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IMPACT OF FUNGAL ANTHRACNOSE ON MICROMINERAL CONCENTRATION IN GUAVA (*PSIDIUM GUAJAVA* L.): A COMPARATIVE STUDY ACROSS DISTRICTS OF PUNJAB, PAKISTAN

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Abstract

The present study was carried out to assess the differences in the concentration of two metals in various guava varieties affected by the fungal disease, Anthracnose. The study was carried out in five districts of Punjab, namely Sargodha (S1), Jhang (S2), Sheikhupura (S3), Sahiwal (S4), and Faisalabad (S5). Fruit samples were collected from both healthy and diseased plants of each variety, with five replicates, amounting to a total of 150 samples. The metal concentrations were quantified using Atomic Absorption Spectrophotometry. The concentration of zinc in healthy fruits varied from 0.212 to 0.256 mg/100g, but in diseased fruits varied from 0.136 to 0.218 mg/100g. Similarly, the value of iron in healthy fruits wavered between 0.212 To 0.28 mg/100g of fruit and that of affected fruits between 0.15 and 0.25 mg/100g of fruit. The range of manganese in healthy fruits is from 0.118 to 0.214 mg/100g of fruit and in anthracnose affected fruits this range was from 0.078 to 0.144 mg/100 g of fruit. These findings clearly suggest the reduction of the metal content in the diseased guava fruits.

Key words: Guava; Anthracnose; Zinc, Iron; Manganese; Fungal disease

Introduction

Fruits distinguished among our consumables are known for their nutritional value, which is significantly higher compared to other food items in terms of essential minerals and vitamins (Khan *et al*., 2021). *Psidium guajava*, commonly known as guava, is a tropical fruit tree from the *Myrtaceae* family, thriving in regions like South Asia, China, and Central America (Kumar *et al*., 2021). Guava is known as the "Poor Man's Apple of the Tropics" and is rich in key minerals, making its leaves a valuable source of nutrition for humans and animals (El-Feky & Abdelrehman, 2025). Pakistan, with an annual production of 938 thousand tons, is the fourth-largest guava producer in the world (Anon., 2023).

Disease and pest infections have a significant impact on guava quality and nutritional value. Guava is attacked by 10 diseases, with anthracnose, caused by *Colletotrichum gloeosporioides*, being the second most important (Zakaria, 2021; Guan *et al*., 2022). This common disease causes discoloration, rotting and loss of nutrients in fruits (Begum *et al.,* 2022). The disease starts with small, brown spots on fruits that grow into bigger, sunken spots surrounded by acervuli and conidia (Mohammed *et al.,* 2022). The complexity of anthracnose lies in the presence of various species of *Colletotrichum*, genetic variability, and environmental factors (Bano *et al*., 2023) (Fig. 1).

The micronutrients move into the root cells through apoplastic or symplastic transport and are carried by phloem sap to the vegetative and reproductive parts, with zinc (Zn) showing high mobility (Holland *et al.,* 2021; Stanton *et al*., 2022). Zn is an essential element in the synthesis of chlorophyll, activation of enzymes, and biosynthesis of auxin and its deficiency causes chlorosis and poor root growth (Mahmood *et al*., 2021; Byrne & Murphy, 2022; Zhao *et al*., 2022). Sodium (Na^+), potassium (K^+), and magnesium (Mg^{2+}) are also important for solute balance (Ghafar *et al*., 2022; Ahmad *et al*., 2024).

Limited research on fungicides and the lack of coordination between policymakers and researchers make the situation worse (Prusky & Romanazzi, 2023; Haile, 2025). Decline of guava production in Pakistan, particularly in Punjab, has been reported by Abbas *et al*., (2021) and Shah *et al*., (2022). It is well known that anthracnose infections lower internal fruit quality characteristics, such as biochemical and physicochemical parameters that are vital for human health and market value, in addition to visual quality. The nutritional effects of fungal infection in tropical fruits like guava are still not well understood despite of the fact that a large portion of current research has been concentrated on temperate and subtropical fruits like mango, citrus, and berries (Law *et al*., 2025).

Notwithstanding this expanding corpus of research, there is little quantitative data on micronutrient depletion (such as zinc, iron, and magnesium) in guava fruits in South Asian agro-ecosystems, especially Punjab, Pakistan, where anthracnose is present (Silva *et al*., 2025). Given that guava is a staple fruit with significant nutritional value for the local population, this knowledge gap is especially significant. To close this gap, the current study of micronutrient decline in anthracnose-affected guava across major production districts of Punjab was undertaken.

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There are a number of studies that reported decline in guava due to anthracnose but they have focused on fungal identification and in-vitro control of the disease (Ansar *et al*., (1994), Bokhari *et al*., (2008), Haq *et al*., (2013), and Abbas *et al*., (2014). These surveys emphasized the damage to orchards but pointed out that there was no information available on the sensitivity of the Round (Gola) and Pyriform (Surahi) guava strains. This dearth of data regarding these two strains made the control of disease difficult (Fig. 1). It is essential to fill this knowledge gap to develop resistant varieties and improve disease control measures in guava-growing areas of Pakistan.

Materials and Methods

Selection of site: Five? different districts in Punjab were included in the current investigation: Sargodha (S1), Jhang (S2), Sheikhupura (S3), Sahiwal (S4), and Faisalabad (S5). Samples from three? distinct diseased guava varieties—Gola sufaid (V1), Chittidar (V2), and Surahi (V3)—were collected in the spring of 2021 and 2022. The selection of these five districts was made in a way that it encompasses different farming practices and soil types, which enable a thorough examination of the effects of anthracnose on the micronutrient content of guava fruits. The regions are high guava producers and diverse in terms of farming practices that may be influencing the intensity of the disease and the depletion of nutrients in guava.

Collection of samples: For every variety, ten samples were taken, five from diseased plants and five from healthy plants (Fig. 2). A thorough comparison between infected guava plants and healthy plants were made possible by this sampling technique. All of the samples were then taken to the University of Sargodha's Department of Botany for additional, in-depth analysis and inspection.

Treatment of samples: Ten grams of each sample were first allowed to air dry before being oven-dried at a temperature of roughly 70 to 75°C. To eliminate all of the moisture, the samples were kept in an oven at a constant temperature for 7 days. Completely dried samples were ground into a fine powder using a mortar and pestle before being sieved. Following sample digestion, the total metal contents in guava samples were examined. About 2.5 mL of nitric acid, 0.5 mL of 30% hydrogen peroxide, and 7.5 mL of hydrochloric acid were used (Ashfaq *et al*., 2022). A calibration curve was created by examining a reference metal solution for sample inspection. Following wet digestion, samples were subjected to mineral analysis using the Perkin-Elmer AAS-5000 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp, 1980). The use of such complex instruments and precise methodologies ensured accurate and detailed mineral profiling of the plant samples.

Statistical analysis: In line with the methodology outlined by Steel *et al*., (2006), the statistical analysis of the data acquired using the aforementioned methods involved the application of Three-way Analysis of Variance (ANOVA) using the SPSS software. The Least Significant Difference (LSD) test was used after the ANOVA results. With the help of this extra post-hoc test, particular group differences could be examined in greater detail, providing information about the significance of observed variations in the data that was gathered. ANOVA and the LSD test were used in tandem to provide a thorough statistical evaluation of the experimental results.

Results and Discussions

Zinc (Zn^{2+}): The analysis of variance showed that zinc values were significantly ($p<0.05$) affected by site, variety, treatment, site x variety, site x treatment and site x variety x treatment (Table 1). The concentration of zinc in healthy fruit ranged from 0.212 to 0.256 mg/100g and that of disease affected fruits from 0.136 to 0.218 mg/100g. The healthy fruits of V1 collected from site 4 were with the highest zinc concentration and the diseased fruits of V1 from site 1, V2 from site 2 and V3 from site 4 were with the lowest concentration of zinc (Fig. 3; Table 2).

The LSD All-Pairwise Comparisons Test offers comprehensive information on zinc (Zn) concentrations in various crop types, treatments, and agricultural locations. It highlights the necessity of site-specific zinc management by separating the sites into three different groups. In a similar vein, crop varieties were divided into two categories, highlighting the significance of variety selection in affecting zinc levels. Two groups were formed as a result of the significant effects of treatment practices. The test also highlighted the complex relationships between these factors by identifying 6 groups from sites and treatments and 5 groups from the interaction between sites and varieties. Finally, the three-way interaction among sites, varieties, and treatments resulted in nine homogeneous groups, offering a comprehensive understanding of how these elements shape zinc dynamics and guiding tailored agricultural strategies.

Iron (Fe^{2+}): According to the results obtained by analysis of variance, site, variety, treatment, site x variety and site x treatment, variety x treatment and site x variety x treatment significantly ($p<0.05$) affected the values of iron in different samples of healthy and anthracnose affected guava fruits (Table 1). The value of iron in healthy fruits ranged between 0.212 To 0.28 mg/100g of fruit and that of affected fruits between 0.15 and 0.25 mg/100g of fruit. The highest value was recorded from fruits of V3 from site 5 and the lowest from disease affected fruits of V1 obtained from site 4 (Fig. 4; Table 2).

The LSD All-Pairwise Comparisons Test for iron (Fe) content showed that site, crop variety, and treatment significantly affected Fe levels in agriculture. Sites, varieties, and treatments were clearly separate groups, and this underlined the importance of developing specific management practices. Variations in site (three groups), variety (three groups), and treatment (two groups) illustrated the importance of choosing the appropriate combination for optimal Fe management. Interactions between sites, varieties, and treatments also showed specific relationships, and the most complex analysis showed 15 groups, and this underlined the importance of accurate site-specific management of Fe.

Manganese (Mn^{2+}): The analysis of variance showed that manganese values were significantly ($p<0.05$) affected by site, variety, treatment, site x variety, site x treatment, variety x treatment and site x variety x treatment (Table 1). The range of manganese in healthy fruits was from 0.118 to 0.214 mg/100g of fruit and in anthracnose affected fruits it ranged was from 0.078 to 0.144 mg/100 g of fruit with the lowest value recorded from diseased V1 fruits collected from site 2 and the highest value was from healthy V2 fruits from site 5 (Fig. 5; Table 2).

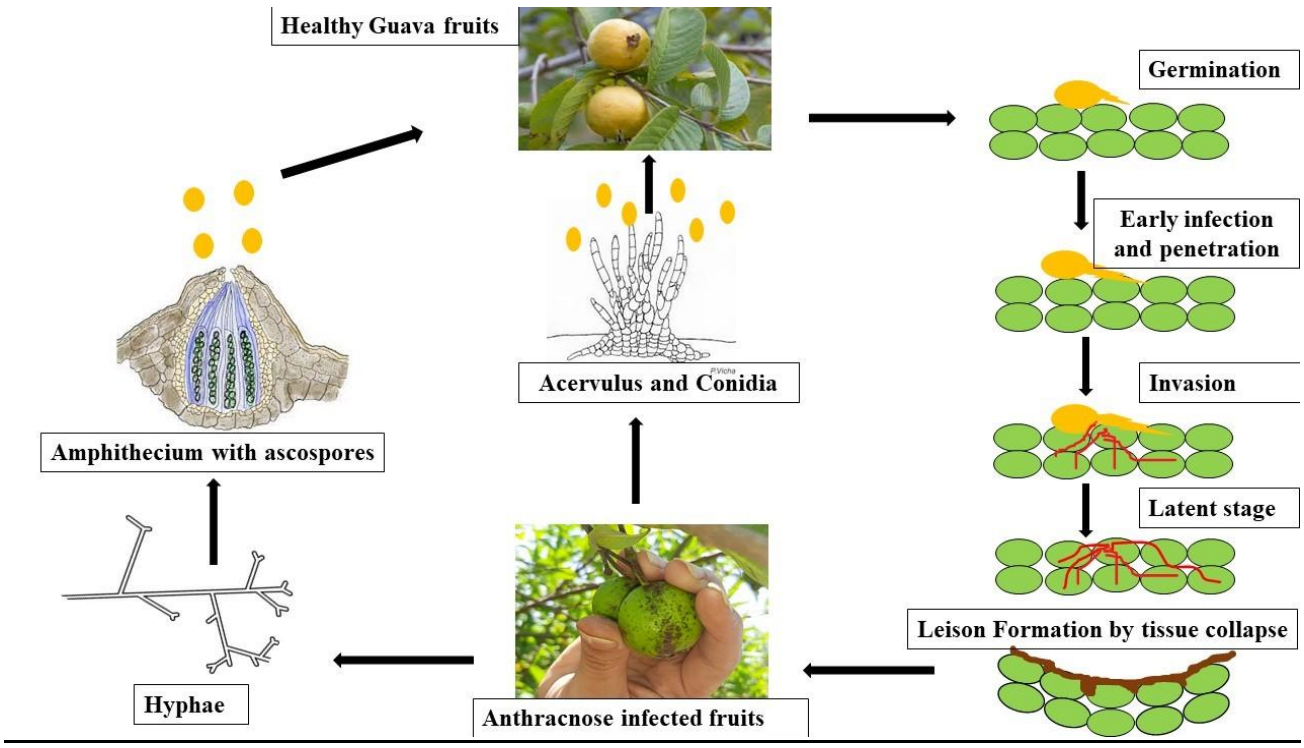

Fig. 1. Mechanism of anthracnose disease development in guava.

Fig. 2. Geographic locations and collection points of guava samples from five districts of Punjab, Pakistan: Sargodha (S1), Jhang (S2), Sheikhupura (S3), Sahiwal (S4) and Faisalabad (S5).

Table 1. Analysis of variance for metal concentrations of healthy and anthracnose affected guava fruits.

Metals	Site	Variety	Treatment	Site x variety	Site x Treatment	Variety x Treatment	Site x Variety x Treatment	Error
Df	**4**	**2**	**1**	**8**	**4**	**2**	**8**	**120**
Zn	0.00249***	0.00193***	0.15424***	0.00500***	0.00040**	0.00029ns	0.00152***	0.00012
Fe	0.00498***	0.01542***	0.05442***	0.00389***	0.00972***	0.00393***	0.00262***	0.00010
Mn	0.00568***	0.00252***	0.11103***	0.00377***	0.00031*	0.00049**	0.00024**	0.00007

***=Significant at 0.001 levels, **=Significant at 0.01 levels, *=Significant at 0.05 levels, ns=Non-significant at above 0.05 levels

Table 2. Mean values of Na^+ and Zn^{2+} in different varieties of healthy and anthracnose affected guava fruits.

Sites	Varieties	Treatment	Zn (Mean)	Zn (Max.)	Zn (Min.)	Fe (Mean)	Fe (Max.)	Fe (Min.)	Mn (Mean)	Mn (Max.)	Mn (Min.)
Site 1	V1	Healthy	0.228	0.25	0.21	0.228	0.24	0.21	0.144	0.15	0.13
		Diseased	0.136	0.14	0.12	0.158	0.17	0.15	0.106	0.12	0.09
	V2	Healthy	0.222	0.24	0.21	0.27	0.28	0.26	0.168	0.18	0.16
		Diseased	0.159	0.175	0.13	0.2	0.21	0.19	0.118	0.13	0.11
	V3	Healthy	0.226	0.24	0.21	0.242	0.26	0.23	0.17	0.18	0.15
		Diseased	0.168	0.19	0.15	0.218	0.23	0.21	0.098	0.11	0.08
Site 2	V1	Healthy	0.25	0.26	0.24	0.262	0.27	0.25	0.118	0.13	0.11
		Diseased	0.202	0.21	0.19	0.224	0.24	0.21	0.078	0.09	0.07
	V2	Healthy	0.228	0.25	0.21	0.234	0.25	0.22	0.165	0.175	0.16
		Diseased	0.136	0.15	0.12	0.25	0.26	0.23	0.116	0.13	0.11
	V3	Healthy	0.242	0.26	0.23	0.178	0.19	0.17	0.136	0.14	0.13
		Diseased	0.218	0.23	0.21	0.2786	0.29	0.26	0.084	0.09	0.07
Site 3	V1	Healthy	0.224	0.24	0.21	0.216	0.23	0.21	0.1872	0.196	0.18
		Diseased	0.159	0.175	0.13	0.163	0.17	0.15	0.13	0.14	0.12
	V2	Healthy	0.254	0.26	0.25	0.274	0.28	0.26	0.16	0.17	0.15
		Diseased	0.212	0.22	0.2	0.22	0.23	0.21	0.114	0.12	0.11
	V3	Healthy	0.226	0.23	0.21	0.224	0.23	0.21	0.166	0.17	0.16
		Diseased	0.148	0.17	0.14	0.159	0.175	0.13	0.118	0.13	0.11
Site 4	V1	Healthy	0.256	0.27	0.25	0.212	0.22	0.2	0.164	0.17	0.16
		Diseased	0.2	0.21	0.19	0.15	0.16	0.14	0.118	0.13	0.11
	V2	Healthy	0.222	0.23	0.21	0.25	0.26	0.24	0.142	0.15	0.13
		Diseased	0.154	0.16	0.14	0.202	0.21	0.19	0.086	0.09	0.08
	V3	Healthy	0.228	0.25	0.21	0.23	0.24	0.22	0.17	0.18	0.15
		Diseased	0.136	0.15	0.12	0.182	0.19	0.17	0.098	0.11	0.08
Site 5	V1	Healthy	0.246	0.25	0.24	0.212	0.22	0.2	0.15	0.16	0.14
		Diseased	0.166	0.17	0.16	0.17	0.18	0.16	0.09	0.1	0.08
	V2	Healthy	0.212	0.22	0.2	0.25	0.26	0.24	0.214	0.22	0.21
		Diseased	0.15	0.16	0.14	0.192	0.2	0.18	0.144	0.15	0.14
	V3	Healthy	0.212	0.22	0.2	0.28	0.29	0.27	0.184	0.19	0.18
		Diseased	0.17	0.18	0.16	0.224	0.23	0.22	0.124	0.13	0.11

Table 3. Heat map for metal concentrations in healthy and anthracnose affected guava fruits.

Fe	Zn	Mn
0.228	0.228	0.144
0.158	0.136	0.106
0.27	0.222	0.168
0.2	0.159	0.118
0.242	0.226	0.17
0.218	0.168	0.098
0.262	0.25	0.118
0.224	0.202	0.078
0.234	0.228	0.165
0.25	0.136	0.116
0.178	0.242	0.136
0.2786	0.218	0.084
0.216	0.224	0.1872
0.163	0.159	0.13
0.274	0.254	0.16
0.22	0.212	0.114
0.224	0.226	0.166
0.159	0.148	0.118
0.212	0.256	0.164
0.15	0.2	0.118
0.25	0.222	0.142
0.202	0.154	0.086
0.23	0.228	0.17
0.182	0.136	0.098
0.212	0.246	0.15
0.17	0.166	0.09
0.25	0.212	0.214
0.192	0.15	0.144
0.28	0.212	0.184
0.224	0.17	0.124

Manganese toxicity varies depending on the plant component. The key elements affecting manganese toxicity are environmental factors and plant species. Manganese toxicity is more prevalent in plants grown in acidic soil. Manganese toxicity causes a reduction in plant growth, brown spots to appear on adult leaves and chlorotic dots to appear on the tips of young leaves (Skórka *et al*., 2023). According to Delfim *et al.,* (2022), the guava fruits contain enough of the micronutrients Cu, Fe, Mn and Zn for human daily needs.

The LSD All-Pairwise Comparisons Test for manganese (Mn) concentration showed that sites, crop varieties, and treatments were significant factors in determining Mn levels in agriculture, and there were homogeneous groups for each factor. This is an important aspect because it shows that agricultural practices should be tailored to specific sites, crop varieties, and treatments to manage Mn levels. The LSD All-Pairwise Comparisons Test not only provides information on how to manage Mn levels but also tells a story about the agricultural environment, and agricultural practitioners should be storytellers in managing manganese levels for a smooth agricultural performance.

Concentration of metals in healthy and anthracnose affected guava fruits of three varieties at five sites has been presented in the form of a heat map. The maximum values are represented as red and the lowest as green (Table 3).

Due to dual significance in plant physiology and human nutrition, zinc (Zn), iron (Fe), and manganese (Mn) were chosen for the current study among the essential micronutrients found in guava. Iron is essential for human oxygen transport and plant chlorophyll synthesis, while zinc is essential for immune system function, gene

expression, and enzyme activation (Arinaitwe *et al*., 2026). Manganese plays a role in metabolic regulation, antioxidant defense systems, and photosynthetic processes. In addition to their nutritional importance, these micronutrients are intimately linked to stress reactions and plant defense systems. Zn, Fe, and Mn are sensitive biochemical markers of disease-induced changes in fruit nutritional quality because fungal infections can interfere with mineral uptake, translocation, and metabolic allocation within host tissues (Sun *et al*., 2025).

The fungus species *Colletotrichum gloeosporioides* causes anthracnose in guava, affects the nutritional value of the fruit through various routes. The disease mainly affects the fruit, causing lesions that reduces the physiological processes of the plant. The fungus competes with the plant for nutrients such as zinc, iron and manganese, resulting in the reduction of these micronutrients in the fruits (Zakaria, 2021).

The notable differences in Zn, Fe, and Mn concentrations between districts raise the possibility that micronutrient dynamics are impacted by site-specific soil chemistry and orchard management techniques. The pH of Punjabi soils varies greatly and is primarily calcareous, which significantly affects the solubility of micronutrients. By lowering their solubility and mobility in the rhizosphere, high $CaCO_3$ and elevated soil pH lower the bioavailability of iron and zinc (Mishra *et al*., 2025). Fe solubility can be significantly reduced by even small increase in soil pH, which limits the amount of Fe that plant roots can absorb. These effects might be amplified even more by orchard management techniques. Both nutrient availability and the severity of anthracnose may be impacted by variations in fertilization schedules, foliar micronutrient sprays, irrigation frequency, canopy density, and disease control techniques. *C. gloesporioides* growth is favored by higher humidity and inadequate canopy aeration, which may raise fungal nutrient demand and hasten micronutrient depletion (Rusu *et al*., 2025).

Guava contains a fair amount of zinc alongwith other microminerals (Moreira *et al*., 2023). The presence of zinc in guava was also reported by Ndoro *et al*., (2023), but their reported concentration (mean= 0.08mg/100g) was far less as compared to the present study. The results obtained in the present study coincide with that of Bogha *et al*., (2020). The values (0.94 mg/100g) reported by Muñoz-Arrieta *et al*., (2021) were higher as compared to the present study.

Their higher concentration may be due to the high nutritional profile of Costa Rican guava. As reported in the present investigation, the concentration of zinc is declined when plant is infected by *C. gloeosporioides* which causes fungal anthracnose disease. However, no similar study was found in the earlier literature. According to Kumar *et al*., (2025), Zn performs numerous key activities in plants, including photosynthesis, membrane structure, gene expression and regulation, lipids and nucleic acid metabolism, phytohormone action, protein synthesis and drought and disease resistance. The primary causes of the Zn level in plants being low are manganese and phosphorus. Zn deficiency has a significant negative impact on fruit growth because it decreases plant growth and yield (Gentile *et al*., 2022). Zn is present in calcareous soil but not available to plants, alkaline calcareous soil reduces the availability of Zn to crops from soil (Pinar, 2023).

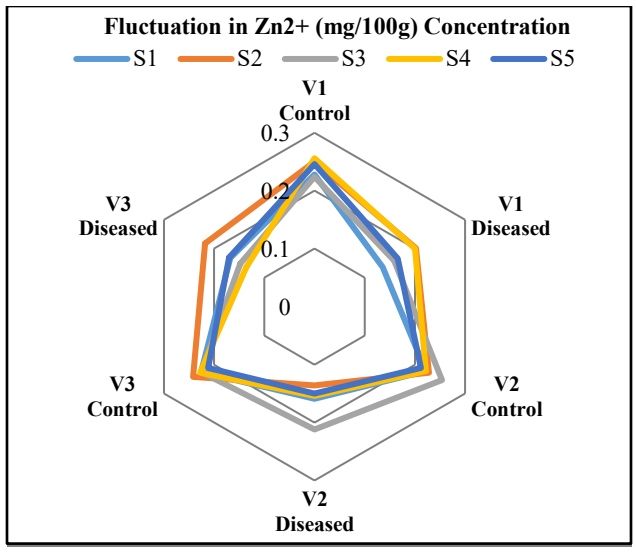

Fig. 3. Mean concentration of zinc in healthy and anthracnose affected guava fruits of different varieties.

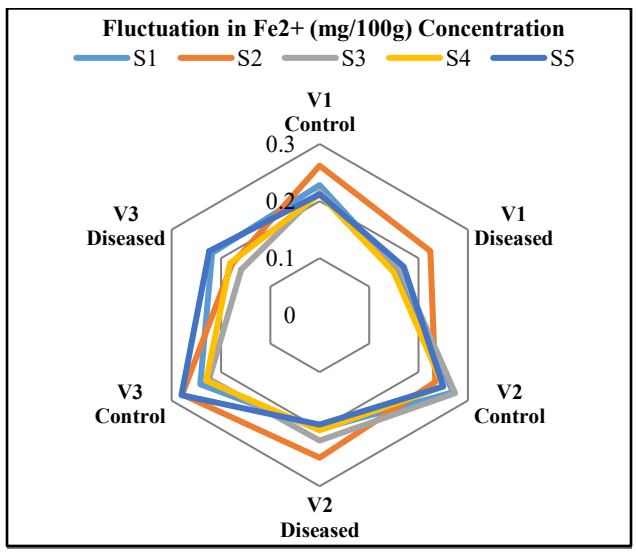

Fig. 4. Mean concentration of Iron in Healthy and anthracnose affected guava fruits of different varieties.

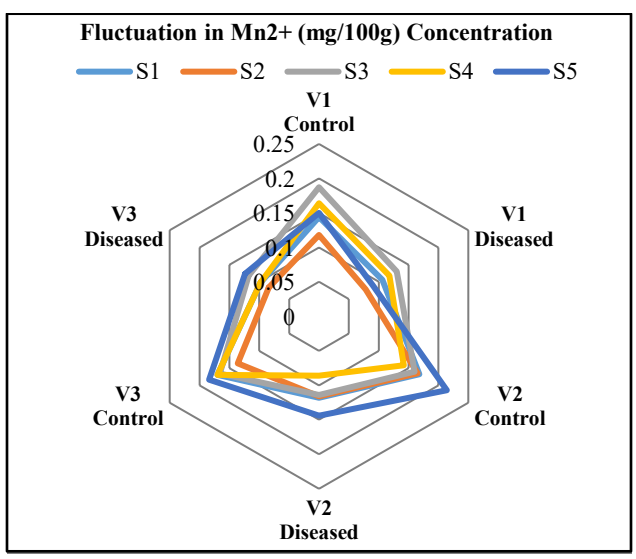

Fig. 5. Mean concentration of manganese in healthy and anthracnose affected guava fruits of different varieties heat map description.

The presence of iron alongside other microminerals in guava is reported by a number of studies including Bogha *et al*., (2020) and Kumari *et al*., (2020). The concentration of iron reported in the present study was in close concordance with those of Bogha *et al.,* (2020) and lower compared to the values reported by Kumari *et al*., (2020). This difference may be due to ecological, climatic, fertilizing, genetic, irrigation and soil condition differences. The cultivar under study also adds up to the difference. The onset of anthracnose disease decreases the concentration of iron in fruits due to the requirement of minerals by fungus.

Only the soil in which plants grow can cause a Fe deficiency in plants. The high levels of calcium and bicarbonate are to blame for the soil's lack of iron. Because a single unit increase in pH can 1000-fold decrease the solubility of iron, high levels of $CaCO_3$ and pH may also contribute to reduced iron availability to plants (Alharbi *et al*., 2022). Young leaves show as the first evidence of a shortage since Fe is immobile in plants. Chlorosis is another effect of Fe shortage in early leaves. Interveinal chlorosis is a minor iron shortage that might be mistaken for a manganese deficiency (Merry *et al*., 2022). Plants experience a lack of Fe despite the fact that it is abundant in soil due to its poor solubility (Liang, 2022).

Manganese is considered among the important micronutrients found in guava fruits (Irshad *et al*., 2020; Tadeka *et al*., 2023). The concentration of Mn found in the present study was in concordance with the results of Pandey *et al.,* (2023) and lower in comparison with the values reported by Muñoz-Arrieta *et al*., (2021). There was a significant decline in the amount of manganese when fungus attacked the fruits. However no earlier studies in support of this decline could be found. It is important to infer from the present study about the antagonistic effect of Ca and Mg on Mn uptake, which probably happened in the present experiment (Saha & Pathak, 2021).

Manganese availability to plants is influenced by plant species, manganese's interactions with other metals and the chemical composition of the soil. Mn toxicity varies depending on the plant component. The main determinants of Mn toxicity are environmental factors and plant type. Manganese toxicity is more prevalent in plants growing in acidic soil. Manganese toxicity causes a reduction in plant growth appearance of brown spots on adult leaves and chlorotic dots on the tips of young leaves (Skórka *et al*., 2023). Manganese uptake in plants and is much lower than that of calcium and magnesium. Due to the detrimental effects of pH and Ca, magnesium and lime in soil produce a decrease in the uptake of manganese by plants (Sharma *et al*., 2024).

The way that manganese interacts with iron affects manganese uptake as well. High concentrations of Fe inhibit Mn accumulation and availability to plants, while high Mn levels in soil restrict the availability of Fe to plants (Lilay *et al*., 2024). Magnesium deficit is extremely susceptible to chloroplast. Manganese deficiency has a significant and irreparable impact on the chloroplast's structure. Due to manganese shortage, photosynthesis activity and chlorophyll content are also disrupted (Fiorentini *et al*., 2021).

Conclusion

In five districts of Punjab under study, anthracnose infection in the guava fruits has dramatically decreased the amounts of Zn, Fe and Mn. This decreases correlates that the disease affects the fruit's nutritional value in addition to yield and appearance. Such decreases could affect consumer health and food security because guava is a significant dietary source of micronutrients. The results emphasize the necessity of creating and promoting guava cultivars resistant to anthracnose that could preserve nutritional stability in the face of pathogen stress. To maintain the nutritional and financial worth of Punjab's guava industry, regional agricultural policies and integrated disease management must be strengthened.

Future Prospects: The incorporation of genetic traits that impart resistance to *Colletotrichum gloesporioides* could aid in lessening the severity of the disease damage and hence will maintain the yield and quality of the fruit. Site-specific approaches to soil management, such as the use of soil acidifiers, micronutrient application or organic matter amendment could aid in countering the negative effects of soil chemistry on plant health and disease development specifically in fruits like guava.

Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

Author's Contribution: The study conception and design were laid out by Kafeel Ahmad and Zafar Iqbal Khan. Material preparation, data collection and analysis were performed by Sonaina Nazar. Humayun Bashir, Muhammad Adeel Ghafar and Muhammad Akram performed the statistical analysis and prepared the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials: Data and material are available for research purpose and for reference.

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