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Research Article

Impact of foliar spray of nano-Zn and nano-Cu on biochemical characteristics of guava cv. Allahabad Safeda

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Abstract
Foliar spraying of nanoparticles (NPs) improves the absorption of plant nutrient application compared to traditional soil–root application, and it also enhances the yield and quality of fruits. The present study aimed to evaluate the qualitative effects of foliar sprays of two concentrations of nano-zinc and nano-copper (40 ppm, 60 ppm; 20 ppm, 30 ppm respectively), in comparison to ZnSo4 (recommended by, Punjab Agriculture University, Ludhiana) and control (foliar spray of water) on the guava crop (var. Allahabad Safeda). The experiment was conducted at Lovely Professional University Research farm, Phagwara, Jalandhar (Punjab) by applying a simple randomized block design, with ten treatments applied as T_1: control, T_2: nano-Zn_1, T_3: nano-Zn_2, T_4: nano-Cu_1, T_5: nano-Cu_2, T_6: nano-Zn_{1+} nano-Cu_1, T_7: nano-Zn_{1+} nano-$Cu_{2,}$ T_8: nano-Zn_2 + nano-Cu_1, T_9: nano-Zn_2 + nano-$Cu_{2,}$ T_{10}: $ZnSO_4$ (PAU recommendation) in three replications. The treatments were sprayed two times, first at the flowering stage and second when the fruit reached pea size. The nutrient spray increases the concentration of nutrients in the leaves while also affecting the biochemical parameters. The performance for total soluble solids (9.89°B), total sugars (8.74%), titratable acidity (0.98%), antioxidants (7.49%), firmness (5.71kg/cm2), non-reducing sugars (3.32%), Vitamin-C (268.90mg/100g pulp), pectin content (2.13%), reducing sugars (5.46%), and TSS/acid ratio (10.06) was superior with the application of nano-Zn_2 + nano-Cu_2 (T9). The application of nano-micronutrients (zinc and copper) in combination is favorable for the quality of guava fruit (Allahabad Safeda).

Keywords: Foliar application, Guava, nano-Cu, Nano-nutrients, Nano-Zn

INTRODUCTION

Guava is a fruit that is native to Central and South America. The distribution of guava was aided by humans and other living organisms (Hussain *et al.*, 2021). The fruit is rich in vitamin C, lycopene, polyphenols, sugars, fiber, calcium, minerals, and calories (Anand *et al.*, 2020). Due to its high nutritional value and inexpensive availability, it is known as the poor man's apple (Mali *et al.*, 2023). Although guava fruit is generally consumed in its fresh form, it is used to make many different items, including jam, jelly, canned fruit, and sharbat (A. Singh *et al*., 2019). Fruits are a crucial component of a healthy diet; consuming them can help prevent serious illnesses. Unfortunately, eating too little fruits and vegetables, especially in poorer nations, is one of the risk factors for death. According to estimates from the Global Burden of Disease study, eating less fruit is associated with close to 3.4 million deaths (Mokdad *et al*., 2016). The population of the globe is expected to reach 9.1 billion people by 2050, placing us under ongoing population pressure (Fao, 2009). In the end, it is noted that the demand for food also increases as the population (Kumar *et al*., 2019). The degradation of land, which is brought on by resource constraints and urbanization, is a substantial obstacle to agricultural production. For the past 50 years, farmers have requested pesticides fertilizers, and disease-free cultivars to solve this problem (Yadav, 2014).

In the absence of the macro-nutrients nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur, the guava is a nutrient-responsive crop and is said to exhibit recognizable symptoms of malnutrition. Micronutrient deficiencies are also reportedly present. As a result, field trials and leaf nutrient content have been advised for monitoring guava's nutritional needs (Mahaveer and Sangma, 2017). In actuality, fertilizers have been shown to be crucial in increasing the output of field crops in general and fruits in particular. Although, the overuse of chemical fertilizers has led to a decline in soil health and food quality (Baweja *et al*., 2020). The increase in agricultural productivity by nanotechnology has great potential to contribute to long-term food security. Qureshi *et al*., (2018) defined nano-fertilizers as nanomaterials with a 1–100 nm diameter that provide plants with at least one type of nutrient. These characteristics include large surfaces, high absorption capacities, smooth delivery systems, and regulated release kinetics to active areas (Solanki *et al*., 2015). Numerous nanomaterials have demonstrated a significant potential for improving the quality and production of horticulture crops, their shelf life, and post-harvest damage (Rana *et al*., 2021).

The trace element zinc (Zn) is essential for normal plant metabolic and physiological activities. Zn availability is confined to the rhizosphere, so the maximum farmed land remains Zn deficient, restricting plant nutrient absorption. Because of their tiny size and wide surface area, Zn nanoparticles are efficiently transported into plant systems (Chandrakala *et al*., 2022). Copper (Cu) is an active essential micronutrient for plant physiology and metabolic enzymatic reactions. In addition, it contributes to the transfer of electrons in the redox reaction. That is why copper is used as an essential nutrient and needs to be supplied through fertilizer in plants (Francis *et al*., 2022).

Nano-fertilizers are substances on the nanoscale that include micronutrients and macronutrients and are used for supplying nutrients to plants (Fatima *et al*., 2021; Adisa *et al*., 2019; Bisma *et al*., 2020). They are more reactive and penetrate the soil and plant deeper due to their increased surface area to volume ratio (Manjunatha *et al*., 2016). Additionally, nano-fertilizers are needed in tiny amounts because of their steady and prolonged material release, which increases plant productivity and encourages effective nutrient use that can be considered climate-friendly. The features for focused delivery can support precision and sustainable agriculture in their potential (Iqbal, 2019; Zulfiqar *et al*., 2019).

MATERIALS AND METHODS

The study was conducted on guava plants (var. Allahabad safeda) that were 4-5 years of age, having uniform shape, size, and Vigor, at Horticulture Farms, Department of Horticulture, Lovely Professional University, Punjab. Three replications of a randomized block design were employed for the experiment. N:P:K and Farm yard manure were applied to the respective treatments as per the recommendation in the Package of Practice (PAU) for the guava plants (July, 2021). Foliar application of different levels of nano zinc and nano copper were used and the results were compared to the recommended foliar application of micronutrients. A total of 10 treatments replicated thrice were evaluated in a randomized block design: T_1: control, T_2: nano-Zn_1, T_3: nano-Zn_2, T_4: nano-Cu_1, T_5: nano-Cu_2, T_6: nano-Zn_{1+} nano-Cu_1, T_7: nano-Zn_{1+} nano-$Cu_{2,}$ T_8: nano-Zn_2 + nano -Cu_1, T_9: nano-Zn_2 + nano-$Cu_{2,}$ T_{10}: $ZnSO_4$ (PAU recommendation). The first dose of nano-nutrients was applied at the flowering stage (June) and the next dose after the fruit came into the pea stage (i.e.; the third week of July). Nano-Zn_1 is 40ppm, nano-Zn_2 is 60ppm, nano-Cu_1 is 20ppm, nano-Cu_2 is 30ppm, and 1% solution of zinc sulphate as per treatments. The solution of 4-5liters of water was prepared for spraying each plant. Fruits were harvested in the first month of November to analyze quality parameters (total sugars, reducing sugars, non-reducing sugars, Vit C, pectin content, total soluble solids (TSS), Titratable Acidity, and TSS: TA ratio, antioxidants and firmness).

Collection and biochemical analysis of guava fruit samples

The collection and detailed analysis of guava fruit samples were performed to investigate various physiochemical characteristics. Ten fruits were systematically selected from each treated guava plant, and the following parameters were assessed according to the protocol outlined by Ranganna, (1986).

Total soluble solids (°Brix)

A digital refractometer (0-32 °Brix) was employed to determine the total soluble solids content. A few drops of ripe fruit juice were placed on the refractometer's prism, with prior calibration using purified water, and the results were expressed in °Brix units.

Titratable acidity (%)

The titratable acidity of the fruit juice was assessed using the method described by (Ranganna, 1986). This involved the combination of 5 mL of fruit extract with 4–5 drops of 1% phenolphthalein indicator, followed by titration with 0.1 N NaOH. The endpoint of the titration was indicated by the appearance of a light pink color, which was maintained for at least 50 seconds. Titratable acidity was calculated using the formula:

Titratable acidity (%) = (56.1 × (V × N)) / W Eq. 1

Where:

V = Volume in mL of standard sodium hydroxide used.

N = Normality of the sodium hydroxide solution.

W = Weight in grams of the sample.

Antioxidants (%)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, based on the Chen *et al*., (2013)method, was slightly adapted. A stock solution of 24 mg DPPH in 100 mL of methanol was prepared and maintained at 20°C. A working solution was created by combining 10 mL of the stock solution with 45 mL of methanol, resulting in an absorbance of 1.17 ± 0.02 units at 515 nm. Fruit extracts (150 mL) were allowed to react with 2850 mL of the DPPH solution in the dark for 24 hours, and absorbance was measured at 515 nm. Antioxidant results were expressed in mM TE (Trolox Equivalents) per gram of fresh mass.

Total sugars (%)

The estimation of total sugars involved the use of anthrone reagent with fruit juice. After subjecting the sample to a water bath at 100°C for 8 minutes and measuring the optical density (O.D.) at 630 nm, the total sugar content was calculated using the formula:

Total sugars (%) = ((mg of glucose) / (Volume of the test sample)) × 100 Eq. 2

Reducing sugars (%)

The Nelson–Somogyi method was employed to determine how to reduce sugars. By taking 1 mL of guava juice, making the volume up to 3 mL with distilled water, and using DNS reagent, reducing sugars were quantified based on a standard curve established using glucose (0-500 µg).

Non-reducing sugars (%)

Non-reducing sugars were calculated by subtracting the reducing sugar content from the total sugar content.

TSS/Acidity Ratio

This ratio, indicating the balance between sugar content (TSS) and acidity, was calculated by dividing TSS (in °Brix) by titratable acidity.

Firmness (kg/cm²)

The firmness of selected fruits was measured using a penetrometer, which provided readings displayed on a dial.

Vitamin C (Ascorbic acid) (mg/100g Pulp)

Ascorbic acid content was determined via a titration method using a standard dye solution.

Pectin content (%)

Pectin content in guava fruit was calculated using a modified version of Ranganna, (1977) method, where pectin precipitates as calcium pectate from an acidified solution. The quantification of pectin content was expressed as a percentage.

Statistical analysis

Data were statistically assessed using analysis of variance (ANOVA) to determine the importance of the major components and the relevance of interactions. The combined analysis of variance was conducted on the assumption that the environment (years and blocks) and treatments were fixed variables. The statistical analysis system, SAS (SAS Institute Inc., Cary, NC, USA), base 9 software was used to analyze the data. Means were compared using Duncan's multiple range test at the p 0.05 level. The data were statistically analyzed using the SPSS V. 23 program, and homogenous subgroups were found to test the level of significance between various treatments.

RESULTS AND DISCUSSION

The foliar application of nano-Zn and nano-Cu was observed to have a significant impact on the quality of guava fruits (Allahabad Safeda), affecting parameters such as total soluble solids (TSS), titratable acidity, ascorbic acid content, antioxidants, firmness, pectin content, total sugars, reducing sugars, and non-reducing sugars as displayed in Table 1. The results indicated that the application of nano-zinc and nano-copper, either individually or in combination, led to an improvement in the quality of guava fruits. In contrast, untreated plants produced smaller-sized and comparatively lighter fruits. The foliar application of nano-zinc and nano-copper on guava trees unequivocally enhanced fruit quality compared to untreated trees.

The combination of both nano-zinc and nano-copper at concentrations of 60 ppm and 30 ppm, respectively, resulted in notably higher levels of non-reducing sugars

(3.32%), total sugars (8.74%), vitamin C (268.90 mg/100 g fruit), and reducing sugars (5.46%) (Table 1). These findings align with the results obtained by Mahaveer and Sangma, (2017) in their study on sweet oranges application of zinc sulphate 0.75% found in their findings.

The zinc plays a pivotal role in oxidation-reduction reactions and catalyzes sugar metabolism, affecting the quantities of total sugars, reducing sugars, and non-reducing sugars in treated plants. This is attributed to zinc's involvement in nucleic acid and starch metabolism and its influence on various enzymes engaged in these biochemical processes. Additionally, copper, which was applied alongside zinc, demonstrated a positive correlation with the soluble solids content and total sugars in guava fruits, consistent with findings in tomato fruits with a concentration of 10 mg Cu NPs, as Hernández *et al*., (2017) mentioned.

The study sheds light on the potential absorption mechanisms of nanoparticles (NPs) by plants. It is suggested that the penetration of NPs through stomata on the leaf surface plays a vital role in their absorption. NPs, due to their small size, can interact with membrane transport proteins and gain entry into plant cells, impacting plant physiology at very low concentration thresholds. Newly formed leaves are particularly efficient at nutrient absorption due to their thinner wax layer and relatively undeveloped nature (Ilyas *et al*., 2015).

Studies have also revealed the effect of NPs on the vascular tissues, epidermis, and mesophyll of leaves (Manjunatha *et al*., 2016). They can even migrate from the foliage to other plant parts, such as roots and recently formed leaves. The entry of NPs into plant cells can occur through various mechanisms, including ion channels, endocytosis, or water molecular pathways. It may trigger redox and other processes that alter the morphology of NPs. Some foliar NPs can even create new entry points in the plant cell walls to facilitate their entry into cells. These findings correlate well with the results of Bisma *et al*. (2020), who provide valuable insights into the interactions and absorption of NPs in plant systems.

Changes in Biochemical properties of fruit

In horticultural research, the influence of foliar application of nano-zinc (nano-Zn) and nano-copper (nano-Cu) on guava fruit quality emerged as a compelling subject. The findings revealed a marked improvement in the quality of guava fruits when nano-Zn and nano-Cu were applied through foliar application, both separately and in combination. Importantly, plants that were not treated produced smaller and lighter fruits by comparison. This robust improvement in fruit quality was corroborated by various analytical data in Table 1.

In particular, combining nano-Zn and nano-Cu at concentrations of 60 ppm and 30 ppm resulted in a noteworthy increase in various parameters. Non-reducing sugars, an indicator of fruit sweetness, surged to 3.32%, while total sugars, encompassing both reducing and non-reducing sugars, reached 8.74%. The ascorbic acid content, a pivotal factor in fruit nutrition, soared to 268.90 mg/100 g of fruit, signifying a substantial enhancement in the fruit's health-promoting attributes (Sachin *et al*. 2019). Additionally, reducing sugars, an essential contributor to sweetness and flavor, escalated to 5.46%. The present results correlate well with the findings of Mahaveer and Sangma (2017), who observed similar positive outcomes in sweet oranges.

Exploration into the underlying mechanisms revealed that nano-Zn plays a central role in oxidation-reduction reactions and serves as a catalyst in sugar metabolism. This participation in sugar metabolism influences the quantities of total sugars, reducing sugars, and non-reducing sugars in treated plants. Zinc's influence extends to nucleic acid and starch metabolism, affecting various enzymes in these biochemical processes. In parallel, copper, applied alongside zinc, demonstrated a positive correlation with soluble solids content and total sugars in guava fruits, aligning with findings in tomato fruits as reported by (Hipólito *et al*. (2019).

The study also delves into the fascinating world of nanoparticles (NPs) and their potential absorption mechanisms in plants. It is proposed that NPs primarily penetrate the plant through stomata on the leaf surface. Their small size allows them to interact with membrane transport proteins, gaining entry into plant cells and affecting plant physiology even at very low concentrations. Newly formed leaves, characterized by a thinner wax layer and relative biological underdevelopment, exhibit heightened efficiency in nutrient absorption (Singh *et al*., 2023).

Moreover, research suggests that NPs can traverse vascular tissues, epidermis, and mesophyll in leaves exposed to NPs. These NPs are not confined to the foliage but can migrate to other plant parts, including roots and recently formed leaves. The mechanisms of NP entry into plant cells encompass ion channels, endocytosis, and water molecular pathways, potentially initiating redox reactions and other processes that impact NP morphology. Remarkably, some foliar NPs can even create novel entry points in plant cell walls to facilitate their entry into cells (Rajkumar, 2014).

The present findings offer valuable insights into the transformative effects of nano-Zn and nano-Cu on guava fruit quality, coupled with a deeper understanding of how NPs interact with and are absorbed by plants. The potential for enhancing fruit quality through nano-based applications represents an exciting frontier in horticultural science.

Table 1. Impact of nano-micronutrient (Zinc and Copper) on some biochemical parameters in guava fruit (Allahabad safeda)

Treatments	Total sugar (%)	Reducing sugars (%)	Non-Reducing sugars (%)	Vit C (mg/100 g fruit)	Pectin content (%)	TSS (^{0}B)	Titratable Acidity (%)	TSS: TA ratio	Antioxi-dants (%)	Firmness (kg/cm^2)
Control (T_1)	8.12^{a}	4.95^{a}	3.06^{a}	246.14^{a}	1.47^{a}	9.68^{a}	1.23^{f}	7.82^{a}	7.28^{a}	5.00^{a}
nano-Zn_1 (T_2)	8.20^{b}	5.03^{b}	3.12^{b}	252.32^{b}	1.57^{ab}	9.75^{bc}	1.23^{ef}	7.93^{ab}	7.39^{cd}	5.17^{a}
nano-Zn_2 (T_3)	8.24^{bc}	5.08^{c}	3.16^{bc}	251.89^{b}	1.60^{b}	9.72^{ab}	1.22^{ef}	7.95^{ab}	7.41^{de}	5.31^{a}
nano-Cu_1 (T_4)	8.25^{bc}	5.12^{d}	3.17^{bc}	253.17^{b}	1.65^{b}	9.77^{bcd}	1.20^{e}	8.09^{b}	7.3^{ab}	5.38^{a}
nano-Cu_2 (T_5)	8.35^{d}	5.19^{e}	3.22^{de}	259.12^{c}	1.78^{cd}	9.84^{efg}	1.17^{d}	8.38^{c}	7.37^{c}	5.49^{a}
nano-Zn_1+nano-Cu_1 (T_6)	8.26^{c}	5.15^{d}	3.2^{cd}	257.22^{c}	1.68^{bc}	9.79^{cde}	1.20^{e}	8.13^{b}	7.32^{b}	5.39^{a}
nano-Zn_1+nano-Cu_2 (T_7)	8.47^{e}	5.20^{e}	3.25^{ef}	264.13^{d}	1.81^{d}	9.87^{fg}	1.15^{d}	8.53^{c}	7.42^{de}	5.52^{a}
nano-Zn_2+nano-Cu_1 (T_8)	8.64^{g}	5.34^{f}	3.29^{gh}	258.46^{c}	1.96^{e}	9.82^{def}	1.07^{b}	9.17^{e}	7.46^{fg}	5.71^{a}
Nano-Zn_2+nano-Cu_2 (T_9)	8.74^{h}	5.46^{h}	3.32^{h}	268.90^{e}	2.13^{f}	9.89^{g}	0.98^{a}	10.06^{f}	7.49^{g}	5.71^{a}
$ZnSo_4$ (T_{10})	8.54^{f}	5.26^{f}	3.27^{fg}	263.51^{d}	1.89^{de}	9.85^{fg}	1.12^{c}	8.80^{d}	7.44^{ef}	5.59^{a}
S.Em(±)	0.017	0.01	0.015	0.998	0.039	0.02	0.009	0.077	0.012	N/A
C.D.@5%	0.05	0.031	0.045	2.99	0.116	0.06	0.026	0.232	0.036	0.202

Conclusion

In the present study, different nanoparticles, including Zn and Cu, were used to fabricate the Zn-Cu nanocomposite with smaller particle sizes. These smaller particles confirmed the composite's open network structure, which helped in the easy migration of nano foliar spray into the fruit body and maintains the biochemical properties of guava cv. *Allahabad Safeda*. At particular concentrations, the nano foliar spray showed significant influence on various parameters, including total soluble solids (TSS), titratable acidity, ascorbic acid content, antioxidants, firmness, pectin content, total sugars, reducing sugars, and non-reducing sugars. The results revealed an increase in total sugar content of 8.74 %, from which the part of non-reducing and reducing sugar was 3.32 and 5.46 %, respectively, which are the indicators of fruit sweetness. In addition, the ascorbic acid content reached 268.90 mg/ 100g of fruit. The nano-Zn played a vital role as a catalyst in the biochemical processes of fruit for their oxidation-reduction reaction in the sugar metabolism of treated plants. This study also concludes the mechanism of NPs on the absorption process in plants. Due to the smaller size of NPs, they interact with membrane transport proteins and then enter by different pathways like ion channels, endocytosis, and water molecules into plant cells, impacting plant physiology at a very low concentration. Thus, these findings offer valuable insights into the transformative effects of nano-Zn and nano-Cu to sustain the guava fruit quality, coupled with a deeper understanding of how NPs interact with and are absorbed by plants. Therefore, this research could be scaled up as an effective system to improve fruit quality and also opens up an exciting frontier in horticultural science with the application of nanotechnology in agriculture.

Conflict of interest

The authors declare that they have no conflict of interest.

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