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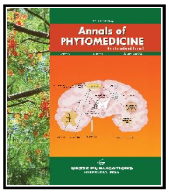

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Effect of foliar micronutrients on the phytochemical composition of the Allahabad Safeda Guava

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Abstract

This study investigated foliar applications' impact of micronutrients on the guava fruits quality. The experiment was conducted in a guava orchard of Lovely Professional University, Agriculture Farm, in Punjab, India, using the Allahabad Safeda cultivar. Seven treatments were applied, including different combinations of boric acid, zinc sulfate, copper sulfate, and calcium chloride, while the control treatment received water only. Different quality parameters such as antioxidant activity, total sugars, total soluble solids (TSS), non-reducing sugars, ascorbic acid, reducing sugars, and titratable acidity were evaluated. The outcomes revealed that the micronutrient treatments had a significant impact on these quality attributes. Treatment T_5, which consisted of boric acid (0.5%), zinc sulfate (0.5%), and calcium chloride (2%), demonstrated the highest TSS content, as well as increased non-reducing sugars, antioxidant activity, reducing sugars, total sugars, and ascorbic acid compared to other treatments. The findings suggest that the foliar application of micronutrients, particularly boric acid (0.5%), zinc sulfate (0.5%), and calcium chloride (2%), can enhance the quality characteristics of guava fruits.

1. Introduction

Guava (*Psidium guajava* L.) is a highly valued fruit known for its delicious flavor, nutritional value, and pectin content (Rawat *et al*., 2010). It is extensively used in the processing industry to produce various products such as fruit butter, jelly, jam, canned goods, toffee, cheese, and guava nectar are some examples of sweets. Guava comes from tropical America but is used commercially and grown in several countries including Brazil, China, Cuba, Hawaii, India, Malaysia, and Mexico. In India, Maharashtra, Uttar Pradesh, Madhya Pradesh, and Bihar are major guava-producing states (Yadav *et al*., 2014). Guava is the sixth most widely cultivated fruit in terms of land area, following papaya, mango, banana, citrus, and apple. India has been cultivating guava since the 17th century. It covers an area of 308,000 hectares in India, with an annual production of 4,582,000 metric tons. Guava also holds a significant position in the Brazilian export market for fruits (Delfim *et al*., 2022). High concentrations of polyphenols and flavonoids in guava are linked to strong antioxidant activity (Pandhi *et al*., 2022).

Micronutrients, including zinc, boron, copper, and calcium are essential for the development and growth of plants. An inadequate supply of these micronutrients can lead to yield limitations and reduced fruit quality in various fruit crops. Micronutrient deficiencies have become a concern in recent years due to widespread soil deficiencies caused by intensive cultivation and insufficient use of organic manure (Dhurve and Maske, 2018; Sau *et al*., 2018). Foliar spray, which involves applying fertilizers directly to the leaves, is an effective technique to ensure prompt nutrient absorption and utilization by plants. It helps to correct nutrient deficiencies, replenish disrupted nutrient supplies, mitigate stress factors, and improve fruit quality and productivity (Yadav *et al*., 2017; Meena and Verma, 2022). Zinc is important for growth stimulation and plays a vital role in various physiological processes. Zinc deficiency in plants can result from low soil zinc content, competition with other nutrients, and soil properties affecting zinc availability. Foliar application of zinc is preferred to correct deficiencies in fruit crops (Haleema *et al*., 2018; Swietlik *et al*., 2001).

Boron is crucial for cell wall synthesis and structural integration in plants. It plays a key role in proper fruit setting and retention percentages. Boron deficiency can lead to lower yields and a decline in crop quality. Boric acid and borax applications have shown favourable impacts on numerous crops' fruit output, quality, yield, and growth (Al-Obeed *et al*., 2018; Bibi *et al*., 2019). Copper is involved in biological processes such as lignin formation, photosynthesis, enzyme functioning, and seed development. Excessive accumulation of copper in cells can lead to various problems. Copper deficiency in plants can result in dieback, chlorosis, and other symptoms. Copper plays a crucial role in various biological processes and serves as a critical cofactor for certain metalloproteinases. Foliar application of copper can help overcome copper deficiencies and promote plant health (Meena and Verma, 2021). Plants require an adequate amount of calcium in soils to support their growth and development. Calcium is a necessary nutrient that is required for the structural, metabolic, and physiological processes of plants. It has significant effects on fruit quality, including texture, storability, and shelf-life. Calcium deficiency can result in

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disorders such as blossom end rot and bitter pit in fruit crops. Foliar application of calcium can help mitigate calcium deficiencies and improve fruit quality.

2. Materials and Methods

The trial was conducted in a guava orchard at Lovely Professional University in Phagwara, Punjab, India, over an 8-month period from June 2022 to January 2023. The experiment utilized the Allahabad Safeda cultivar, known for its upright growth habit, medium to tall size, dense foliage, and high-quality fruits. The climate in Punjab is subtropical, allowing guava to be grown year-round, but the best quality is obtained during Mrig bahar (June-July). Fruiting occurs during the winter (Oct-Dec). The guava orchard was irrigated through drip irrigation, and mechanical weeding was carried out to keep a weed-free environment. A plant protection strategy was implemented, as needed to address pests. The experiment followed a three-replication randomized block design, with trees spaced at 6 m × 6 m.

The study's goal was to determine micronutrient foliar treatments' effects on guava quality during the Mrig bahar crop, with fruit harvesting in December. The experiment included seven treatments: T_1 (water), T_2 (boric acid 0.5% + calcium chloride 2%), T_3 (zinc sulfate 0.5% + calcium chloride 2%), T_4 (copper sulfate 0.5% + calcium chloride 2%), T_5 (boric acid 0.5% + zinc sulfate 0.5% + calcium chloride 2%), T_6 (zinc sulfate 0.5% + copper sulfate 0.5% + calcium chloride 2%), and T_7 (copper sulfate 0.5% + boric acid 0.5% + calcium chloride 2%). The control treatment received no application. Data collection included parameters such as antioxidant activity, titratable acidity, ascorbic acid, total sugars, reducing sugars, and non-reducing sugars are some of the terms used to describe total soluble solids (TSS). Statistical analysis using ANOVA and Tukey's test was performed on the collected data.

2.1 Total soluble solids (°Brix)

Fruits from each treatment were randomly selected and crushed to extract the juice using a pestle and mortar. Using an Erma hand refractometer, the total soluble solids (TSS) of the juice were calculated., which measures Brix values in the 0-32° range. The TSS (°Brix) of the fruit juice was calculated at a temperature of 20°C.

2.2 Titratable acidity (%)

Using phenolphthalein as an indicator, 0.1N NaOH solution was titrated with 5 ml of fruit juice. The titration's endpoint was signaled by the emergence of a pink color. Citric acid content was then calculated as a percentage of 100 g of fruit pulp. This formula was used to calculate the acidity:

$$\text{Total acid} = \frac{\begin{array}{c}\text{(Titration value) x (Normality of NaOH)}\\ \text{x (Volume made up to 100 ml) x 64 x 100}\end{array}}{\begin{array}{c}\left(\text{Aliquot of extract taken for estimation}\right)\\ \text{x}\left(\text{Weight of sample}\right)\text{x 1000}\end{array}} \times 100$$

2.3 Ascorbic acid (mg 100 g^{-1})

To ascertain the level of ascorbic acid in the fruit, 5 g of fruit pulp were ground with 3.0% metaphosphoric acid serving as a buffer. The resulting extract was filtered, and a 100 ml remedy was made. A 5 ml portion of this solution was titrated against a 2,6-dichlorophenol dye until a bright pink color appeared. The outcomes were disclosed in milligrams of per 100 g of fruit pulp of ascorbic acid.

The calculation for the concentration of ascorbic acid is as follows:

$$\text{Ascorbic acid} = \frac{\begin{array}{c}\text{Titration value} \times \text{Dye factor} \times\\ \text{Volume made up to 100 ml}\end{array}}{\begin{array}{c}\text{Aliquot taken} \times\\ \text{Weight of sample taken for estimation}\end{array}} \times 100$$

2.4 Total sugars (%)

In a conical flask, 10 ml of juice, 100 mL of distilled water, and 2 ml of 45% lead acetate were combined. After two days, 1.9 ml of potassium oxalate (22%), along with 250 ml of distilled water, were added. 5 ml of strong HCl was added to 50 ml of this sample, and it was let to stand for 24 h. The sample was neutralized the following day with 40% NaOH to produce a pale pink color, and the total volume was changed to 100 ml. Fehling's solutions A and B, 2.5 ml each, were added to another conical flask and diluted with 50 ml of distilled water. On a hot plate, the liquid was brought to a boil. Methylene blue indicator was then added in little amounts. The juice sample in the burette was used for the titration, with the appearance of a brick red color being the endpoint. The following formula was used to get the total sugar content (%):

$$\text{Total sugars (\%)} = \frac{\begin{array}{c}\text{Fehling's solution factor}\\ \times\text{(Dilution made)}\times 100 \times 100\end{array}}{\begin{array}{c}\text{(Titre volume)}\\ \times\text{Weight of sample taken}\times 50\end{array}} \times 100$$

2.5 Reducing sugars (%)

To determine the concentration of reducing sugars in the fruit juice sample, the following steps were performed. Initially, 10 ml of the fruit juice was added to distilled water to dilute to achieve a total volume of 20-30 ml. Then, the solution was neutralized by adding 1 N NaOH while using phenolphthalein as an indicator. Subsequently, 10 ml of 44% lead acetate solution was added and thoroughly mixed. After a 10 min interval, 10 ml of 22% potassium oxalate solution was introduced to eliminate any excess lead. The resulting solution was then diluted to 250 ml with distilled water and filtered to obtain the aliquot. This aliquot was titrated using boiling Fehling's solution (5 ml of solution A + 5 ml of solution B), while methylene blue served as the indicator. The titration continued until a brick-red color developed.

The following formula was used to determine the quantity of reducing sugars:

Reducing sugars (%) = $(V \times 0.05 \times F \times 100) / W$

where:

V = volume of aliquot used for titration (in ml)
0.05 = conversion factor for Fehling's solution
F = factor for Fehling's solution
W = weight of fruit juice sample used (in grams)

2.6 Non-reducing sugars (%)

To calculate the percentage of non-reducing sugars in the fruit juice, we can subtract the percentage of reducing sugars from the total sugar percentage. This can be expressed using the following formula:

Non-reducing sugars (%) = Total sugars (%) – Reducing sugars (%) × 0.95

By substituting the respective values obtained for total sugars and reducing sugars, we can calculate the percentage of non-reducing sugars in the fruit juice sample.

2.7 Total antioxidants (µmol g^{-1})

5 g pulp was extracted with 20 ml of 60 per cent methanol (0.1% HCl) and kept overnight. Then, it was centrifuged at 10,000 rpm for 15 min at 10°C. The supernatant was taken for analysis.

100 µl of methanolic extract was mixed with 3 ml of solution (1.2 M sulphuric acid, 46 mM sodium phosphate, and 8 mM ammonium molybdate) and was incubated for 90 min at 95°C in the water bath. It was then allowed to cool down to room temperature. Reading of the sample was measured at a wavelength of 695 nm on a spectrophotometer (Double beam SL 210 UV visible spectrophotometer, Ellico, Hyderabad, India) and ascorbic acid was taken as standard. The standard curve was plotted with the absorbance readings of the standard and sample which gave the value of total antioxidants in mg 100 g^{-1}.

3. Result and Discussion

3.1 Total soluble solids (°Brix)

One notable finding from the study is that treatment T_5, which consisted of boric acid (0.5 %), zinc sulphate (0.5 %), and calcium chloride (2 %), demonstrated significantly highest total soluble solids (TSS) content (12.2 °Brix) compared to other treatments. Boron and zinc have been reported to positively influence sugar metabolism in fruits, including guava, leading to increased TSS levels (Pandey *et al.*, 2015; Rahman *et al.*, 2018). Boron promotes sugar synthesis and transportation, enhancing sugar accumulation in fruits, while zinc plays a crucial role in sugar metabolism and transportation within plants (Pandey *et al.*, 2015; Rahman *et al.*, 2018). The combined use of boron and zinc in Treatment T_5 may have a synergistic effect on TSS content. Additionally, the presence of calcium chloride (2 %) in all treatments, including T_5, may contribute to TSS accumulation by regulating metabolic pathways.

3.2 Titratable acidity

Another important observation is the effect of micronutrient treatments on titratable acidity. Treatments T_5 (boric acid (0.5 %), zinc sulphate (0.5%) and calcium chloride (2%)), exhibited the highest titratable acidity values (0.5%). Boron and calcium have been reported to influence acidity levels in fruits, including guava (Pandey *et al.*, 2015; Romero-Aranda *et al.*, 1998). Boron enhances fruit acidity by enhancing citric acid metabolism, while calcium plays a role in maintaining cell wall integrity and regulating enzyme activity related to acidity (Pandey *et al.*, 2015; Romero-Aranda *et al.*, 1998).

3.3 Ascorbic acid (mg)

The study also found that treatment T_5 had the highest ascorbic acid content (242.4 mg/100 g) among all treatments. Boron and zinc have been shown to enhance the vitamin C content in fruits, including guava (Pandey *et al.*, 2015). Ascorbic acid, a water-soluble antioxidant vital for human health, is known to be positively influenced by these micronutrients. Therefore, the combined application of boric acid and zinc sulphate in treatment T_5 likely contributed to the increased ascorbic acid content in guava (Pandey *et al.*, 2015).

3.4 Total sugars (%)

It is evident from the data presented in Table 1 that treatment T_5 exhibited the highest total sugar percentage (8.6%) among all treatments. Boron and zinc have been reported to positively influence sugar metabolism in guava and other plants, leading to increased levels of total sugars (Pandey *et al.*, 2015; Rahman *et al.*, 2018). The combined application of boron and zinc in treatment T_5 may have a synergistic effect on total sugar content. During basipetal sucrose flow, the application of B integrates into the plant through phloem filter plates. It strengthens the nuclear membrane and controls the metabolism of ribonucleic acids, which was crucial for the assimilation of the sugar content (Khan *et al.*, 2022). Additionally, calcium chloride (2 %) present in all treatments, including T_5, may contribute to total sugar accumulation by regulating metabolic pathways (FAO, 2014).

Table 1: Effect of foliar application of micronutrients on the phytochemical composition of guava fruit

Treatments	TSS (°Brix)	Titratable acidity (%)	Ascorbic acid (mg/100g^{-1})	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Antioxidant (imol g^{-1})
T_1	9.30	0.38	206.97	6.45	3.56	2.89	17.24
T_2	11.94	0.50	236.97	8.25	4.40	3.84	29.60
T_3	10.15	0.43	224.40	7.50	3.99	3.51	21.16
T_4	9.98	0.42	216.00	7.08	3.74	3.34	19.44
T_5	12.17	0.54	242.43	8.55	4.57	3.97	31.36
T_6	10.74	0.49	234.43	8.06	4.29	3.77	27.99
T_7	10.36	0.46	232.03	7.82	4.13	3.69	26.37
SE (m)	0.104	0.018	2.670	0.061	0.081	0.096	0.176
CD @ 5%	0.309	0.053	7.889	0.182	0.240	0.286	0.519

3.5 Reducing sugars (%)

Table 1 data reveals that treatment T_5 showed the highest percentage of reducing sugars (4.6%) among all treatments. Boron and zinc have been reported to enhance sugar metabolism in plants, resulting in higher levels of reducing sugars (Pandey *et al*., 2015; Rahman *et al*., 2018). In the oxidation process, zinc functions as a catalytic component that is essential for sugar metabolism (Anitha *et al*., 2023). The combined application of boron and zinc in treatment T_5 may have contributed to the observed increase in reduced sugar content.

3.6 Non-reducing sugars (%)

In terms of non-reducing sugars, treatment T_5 demonstrated the highest percentage (4.0%) among all treatments (Table 1). The combined application of boron, zinc, and calcium chloride likely contributed to the increased non-reducing sugar levels. The creation of cell walls and the integrity of membranes both need the element boron, while calcium uptake may facilitate the movement of sugars. Increased sugar content reducing sugars, non-reducing sugars, and total sugar may be the result of boric acid regulation's effective translocation of photosynthates to the fruits. The boron treatment may have sped up the ripening phase, during which acid decomposition may have taken place, and assisted in halting excessive sugar polymerization and buildup in plant cells (Yadav *et al*., 2023).

3.7 Antioxidants (%)

The data (Table 1) revealed that treatment T_5 exhibited the highest antioxidant activity (31.4%) compared to other treatments. Boron and zinc have been reported to enhance antioxidant activity in fruits by regulating enzymatic and non-enzymatic antioxidant defense systems (Pandey *et al*., 2015). The presence of calcium chloride in treatment T_5 may have further contributed to improved antioxidant defense mechanisms (Gupta *et al*., 2017).

4. Conclusion

In conclusion, the foliar application of micronutrients had a significant effect on the quality attributes of guava fruits. Treatment T_5, which involved the combined application of boric acid (0.5%), zinc sulfate (0.5%), and calcium chloride (2%), showed the most positive impact on various parameters, including acidity, ascorbic acid, total sugars, reducing sugars, non-reducing sugars, and antioxidant activity. These micronutrients influenced sugar metabolism, acidity regulation, vitamin C content, and antioxidant defense systems in guava fruits. The results highlight the potential of micronutrient foliar sprays to improve the quality of guava.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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