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Effect of foliar application of zinc sulphate and plant growth regulators on quality and leaf nutrient status of guava (*Psidium guajava* L.) cv. Hisar Surkha

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ABSTRACT

A field study was carried out at the Experimental Orchard, Department of Horticulture, CCS Haryana Agricultural University, Hisar (Haryana) on six-year-old guava trees cv. Hisar Surkha to determine the effect of foliar application of zinc sulphate and plant growth regulators on the quality and leaf nutrient status of guava. Different treatments were taken i.e. T_1 : $ZnSO_4.7H_2O$ @ 0.25%, T_2 : $ZnSO_4.7H_2O$ @ 0.50%, T_3 : $ZnSO_4.7H_2O$ @ 0.75%, T_4 : $ZnSO_4.7H_2O$ @ 1.0%, T_5 : NAA @ 25 ppm, T_6 : NAA @ 50 ppm, T_7 : NAA @ 75 ppm, T_8 : NAA @ 100 ppm, T_9 : GA_3 @ 50 ppm, T_{10} : GA_3 @ 75 ppm, T_{11} : GA_3 @ 100 ppm and T_{12}: Control (water spray) with three replications under randomized block design. The results of the study revealed a significant increase in the quality and leaf nutrient status of guava fruit. However, foliar application of NAA @ 100 ppm proved to be the best treatment in improving the TSS (12.93°B), TSS/Acid Ratio (39.18), total sugars (6.75 %), reducing sugars (4.05 %) and non-reducing sugars (2.70 %) and foliar application of GA_3 @ 100 ppm improved the pectin content (0.89 %), ascorbic acid content (208.4 mg/100 g pulp) and leaf nutrient status of guava fruit over control.

Keywords: *Hisar Surkha, plant growth regulators, TSS, ascorbic acid, leaf nutrient status.*

Introduction

Guava (*Psidium guajava* L.), "Apple of the Tropics" and "Poor Man's Apple" is an important fruit crop of the country. Widely cultivated in tropical and sub-tropical regions up to 1500 m above sea level, guava thrives in areas with distinct winters, resulting in increased yield and improved quality. It is classified under the genus Psidium covering about 150 species (Hayes, 1970) but only *Psidium guajava* L. has been commercially exploited. It was introduced in India in the 17th century by the Portuguese and became a commercial crop. It is very popular fruit crop and widely grown in tropical and sub-tropical regions up to 1500 m above mean sea-level. It is being cultivated throughout the American tropics, Asia, Africa and Pacific Islands. Though native to Central America, guava has been cultivated and naturalized across tropical regions and is now extending into some subtropical areas due to rising demand (Baloda *et al*., 2023). It is a more income-generating crop without much care and input as it is sturdy, prolific in bearing even on marginal lands. Guava stands out as a rich source of sugars, ascorbic acid (Vitamin C) and pectin. The ascorbic acid content in the pulp ranges from 75-260 mg/100 g, varying based on cultivar, season, location, and maturity stage.

Micronutrients are required by the plants in small quantities and thus, can be applied more safely and easily through foliar application. Application of micronutrients through foliar fertilization has the advantage of lower application rates, uniformity in the distribution of fertilizer materials and quick response to applied nutrients (Parmar *et al*., 2014). Among these micronutrients, zinc is the most important. Zinc plays an important role in starch metabolism, acts as a cofactor for many enzymes and affects photosynthesis, nucleic acid metabolism and protein biosynthesis. Zinc is also involved in regulating protein and carbohydrate metabolism (Jawed *et al*., 2016). Plant growth regulators like NAA play an essential role in plant growth, flower induction, fruit set, fruit growth, yield and quality (Lenka *et al*., 2019). By the application of NAA, total soluble solid as well as vitamin C content of fruit increased and acidity is reduced. The function of GA_3 is to induce flowering and increase the fruit setting and fruit retention percentage (Suman *et al*., 2021). GA_3 helps in accelerating the translocation of metabolites from plant parts to the developing fruits. Keeping in view, this experiment has been planned to study the "Effect of foliar application of zinc sulphate and plant growth regulators on quality and leaf nutrient status of guava (*Psidium guajava* L.) cv. Hisar Surkha".

Materials and Methods

Experimental details

The present investigation was conducted at the Experimental orchard of the Department of Horticulture, CCS Haryana Agricultural University, Hisar during the year 2022-23. The six years old guava cultivar Hisar Surkha was selected as experimental material to examine the effect offoliar application of zinc sulphate and plant growth regulators on quality and leaf nutrient status of guava. The experiment comprised of total 12

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treatments i.e. T_1 : $ZnSO_4.7H_2O$ @ 0.25%, T_2 : $ZnSO_4.7H_2O$ @ 0.50%, T_3 : $ZnSO_4.7H_2O$ @ 0.75%, T_4 : $ZnSO_4.7H_2O$ @ 1.0%, T_5 : NAA @ 25 ppm, T_6 : NAA @ 50 ppm, T_7 : NAA @ 75 ppm, T_8 : NAA @ 100 ppm, T_9 : GA_3 @ 50 ppm, T_{10} : GA_3 @ 75 ppm, T_{11} : GA_3 @ 100 ppm and T_{12}: Control (water spray) with three replications under randomized block design. The treated fruits were analyzed for TSS (°B), Acidity (%), TSS/Acid ratio, Total sugar content (%), Reducing sugar content (%), Non - reducing sugar content (%), Pectin content (%), Ascorbic acid (mg/100 g pulp) and Leaf sample analysis for N, P, K and Zn content.

Observations for evaluation

TSS (°B) : The total soluble solids (TSS) were measured by hand refractometer in the range of 0-32°Brix. The juice was extracted from selected fruits by squeezing through muslin cloth with the hands from each replication and reading was noted by just putting the drop of juice on the prism of the hand refractometer. The refractometer was calibrated with the help of distilled water after each reading and the value was expressed in °Brix.

Acidity (%): Titratable acidity was estimated by using the method given in AOAC (1990).

Reagents prepared.

The following reagents were prepared for further use:

1. Sodium hydroxide 0.1 N
2. Phenolphthalein indicator 1 per cent

Procedure

Mashing of 5g of fruit pulp was done using small amount of distilled water. 2ml of filtrate was pipette out into a beaker and titrated against N/10 sodium hydroxide using phenolphthalein as an indicator. Light pink colour end point is reached. Acidity was expressed in terms of per cent citric acid equivalent after applying the following formula:

$$\text{Acidity (\%)} = \frac{\text{Titrate value x Normality of NaOH x Equivalent weight of citric acid}}{\text{Juice taken (ml) x 1000}} \times 100$$

TSS/Acid ratio: The ratio of total soluble solids to acid was calculated by dividing the total soluble solids value by the acidity.

$$\text{TSS/Acid ratio} = \frac{\text{Total soluble solids}}{\text{Acidity}} \times 100$$

Total sugar content (%): Hulme and Narain (1931) advocated the method for the estimation of sugars.

Reagents

i) Potassium ferricyanide solution

a. Potassium ferricyanide 8.25 g
b. Anhydrous sodium carbonate 10.6 g
c. Final volume adjusted 500 ml

ii) Potassium iodide solution

a. Potassium iodide 12.5 g
b. Zinc sulphate 25 g
c. Sodium chloride 125 g
d. Final volume adjusted 500 ml

iii) 5 per cent acetic acid solution

a. Glacial acetic acid 50 ml
b. Final volume adjusted 1000 ml

iv) Sodium thiosulphate solution (N/ 100)

a. Sodium thiosulphate 2.482 g
b. Final volume adjusted 1000 ml

v) Starch (indicator)

a. Soluble starch 1 g
b. Sodium chloride 20 g
c. Final volume adjusted 100 ml

Extraction

0.2 ml juice was taken in a test tube with pipette and the final volume was adjusted to 20 ml (100 times dilution) with the help of distilled water. These were covered with aluminum foil and kept in a boiling water bath for 30 minute.

Total sugar content (%): In a test tube, 5.0 ml of aliquot was taken, following which 4 ml of HCl (hydrochloric acid) was added to it and kept in a boiling water bath for 15 minute by covering it with aluminium foil. It was neutralized using adding anhydrous Na_2CO_3 (sodium carbonate) till the effervesce stopped completely. Final volume was made to 50 ml using a volumetric flask with the help of distilled water. From this, 5.0 ml of aliquot was taken from it and the same procedure was repeated as used in reducing sugars.

Reducing sugar content (%): In a test tube, 5 ml of aliquot was taken and 5 ml of potassium ferricyanide was added to it. After that, the test tube was covered with aluminum foil and kept in a water bath for 15 minutes. Test tubes were cooled down by keeping under running tap water, and thereafter, 5 ml of potassium iodide and 3 ml of acetic acid were added to it so that it might turn into orange-yellow coloured solution. This solution was titrated against sodium thiosulphate using starch solution as indicator up to the time the milky white colour appeared. The reading from the burette was recorded at this point and at the same time a blank was run parallel. The results were calculated by using the subsequent formula and expressed in gram of sugars per 100 g fresh weight.

X= [(ml of sodium thiosulphate used in blank – ml of sodium thiosulphate used in unknown) + 0.05] x 0.338 = mg of sugar per 5 ml extract.

$$\text{Reducing sugars (\%)} = \frac{\text{X'x dilution factor}}{\text{5 x 1000}} \times 100$$

Non-reducing sugar content (%): Non-reducing sugars was determined by subtracting reducing sugars from total sugars.

Pectin content (%): Ranganna (1979) narrated the method for the estimation of total pectin as calcium pectate in fresh fruits.

Reagents

1. 1N Acetic acid

Glacial acetic acid 30 ml
Volume 200 ml

2. 1N Calcium chloride

Anhydrous calcium chloride 27.5 g
Volume 500 ml

3. 1N Sodium hydroxide
Sodium hydroxide 20 g
Volume 500 ml

4. 1% Silver nitrate
Silver nitrate 1 g
Volume 100 ml

Extraction
For the estimation of pectin content, 25 g of fresh fruit mashed samples were taken in a flask and 200 ml distilled water was added to it and placed on a hot plate for an hour. The water lost during boiling was restored back simultaneously. The flask was then further cooled and the final volume was made up to 250 ml. The contents of flask were then filtered through Whatman filter paper No. 4.

Estimation
To 50 ml part of the filtrate, 50 ml of distilled water and 5.0 ml of 1N NaOH was added and kept overnight. The following day, 25 ml of acetic acid solution was added and after 5 minutes again 12.5 ml of 1N calcium chloride solution was added using string. After permitting it to stand for an hour, it was boiled for a minute and filtered through oven-dried, previously weighed Whatman filter paper No. 4. After that, the precipitates were dried at 100°C overnight, cooled in desiccators and weighed. The amount of pectin was indicated as the percent calcium pectate.

$$\text{Calcium pectate (\%)} = \frac{\text{Weight of calcium pectate x volume of content}}{\text{Volume of filtrate x weight of sample for estimation}} \times 100$$

Ascorbic acid (mg/ 100 g pulp): Ascorbic acid was estimated by the method mentioned in AOAC (1990).
Reagents

a) Metaphosphoric acid solution (3%)
Metaphosphoric acid (HPO_3) 15 g
Glacial acetic acid 40 ml
Final volume adjusted 500 ml

b) 2, 6 dichlorophenol indophenol dye
2, 6-dichlorophenol indophenol dye 50 mg
Sodium bicarbonate 42 mg
Volume adjusted 200 ml

c) Standard ascorbic acid solution
50 mg of ascorbic acid ($C_6H_8O_6$) was dissolved in 50 ml metaphosphoric acid (3%).

Estimation
Grinding of 5g of fruit pulp was done using 25 ml of 3 per cent metaphosphoric acid and filtered through muslin cloth. A 2ml of filtrate was titrated against 2, 6-dichlorophenol dye until a distinctly rose pink colour appeared. Concurrently, 1.0 ml of standard ascorbic acid was also titrated against the dye.

The results were manifested as mg of ascorbic acid per 100 g of fruit pulp. It was determined by the given mathematical formula:

$$\text{Ascorbic acid (mg/100g fruit pulp)} = \frac{\text{Titrate value x total volume}}{\text{Standard reading x ml of sample}} \times 100$$

Leaf sample analysis for N, P, K and Zn contents: N, P, K and Zn were determined. For nutrient analysis oven dried plant material from each tagged plant was grinded separately with a grinder. Nitrogen (Colorimetric or Nessler's reagent method, Lindner, 1944), phosphorus (Vanadomolybdo-phosphoric acid yellow colour method, Koeing and Johnson, 1942), potassium (Flame photometer) and Zn (using atomic absorption spectrophotometer) contents in the sample were analyzed.

Results and Discussion
Foliar application of zinc sulphate and plant growth regulators significantly affected the quality and leaf nutrient status of guava *viz.,* TSS (°B), Acidity (%), TSS/Acid ratio, Total sugar content (%), Reducing sugar content (%), non-reducing sugar content (%), Pectin content (%), Ascorbic acid (mg/100 g pulp) and Leaf sample analysis for N, P, K and Zn content.
It is evident from the data presented in Table 1, the TSS was increased significantly by the foliar application of zinc sulphate and plant growth regulators at various concentrations on guava. Among different foliar applications, the maximum TSS (12.93°B) was recorded in treatment T_8*i.e.* NAA @ 100 ppm which was statistically at par with treatments T_7*i.e.* NAA @ 75 ppm (12.55°B) and T_{11}*i.e.* GA_3 @ 100 ppm (12.73°B). However, the minimum TSS (10.23°B) was recorded in treatment T_{12}*i.e.* control which was comparatively lower than all the other treatments. The results are similar by the findings of Kher *et al.* (2005), Bikash *et al.* (2007), Garasiya *et al.* (2013), Manivannan *et al.* (2015) in guava, Chandra *et al.* (2015) in aonla, Osama *et al.* (2015) in mango and Pandey *et al.* (1999) and Yadav *et al.* (2021) in ber. On the other hand, the acidity of the fruits was not significantly affected by the foliar application of zinc sulphate and plant growth regulators at various concentrations on Hisar Surkha guava plants as per the data shown in table 1. Minimum acidity (0.33%) was recorded under 100 ppm NAA concentration whereas, maximum acidity (0.52%) was recorded in control. The results are similar by the findings of Agnihotri *et al.* (2013), Manivannan *et al.* (2015), Sandeep and Amarjeet, (2017) in guava and Chandra *et al.* (2015) in aonla and Singh and Bons (2020) in sapota. Simultaneously, the foliar application of zinc sulphate and plant growth regulators resulted in higher TSS/Acid ratio over the control (Table 1). The maximum TSS/Acid ratio (39.18) was observed in treatment T_8*i.e.* NAA @ 100 ppm whereas, the minimum TSS/Acid ratio (19.67) was observed in treatment T_{12}*i.e.* control.The outcomes of the present study are in accordance with the results of Kher *et al.* (2005), Bikash *et al.* (2007), Garasiya *et al.* (2013), Manivannan *et al.* (2015) in guava, Osama *et al.* (2015) in mango and Chandra *et al.* (2015) in aonla and Pandey *et al.* (1999) and Yadav *et al.* (2021) in ber.

Table 1: Effect of zinc sulphate and plant growth regulators on TSS (°B), acidity (%) and TSS/Acid ratio of guavacv. Hisar Surkha

Treatments	TSS (°B)	Acidity (%)	TSS/Acid ratio
T_1 $ZnSO_4$ @ 0.25%	11.42	0.46	24.83
T_2 $ZnSO_4$ @ 0.50%	11.90	0.42	28.33
T_3 $ZnSO_4$ @ 0.75%	12.30	0.36	34.17
T_4 $ZnSO_4$ @ 1.0%	12.48	0.35	35.65
T_5 NAA @ 25 ppm	11.92	0.41	29.07
T_6 NAA @ 50 ppm	12.43	0.37	33.59
T_7 NAA @ 75 ppm	12.55	0.36	34.86
T_8 NAA @ 100 ppm	12.93	0.33	39.18
T_9 GA_3 @ 50 ppm	11.93	0.44	27.11
T_{10} GA_3 @ 75 ppm	12.40	0.40	31.00
T_{11} GA_3 @ 100 ppm	12.73	0.39	32.64
T_{12} (Control)	10.23	0.52	19.67
CD @ 5%	0.43	NS	1.14

The data presented in Table 2 depicts that foliar application of zinc sulphate and plant growth regulators at different concentrations influenced the total sugar content of guava. The maximum total sugar content (6.75%) was observed in treatment T_8*i.e.* NAA @ 100 ppm followed by treatment T_7*i.e.* NAA @ 75 ppm (6.60%) and T_{11}*i.e.* GA_3 @ 100 ppm (6.58%). However, the minimum total sugar content (5.04%) was observed in control. However, the reduced sugar content of guava was increased by foliar application of zinc sulphate and plant growth regulators (Table 2). The maximum reducing sugar content (4.05%) was recorded in treatment T_8*i.e.* NAA @ 100 ppm followed by NAA @ 75 ppm (3.92%), GA_3 @ 75 ppm (3.91%) and GA_3@ 100 ppm (3.97%). However, the minimum reducing sugar content (3.04%) was recorded in control. It is apparent from table 2 that the foliar application of zinc sulphate and plant growth regulators had a great impact on the non-reducing sugar content in guava. The highest non-reducing sugar content (2.70%) was recorded in treatment T_8*i.e.* 100 ppm NAA treatment which was followed by treatments T_4, T_7 and T_{11}*i.e.* $ZnSO_4$ @ 1.0 percent (2.59%), NAA @ 75 ppm (2.68%) and GA_3 @ 100 ppm (2.61%) while, the lowest non-reducing sugar content (2.00%) was recorded in control. The outcomes of the present study are in accordance with the results of Kher *et al.* (2005), Garasiya *et al.* (2013), Manivannan *et al.* (2015), Sandeep and Amarjeet, (2017) in guava.

Table 2 : Effect of zinc sulphate and plant growth regulators on total sugars (%), reducing sugars (%) and non-reducing sugars (%) in guavacv. Hisar Surkha

Treatments	Total sugar content (%)	Reducing sugar content (%)	Non-reducing sugar content (%)
T_1 $ZnSO_4$ @ 0.25%	5.14	3.07	2.07
T_2 $ZnSO_4$ @ 0.50%	5.93	3.65	2.28
T_3 $ZnSO_4$ @ 0.75%	6.28	3.80	2.48
T_4 $ZnSO_4$ @ 1.0%	6.46	3.87	2.59
T_5 NAA @ 25 ppm	5.32	3.22	2.10
T_6 NAA @ 50 ppm	6.10	3.77	2.33
T_7 NAA @ 75 ppm	6.60	3.92	2.68
T_8 NAA @ 100 ppm	6.75	4.05	2.70
T_9 GA_3 @ 50 ppm	6.02	3.69	2.33
T_{10} GA_3 @ 75 ppm	6.44	3.91	2.53
T_{11} GA_3 @ 100 ppm	6.58	3.97	2.61
T_{12} (Control)	5.04	3.04	2.00
CD @ 5%	0.28	0.16	0.12

The data concerning the pectin content (%) and ascorbic acid content (mg/100 g pulp) is shown in Table 3. Fruits had a substantially higher pectin content over the control. The maximum pectin content (0.89%) was observed in treatment T_{11}*i.e.* GA_3@ 100 ppm, closely followed by treatment T_{10}*i.e.* GA_3@ 75 ppm (0.86%). However, the minimum pectin content (0.65%) was observed in control. The present results are in line with the findings of Kumar *et al.* (2010) and Sharma and Tiwari (2015) in guava. It is clear from the data presented in Table 3 that ascorbic acid content was significantly increased with foliar application of zinc sulphate and plant growth regulators at different concentrations on guava. Treatment T_{11}*i.e.* GA_3@ 100 ppm had the highest ascorbic acid content (208.4 mg/100 g pulp) followed by treatments T_8, T_9 and T_{10}*i.e.* NAA @ 100 ppm (202.8 mg/100 g pulp), GA_3@ 50 ppm (203.1 mg/100 g pulp) and GA_3@ 75 ppm (206.9 mg/100 g pulp). However, the treatment T_{12}*i.e.* control had the lowest ascorbic acid content (186.4 mg/100 g pulp).The present results are in line with the findings of Tiwari *et al.* (2017) in strawberries, Lal *et al.* (2013), Rajput *et al.* (2015) and Lal and Das, (2017) in guava and Kumar *et al.* (2017) in cape gooseberry and Yadav *et al.* (2021) in ber.

Table 3 : Effect of zinc sulphate and plant growth regulators on pectin content (%) and ascorbic acid content (mg/100 g pulp) in guavacv. Hisar Surkha

Treatments	Pectin content (%)	Ascorbic acid (mg/100 g pulp)
T_1 $ZnSO_4$ @ 0.25%	0.69	191.4
T_2 $ZnSO_4$ @ 0.50%	0.70	193.7
T_3 $ZnSO_4$ @ 0.75%	0.74	195.5
T_4 $ZnSO_4$ @ 1.0%	0.79	198.7
T_5 NAA @ 25 ppm	0.67	197.3
T_6 NAA @ 50 ppm	0.70	199.7
T_7 NAA @ 75 ppm	0.78	201.4
T_8 NAA @ 100 ppm	0.82	202.8
T_9 GA_3 @ 50 ppm	0.83	203.1
T_{10} GA_3 @ 75 ppm	0.86	206.9
T_{11} GA_3 @ 100 ppm	0.89	208.4
T_{12} (Control)	0.65	186.4
CD @ 5%	0.03	6.5

The Table 4 depicts the leaf nutrient status of guava. A brief appraisal of the data clearly indicates that all the treatments were found significant in altering the leaf N, P, K and Zn content over the control. Maximum N content (1.78%), P content (0.23%) and K content (1.31%) was obtained with the treatment T_{11}*i.e.* GA_3 @ 100 ppm and the minimum was observed in treatment T_{12}*i.e.* control. The maximum zinc content (48.02 ppm) was obtained in treatment T_4*i.e.* $ZnSO_4$ @ 1.0 per cent and the treatment T_3*i.e.* $ZnSO_4$ @ 0.75 per cent (46.87 ppm) was at par with the maximum value (48.02 ppm). The minimum zinc content (31.12 ppm) was observed in treatment T_{12}*i.e.* control. Similar results were reported by Wali and Sharma (1997) in Kinnow, Nirmaljit *et al.* (2005) in Kinnow leaves, Prasad *et al.* (2017) in Kinnow mandarin, Lal *et al.* (2000) in guava, Jeyabhaskaran and Pandey (2008) in Banana and Sharma *et al.* (2009) in ber.

Table 4 : Effect of zinc sulphate and plant growth regulators on leaf nutrient content in guavacv. Hisar Surkha

Treatments	N (%)	P (%)	K (%)	Zn (ppm)
T_1 $ZnSO_4$ @ 0.25%	1.63	0.16	1.14	42.41
T_2 $ZnSO_4$ @ 0.50%	1.64	0.16	1.16	44.71
T_3 $ZnSO_4$ @ 0.75%	1.67	0.18	1.17	46.87
T_4 $ZnSO_4$ @ 1.0%	1.69	0.17	1.21	48.02
T_5 NAA @ 25 ppm	1.66	0.16	1.20	39.16
T_6 NAA @ 50 ppm	1.70	0.19	1.22	38.72
T_7 NAA @ 75 ppm	1.67	0.20	1.23	34.69
T_8 NAA @ 100 ppm	1.61	0.22	1.26	34.12
T_9 GA_3 @ 50 ppm	1.71	0.15	1.27	32.85
T_{10} GA_3 @ 75 ppm	1.76	0.18	1.30	35.39
T_{11} GA_3 @ 100 ppm	1.78	0.23	1.31	37.35
T_{12} (Control)	1.61	0.18	1.13	31.12
CD @ 5%	0.07	0.02	0.06	1.69

Conclusion

Micronutrients like zinc and plant growth regulators like NAA and GA_3 play an important role in growth, and development and cause efficient quality and leaf nutrient status improvement. Results revealed that NAA @ 100 ppm improved the TSS, acidity, TSS/Acid ratio, total sugars, reducing sugars and non-reducing sugars content over control. However, GA_3 improved pectin content, ascorbic acid content and leaf nutrient status of guava. So, there is need to disseminate the improved technologies of foliar application of guava among the farmers with effective extension methods like front-line demonstration and others etc.

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