderate positive (R = 0.50–0.70) correlation between Ca and Mg. Different relationships

eISSN Number: 2462-2389

between Ca and Mg may be affected by nutrient imbalance in trees with IFR incidence. Besides that, the leaf samples with IFR incidence showed moderate positive correlations between N and P, and between K and P.

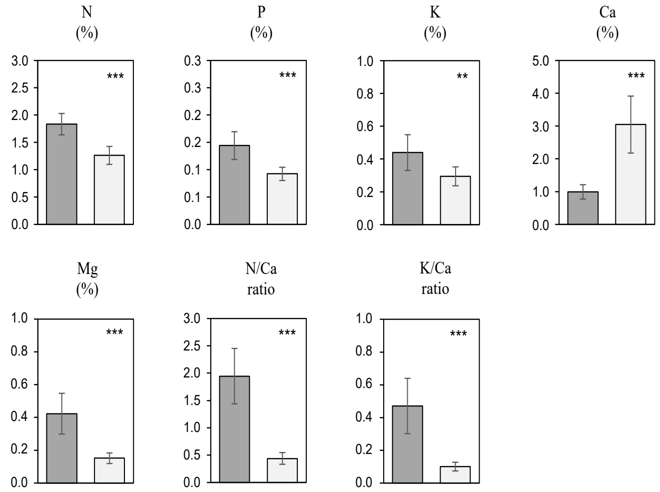

Figure 2: Comparison of leaf nutrient content with (dark grey) and without (light grey) insidious fruit rot incidences

Table 3: The Pearson correlation coefficient among the measured parameters for leaf samples with and without the insidious fruit rot (IFR) incidences.

	N	P	K	Ca	Mg	N/Ca ratio	K/Ca ratio
Harumanis leaves with IFR incidence (n = 10)							
N	1.00						
P	0.68*	1.00					
K	0.47	0.58*	1.00				
Ca	0.19	-0.05	0.39	1.00			
Mg	0.04	0.3	-0.41	-0.59*	1.00		
N/Ca ratio	0.24	0.37	-0.15	-0.89***	0.53	1.00	
K/Ca ratio	0.10	0.49	0.38	-0.70*	0.26	0.76**	1.00
Harumanis leaves without IFR incidence (n = 12)							
N	1.00						
P	0.53	1.00					
K	0.35	0.88***	1.00				
Ca	-0.35	-0.42	-0.48	1.00			
Mg	-0.79**	-0.8**	-0.70*	0.65*	1.00		
N/Ca ratio	0.55	0.44	0.42	-0.95***	-0.73*	1.00	
K/Ca ratio	0.30	0.69*	0.80**	-0.87***	-0.70*	0.82**	1.00

4. DISCUSSION

The optimum levels of leaf N, P, K, Ca, Mg, N/Ca ratio, and K/Ca ratio mentioned in this study were based on the guidelines by the Malaysian Agricultural Research and Development Institute (Sabdin, 2024) and the Queensland Department of Agriculture and Fisheries (QDAF, 2022). The optimum leaf N content of Harumanis is around the range of other Asian cultivars, 1.2%–1.4% (QDAF, 2022). Excessive N content in either leaves or soil, associated with internal disorders in mangoes, has been reported in many studies (Ullah et al., 2024; Kamarudin et al., 2023; Silber et al., 2022). Nitrogen is required to stimulate vegetative growth of mango (Silber et al., 2022) and increase fruit yield and quality (Asis et al., 2025). However, relatively high N levels may stimulate new flushes at the beginning of the fruiting stage, thereby reducing fruit yield (Silber et al., 2022). Therefore, reducing the frequency or temporarily stopping N fertiliser applications during certain growth periods may help limit excessive N uptake by Harumanis trees with IFR incidence, particularly when soil N levels are already moderate (Kamarudin et al., 2023).

Calcium is mainly taken up by the root system through the soil and transported to leaves and fruits via the transpiration stream (Ma et al., 2023). Relatively high N levels in mango trees may change the direction of Ca influx from the fruit to the high transpiration rates of young leaves (Ullah et al., 2024). Calcium uptake by mango fruit is often lower than required due to low transpiration during fruit development (Ullah et al., 2024; Ma et al., 2023). Calcium contributes to membrane stability and cell wall strength by cross-linking pectin and carboxylic groups (Ullah et al., 2024; Hocking et al., 2016). Therefore, relatively low Ca levels in mango leaves with IFR incidence, due to low soil Ca content (Kamarudin et al., 2023), may soften the cell walls and render the fruit susceptible to breakdown during post-harvest rigours and stresses (Ullah et al., 2024). Hofman and Whiley (2010) recommended maintaining leaf N and Ca concentrations at 1.0%–1.5% and 2.0%–3.5%, respectively, before flowering for optimum fruit quality, given that the N/Ca ratio is less than 0.5. To increase Ca content during the fruiting stage, Ca foliar applications can be made to the mango tree at an earlier stage to reduce internal fruit disorders (Bitange et al., 2020).

Phosphorus is a structural element in nucleic acids, and as a component of adenosine phosphates, plays an important role in energy transfer and is also essential for carbohydrate transport in leaf cells (Hawkesford et al., 2012). Potassium plays a unique role in stimulating starch accumulation, blooming, and fruit development (Farooq et al., 2014). However, increased K rates in plants can lead to an imbalance between Mg and Ca (Nahar et al., 2015). Meanwhile, Mg is a component of chlorophyll, and it is required for photosynthesis and protein synthesis (Hawkesford et al., 2012). A significantly higher concentration of P, K, and Mg was found in affected internal fruit disorders of Keitt and Beverly mangoes compared to healthy fruits (Ma et al., 2022; Burdon et al., 1991). Ma et al. (2023) also reported excess Mg in the spongy tissues (i.e., internal disorders) of Keitt mango in China. However, there have been inconsistent reports on the association of P, K, and Mg with internal disorders (Ullah et al., 2024). For instance, Andrade et al. (2022) reported Mg deficiency in the spongy tissues of Keitt mangoes in Brazil. Inconsistent reports on the association of P, K and Mg may be attributed to variations in management practices, soil fertility status, environmental conditions, plant growth stages, plant cultivars and/or sampling methods. Such variations can influence nutrient availability, uptake, and partitioning within the plant, reflecting the complex interactions of the soil–plant system.

However, a consistent trend of higher N/Ca and K/Ca ratios in fruit associated with internal disorders compared to healthy fruit has been observed (Ma et al., 2023; Andrade et al., 2022; Ma et al., 2022). These ratios are important parameters for monitoring the proportion and balancing of N to Ca and K to Ca and have been reported in previous studies (Ma et al., 2023; Tarmizi et al., 1993). Therefore, maintaining a balance between N and Ca, and between K and Ca, is important to achieve optimal fruit quality. The antagonistic effect of the accumulation of N and Ca in mango leaves and fruits has been well established (Ullah et al., 2024; Ma et al., 2023; Tarmizi et al., 1993). Tarmizi et al. (1993) reported that the N/Ca ratio in the Harumanis leaf was positively correlated with IFR incidence rather than N and Ca alone. An increase in leaf N would increase IFR incidence if leaf Ca remained unchanged (Tarmizi et al., 1993). In this study, the N/Ca ratio was significantly affected by higher N and lower Ca levels in the Harumanis orchard with IFR incidence. Therefore, monitoring the N/Ca ratio is important, and reducing or temporarily suspending N fertiliser application while increasing Ca fertiliser application may help reduce or minimise IFR incidence.

From a management perspective, these findings highlight the importance of adopting site-specific nutrient management strategies rather than relying on generalised fertiliser recommendations. Regular leaf and/or soil nutrient monitoring can help growers optimise fertilisation practices, maintain balanced nutrient supply, and potentially reduce the risk of nutrient-related fruit disorders.

5. CONCLUSION

In conclusion, there were significant differences in N, P, K, Ca, Mg, N/Ca ratio, and K/Ca ratio in the leaf samples with and without IFR incidence. Significantly higher N, P, K, Mg, N/Ca ratio, and K/Ca ratio (0.57%, 0.05%, 0.15%, 0.27%, 1.50, and 0.37) respectively, and a significantly lower Ca content (2.05%) were observed in leaf samples with IFR incidence. A high correlation was found between Ca and the N/Ca ratio, and between Ca and the K/Ca ratio, in leaf samples with IFR incidence. To further this study, it is suggested that leaf micronutrients, such as copper, boron, molybdenum, manganese, and zinc, be studied.

ACKNOWLEDGMENTS

Special thanks to Mr Ramli Abu Seman and Mr Hasni Halim for their permission to conduct this study in their mango orchard and for the information they shared with the authors. The authors extend their gratitude to Dana Pembudayaan Penyelidikan Dalaman (600-UiTMPs (PJIM&A/PI-DPPD 08), Universiti Teknologi MARA Perlis Branch, Arau Campus, Malaysia, for the funding support. The authors also extend their gratitude to the students for their assistance during the field survey and to Universiti Teknologi MARA Perlis Branch, Arau Campus, for the facilities.

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