PLANT SCIENCE TODAY
Vol 12(sp1): 01–13
https://doi.org/10.14719/pst.8851

eISSN 2348-1900

RESEARCH ARTICLE

Optimization of zinc oxide nanoparticle dosage in guava (*Psidium guajava* L.) for enhanced yield, nutritional quality, antioxidant activity and economic performance

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Received: 13 April 2025; Accepted: 29 June 2025; Available online: Version 1.0: 22 September 2025

Cite this article: Sampurna NS, Navin S, Alok SJ, Kuldeep KS, Pawan S, Kusum K, Chinmaya J, Usha S, Pavan S. Optimization of zinc oxide nanoparticle dosage in guava (*Psidium guajava* L.) for enhanced yield, nutritional quality, antioxidant activity and economic performance. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.8851

Abstract

Zinc (Zn) is the most deficient micronutrient among all and it also holds importance in human nutrition. The role of nanoparticles in meeting the micronutrient demands of horticultural crops is well known. In view of this, five levels of Zinc oxide nano particles (ZnONP), including a control, were used, with two varieties (i.e., VNR Bihi and Pant Prabhat), in a randomised block design replicated three times. VNR Bihi recorded significantly higher yield attributing characteristics and fruit yield compared to Pant Prabhat, whereas an opposite trend was recorded for the quality and biochemical parameters. Among all the levels of ZnONP, 150 ppm recorded the highest values of fruit yield; however, it remained statistically at par with 100 ppm. Increasing dosage to 200 ppm recorded loss in the fruit yield by 6.3 % reduction compared to 100 ppm. TSS, total sugars, ascorbic acid, total phenols, flavonoids and antioxidant activity were recorded as highest at 150 ppm, comparable to 100 ppm. The economic analysis revealed that a 100 ppm ZnONP spray can earn USD 961 ha^{-1} more than the control (no spray). B:C ratio (1.69) also recorded markedly highest value with 100 ppm. The study has shown that a ZnONP spray at a dosage of 100 ppm is the most optimal for guava production in India.

Keywords: antioxidant activity; economics; guava; quality; yield; zinc oxide nano particles

Introduction

Guava (*Psidium guajava L*), also known as the “Apple of the tropics”, being originated in the Tropical America; however, it has naturalized across the world owing to its ability to withstand the extremes of the weather, except extreme frost (1).The crop is preferably grown in a pH range of 4.5-9.4 (2).The nutritional quality of fruit includes higher vitamin C content ranging from 50-300 mg 100 g^{-1} of fresh fruit, rich source of thiamine, potassium, B -complex vitamins, riboflavin, carbohydrates, magnesium, copper, manganese, pectin, fibres and antioxidants (3-5). The higher pectin content of the fruit makes enhances its suitability for the preparation of jam, jelly, nectar, cider, ketchup, juices, powder and other processed products. Biologically active compounds in the fruit has tremendous therapeutic and medicinal potential and even barks, leaves and roots are utilized for the curing of cough, vomiting, gastroenteritis, wounds, diarrhoea, sore throat, toothache and inflamed gums and to increase the platelets count in the patients suffering from dengue (6, 7).The total production of guava in India stands at 4.6 MT, with Uttar Pradesh, Madhya Pradesh, Bihar and Andhra Pradesh contributing more than 50 % of the total production (8). Although India is the worlds’ largest producer of guava, accounting for 45 % of the total production, it is still hindered by various constraints that impede its productivity. It owes to the various issues faced by growers, ranging from the availability of improved planting material to the marketing of the harvest. Among the various studies conducted, plant nutrition (non-availability of fertilisers or lack of knowledge about their application) has been identified as one of the major constraints (9-11). Nitrogen (N), phosphorus (P) and potassium (K) are the most important nutrients required in higher quantities; however, nowadays, deficiency of micronutrients is becoming more common in Indian soils (12). These include Zinc (Zn), iron (Fe), manganese (Mn), boron (B), copper (Cu) and molybdenum (Mo), of which first three posing threats to sustenance of food productivity (13). Additionally, it has been observed that guava exhibits a notable response to the application of Zn, B, Cu and Mn in terms of yield and quality (14).

Zinc plays an essential role in enzyme activation, is a structural component of the ribosome, thus facilitating protein synthesis and regulates auxin activity and carbohydrate metabolism (15-17). Additionally, it exhibits synergistic interactions with N, K and Mn in plants (18, 17). In guava, Zn deficiency is manifested in form of leaf size reduction, interveinal chlorosis, growth retardation and dieback (19, 20). Almost 50 % of the worlds' soils are deficient in Zn, which is the most deficient micronutrient in Indian soils and is now recognised as the fourth most important yield-limiting nutrient, after the three primary nutrients (N, P and K) (21, 22, 12).The current study location has previously been classified among the highly deficient districts (18 -20 %) of the state (15). The essentiality of this nutrient and the simultaneous deficiency call for its external supply to meet its requirement. Foliar application of micronutrients is effective for maintaining plant status during the growing season, thus correcting immediate deficiencies and avoiding yield or quality loss. It also allows for multiple applications after planting (19). Foliar application of Zn leads to an increase in yield parameters, fruit yield, leaf NPK and Zn content and quality parameters of guava, including total soluble sugars, sugar-to-acid ratio and pectin (14, 23-25). Nanofertilizers represent a new dimension in nutrient delivery for agriculture, being not only cost-effective but also environmentally friendly (26, 27). It owes its benefits to its high surface area-to-volume ratio, higher penetration ability, controlled and timely release, higher stability, solubility, targeted delivery at optimal concentration, safe and easy disposal and reduced toxicity (28, 29).In horticultural crops, nano-fertilisers are reported to enhance the quality of the harvest. Zinc oxide nanoparticles (ZnO NPs) are reported to increase crop yields, as well as fruit quality, total soluble sugars (TSS), antioxidant activity, total protein content, biomass accumulation and chlorophyll content (26, 30).

There is considerable work done on the effect of foliar spray of zinc fertilizers on guava in terms of the fruit yield and quality. However, the work on the use of Zn/ZnO nanofertilizers is generally lacking and this is particularly true for the Tarai region (experimental location). Recently, research indicates that nano Zn on guava crop encourages the growth and quality characteristics of guava (31, 32). ZnONP is suggested to provide the near-required Zn ions to the crop; however, optimising its dosage for a particular crop is still needed (33). To address this gap, the current experiment was designed with two popular cultivars *i.e.*, VNR Bihi and pant Prabhat combined with four graded levels of ZnO nanoparticles *i.e.*, 50 ppm, 100 ppm, 150 ppm and 200 ppm, so that their effect on the yield and quality of the guava crop can be analyzed and an economic dose can be recommended for the growers of the region.

Materials and Methods

Experimental site

The research was conducted at the Horticulture Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, during the years 2021-22 and 2022-23. The experimental site is located in a humid sub-tropical climate experiencing weather extremes, with an average annual rainfall of 1450 mm and maximum temperatures of 40.2 ˚C and 4.3 ˚C, respectively. The weather conditions during the flowering to fruit harvest are presented in Fig. 1. It shows that the average temperature varied from 12.1 to 31 ˚C and from 9.8 to 30.2 ˚C during the 2021-22 and 2022-23 periods, respectively. The total rainfall received was 1153.3 mm and 1809.1 mm during the 2021-22 and 2022-23 seasons.

Experimental design and details

The experiment was laid out in a randomised block design with a factorial arrangement, with two factors: one with two levels, i.e., cultivars VNR Bihi and Pant Prabhat and another with five levels of ZnONP, i.e., control (No ZnONP treatment), 50 ppm, 100 ppm, 150 ppm and 200 ppm. The treatments were replicated thrice. ZnONP was procured from Nano Research Laboratory (NRL), Jamshedpur, Jharkhand. The experiment involved 6–7-year-old guava cultivars planted at a 5m x 3m spacing under a high-density planting system with a ring basin irrigation system. Fertility management and other agricultural practices were consistent across all trees. Only healthy, uniform and vigorously growing guava plants were selected for foliar application and were considered as part of the experiment. ZnONP was sprayed

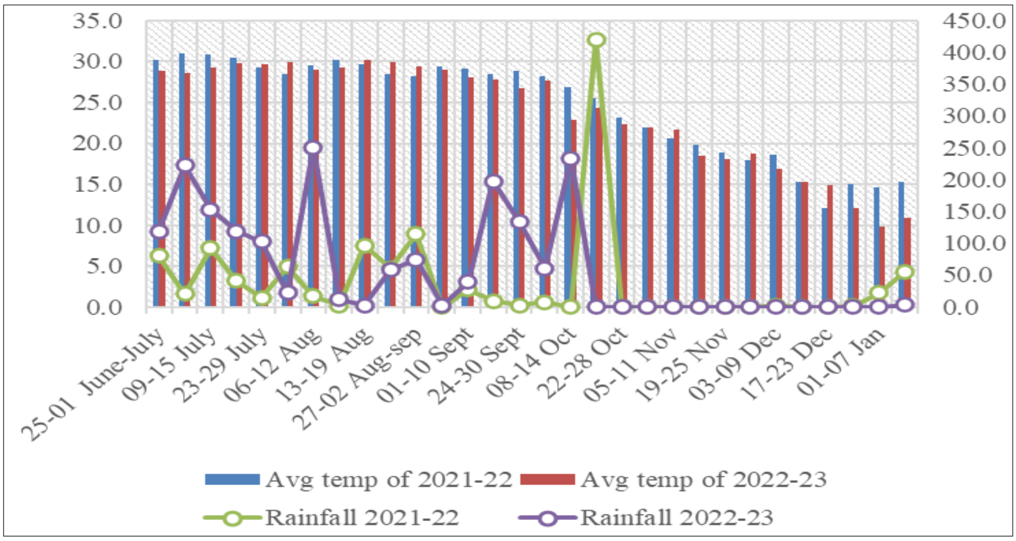


Fig. 1. Weekly weather data for the cropping season Rabi 2021-22 and Rabi 2022-23. Standard meterological week on X-axis. Temperature on Y axis (left) and Rainfall on Y axis (right).

at the pea stage (21st September and 28th September during the first and second years of experimentation, respectively) at a rate of 6 L tree^{-1}. The fertilisation solution was prepared by mixing a commercially available product with the well water available at the experimental site.

Fruits and leaves sampling

Mature leaves of guava trees were collected from the middle part of the fruiting shoot during first week of December during both years of experimentation. Guava fruits were harvested on December 24th and December 29th during the first and second year of the experiment, respectively. The harvested fruits and leaves were then taken to the laboratory for analysis of their physical, chemical and nutritional quality.

Leaf and fruit nutrients

Each sample (both leaf and fruit) was washed with tap water, dilute hydrochloric acid and distilled water, followed by shade drying, oven drying at 72 °C for 24 hr, ground to fine powder. These were further passed through 1 mm sieve and mixed homogenously. The sample were digested in sulfuric acid and digestion mixture (K_2SO_4 + $CuSO_4$ + Se powder) for N estimation and with diacid mixture (HNO_3: $HClO_4$ in 4:1) for estimation of other nutrients. N, P and K were estimated following Micro-kjeldahls' method, Molybdophosphoric acid method and flame photometric method, respectively (34). Zn, Fe, Mn and Cu were estimated using the absorption spectrophotometer method (AAS) (35).

Yield and physical properties of fruits

To calculate the fruit drop, fruit retention was first calculated as per the standard procedure (36):

$$\text{Fruit retention (\%)} = \frac{\text{Total number of fruits harvested} \times 100}{\text{Total number of fruits set}}$$

(Eqn. 1)

Thereafter, fruit drop was calculated as:

Fruit drop (%) = 100 - fruit retention (%) (Eqn. 2)

The total number of fruits tree^{-1} was counted manually at the time of harvesting. The yieldtree^{-1} was then calculated by multiplying the number of fruittree^{-1} with the average weight of each fruit (g). This was finally expressed in kg tree^{-1}.

Five fruits were selected from each tree to assess their physical properties. The weight of the fruit was measured using an electronic balance. The fruit volume of ten randomly collected fruits from each tree in every replication was measured using the water displacement method. The resulting data was used to calculate the average fruit volume, expressed in cubic centimeters (cm^3).

Fruit quality parameters

The fruit quality parameters analyzed were total soluble solids (TSS), total sugars, ascorbic acid content and titratable acidity. Fruits were selected for extracting juice by using a muslin cloth. TSS content of the guava juice was measured using an Erma Hand Refract meter and expressed in °B (degrees Brix). The TSS values were adjusted for a temperature of 20 °C using the temperature correction chart specified in the (37).The process for calculating total sugars followed the standard methodology of (37).The fresh juice was dilute ten times, followed by precipitation with 2 ml lead acetate (45 %). The excess lead was removed with potassium oxalate, followed by acid hydrolysis of solution overnight with hydrochloric acid and neutralization with 40 % sodium hydroxide. This neutralized solution was titrated against the Fehling solutions (A & B) and methylene blue was added as an indicator. The endpoint reading was noted when the solution turned brick-red in color. Total sugar was calculated as following:

$$\text{Total Sugar (\%)} = \frac{\text{Fehlings factor (0.05)} \times \text{final volume made}}{\text{Titre value} \times \text{Weight of sample}} \times 100$$

(Eqn. 3)

Titratable acidity was determined by visual titration method described by (38). The ascorbic acid content of guava fruits was measured using the 2,6-dichlorophenol indophenol visual titration method (38).

Total antioxidant activity

Total antioxidant activity included total phenol content, flavonoid content and antioxidant activity, The total phenolic content of fresh samples of guava fruit was measured using Folin -Ciocalteau method, while the total flavonoid content was measured according to the procedure described by (39, 40). The antioxidant activity was assessed using the 2, 2-diphenyl -1-picrylhydrazyl (DPPH) followed by absorbance estimation at 517 nm using spectrophotometer (41). Antioxidant activity was measured using the following formula:

$$\text{Anitoxidant activity (\%)} = [1 - \frac{\text{Sample absorbance}}{\text{Control absorbance}}] \times 100$$

(Eqn. 4)

Economic analysis

Economic analysis was carried out for each treatment. The cost of cultivation included the expenses towards the various agronomic operations, pest management, rental cost of orchard for one year, cost of treatment. Gross returns were calculated on the basis of average retail price of two varieties prevailing in particular harvest season in the growing area. Net returns and Benefit-cost ratio (B:C ratio) were calculated using the following formulae:

Net returns (USD ha^{-1}) = Gross returns (USD ha^{-1}) - Cost of cultivation (USD ha^{-1})

$$\text{B:C ratio} = \frac{\text{Net returns}}{\text{Cost of cultivation}}$$

(Eqn. 5)

Partial budget analysis was carried out for treatments having ZnONP spray *w.r.t.* control (41). Fixed cost included the cost which were basic to each treatment and the variable cost included the cost of ZnONP usage and associated labor cost for spray. Rate of return (RR) was calculated as:

$$\text{RR} = \frac{\triangle \text{NR}}{\triangle \text{VC}}$$

(Eqn. 6)

$\triangle$NR= Change in net return

$\triangle$VC= Change in variable cost

Acceptance for a treatment was give when RR>1.

Statistical analysis

The data from both the years were pooled, statistical analysis was carried out following standard procedure of "Analysis of variance (ANOVA)" as outlined by (42). Least significant difference (LSD) test was carried out for determining significance of mean values using R Studio version 2022.07.1. The graphical presentation of the data is done utilizing R Studio version 2022.07.1

Results

Leaf nutrient content

The nutrient content was significantly affected by the variety selection (Table 1). For all the nutrients analysed, i.e., N, P, K and Zn, significantly higher values were recorded with Pant Prabhat. Among the factors B, the highest values of N and K content were recorded with the spray of 150 ppm ZnONP, whereas the highest dosage of 200 ppm recorded the significantly highest value of Zn content in the leaves. Each increment of 50 ppm concentration resulted in a significant increase in Zn content in the leaf.

Fruit drop and number of fruits per tree

The fruit drop was inversely proportional to the dosage of ZnONP for both the varieties, except 200 ppm which recorded values more than 100 and 150 ppm (Fig. 2). Significantly higher fruit drop was recorded VNR Bihi (39.9 %), compared to Pant Prabhat (27.6 %) (Fig. 3). Fruit drop was inversely proportional to the level of ZnONP spray upto 150 ppm, following trend as 150ppm (30.0 %) <100ppm (31.6 %) <200ppm (32.8 %) <50ppm (34.8 %) < Control (39.6 %). The two lowest values were statistically equivalent to each other.

The interaction effect was significant for the number of fruits per tree (Fig. 4). The variety 'Pant Prabhat' recorded significantly higher values with all the levels of ZnONP compared to the variety "VNR Bihi". Pant Prabhat with foliar spray of 150 ppm ZnONP recorded the significantly highest value, followed by spray of 100 ppm ZnONP with same variety, both being statistically at a par with each other. Pant Prabhat + 150 ppm ZnONP recorded 39 % higher value compared to the VNR Bihi + 150 ppm ZnONP, even the former without any ZnONP spray (control treatment) recorded 3.3 % higher value compared to latter. Both varieties, with 150 ppm ZnONP spray, recorded 34.3 % and 17.2% higher values compared to the control treatment for Pant Prabhat and VNR Bihi, respectively.

Fruit weight, volume and yield

Fruit weight, volume and yield were markedly influenced by the choice of variety as well as different levels of ZnONP spray (Fig. 5). All the three parameters had significantly higher value with VNR Bihi compared to Pant Prabhat. The corresponding values of fruit weight (534.4 g), volume (477.5 cm^3) and yield (43.0 kg $tree^{-1}$) for VNR Bihi were 134.6 %, 168.6 % and 74.8 % higher compared to those with Pant Prabhat (227.8 g, 177.8 cm^3, 24.6 kg $tree^{-1}$), respectively.

Among the different levels of ZnONP, 150 ppm recorded highest values of the fruit weight (396.7 g), volume (345.0 cm^3) and yield (38.0 kg $tree^{-1}$), however remained at par with 100 ppm. Compared with these two, significantly lower values were observed with 200 ppm spray which were 3.5 %, 3.7 % and 10.3 % lower for fruit weight, volume and yield, respectively. The fruit yield increased over control treatment (No spray of ZnONP) by 16.5 %, 30.9 %, 36.7 % and 22.7 %, with 50 ppm, 100ppm, 150

Table 1. Effect of different varieties and different levels of Zn application on the leaf N, P, K and Zn content

Treatments	Leaf N content (%)	Leaf P content (%)	Leaf K content (%)	Leaf Zn content (ppm)
Factor A (Varieties)				
Pant Prabhat	2.27^{a}	0.24^{a}	1.21^{a}	54.2^{a}
VNR Bihi	2.20^{b}	0.23^{b}	1.17^{b}	51.2^{b}
LSD (p= 0.05)	0.043	0.003	0.023	0.84
Factor B (Levels of Zn application)				
Control	2.02^{d}	0.23	1.05^{d}	46.4^{e}
50 ppm	2.18^{c}	0.24	1.17^{c}	50.5^{d}
100 ppm	2.33^{ab}	0.23	1.25^{ab}	53.1^{c}
150 ppm	2.37^{a}	0.23	1.27^{a}	55.6^{b}
200 ppm	2.26^{b}	0.23	1.21^{b}	57.8^{a}
LSD (p= 0.05)	0.068	NS	0.037	1.33

*Data is represented as a mean. Different letters in a column represent significant difference (higher to lower as letters occur in the alphabet a-z) among the treatments, at P < 0.05. N: Nitrogen; P: Phosphorus; K: Potassium; Zn: Zinc; ppm: parts per million

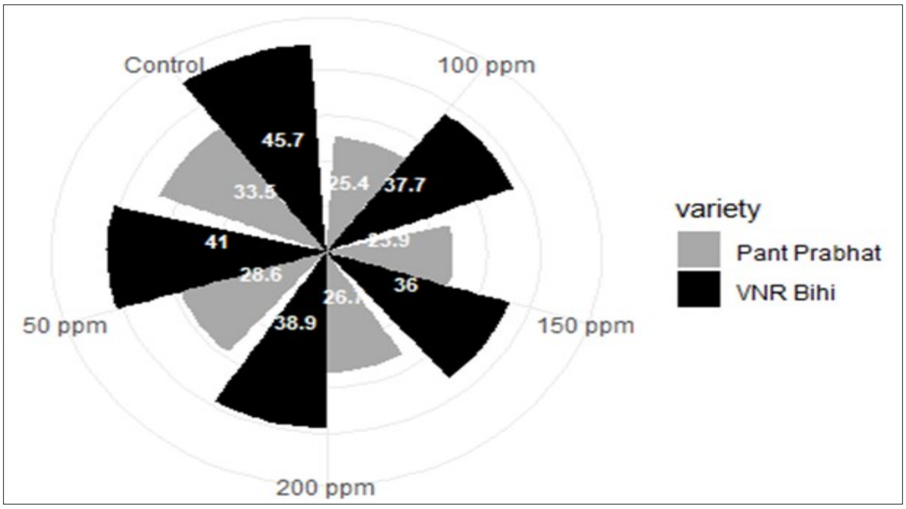


Fig. 2. Polar bar chart showing fruit drop (%) as influenced with the ZnONP dosage for the two varieties.

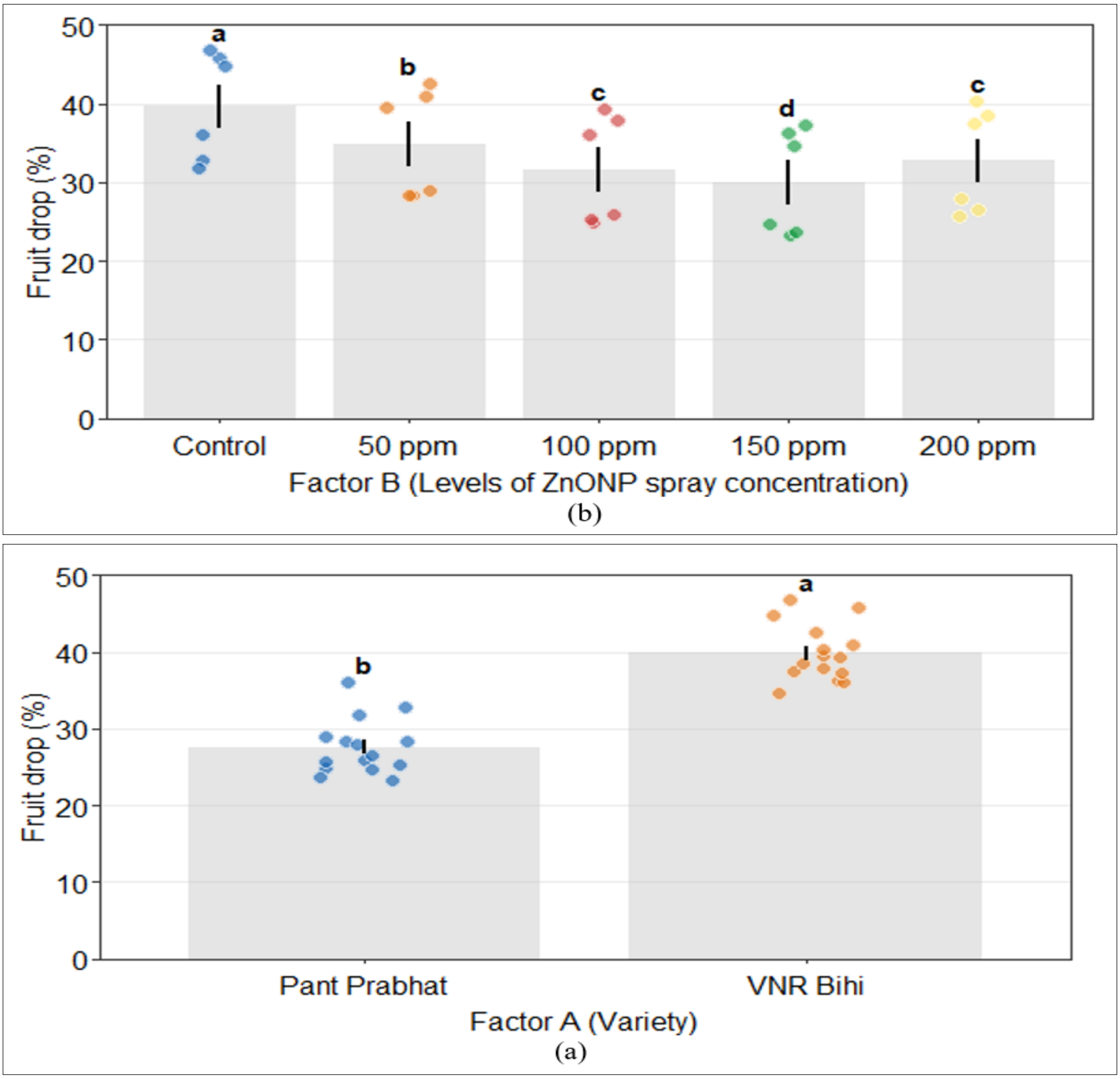


Fig. 3. Fruit drop (%) as influenced by (a) different varieties (b) different levels of ZnONP spray concentrations. Error bars represent standard error of mean.

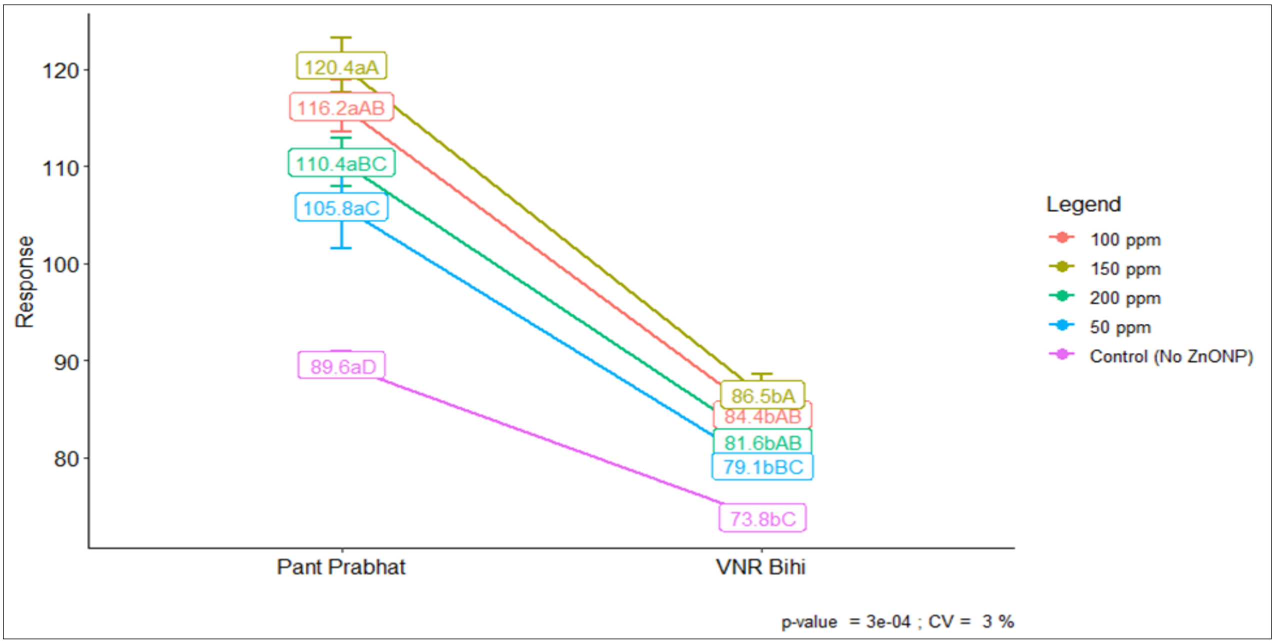


Fig. 4. Interaction between Factor A (varieties) and Factor B (Levels of ZnONP spray concentration) on the number of fruits per tree. Response represents "Number of fruits per tree". Error represents standard error of mean.

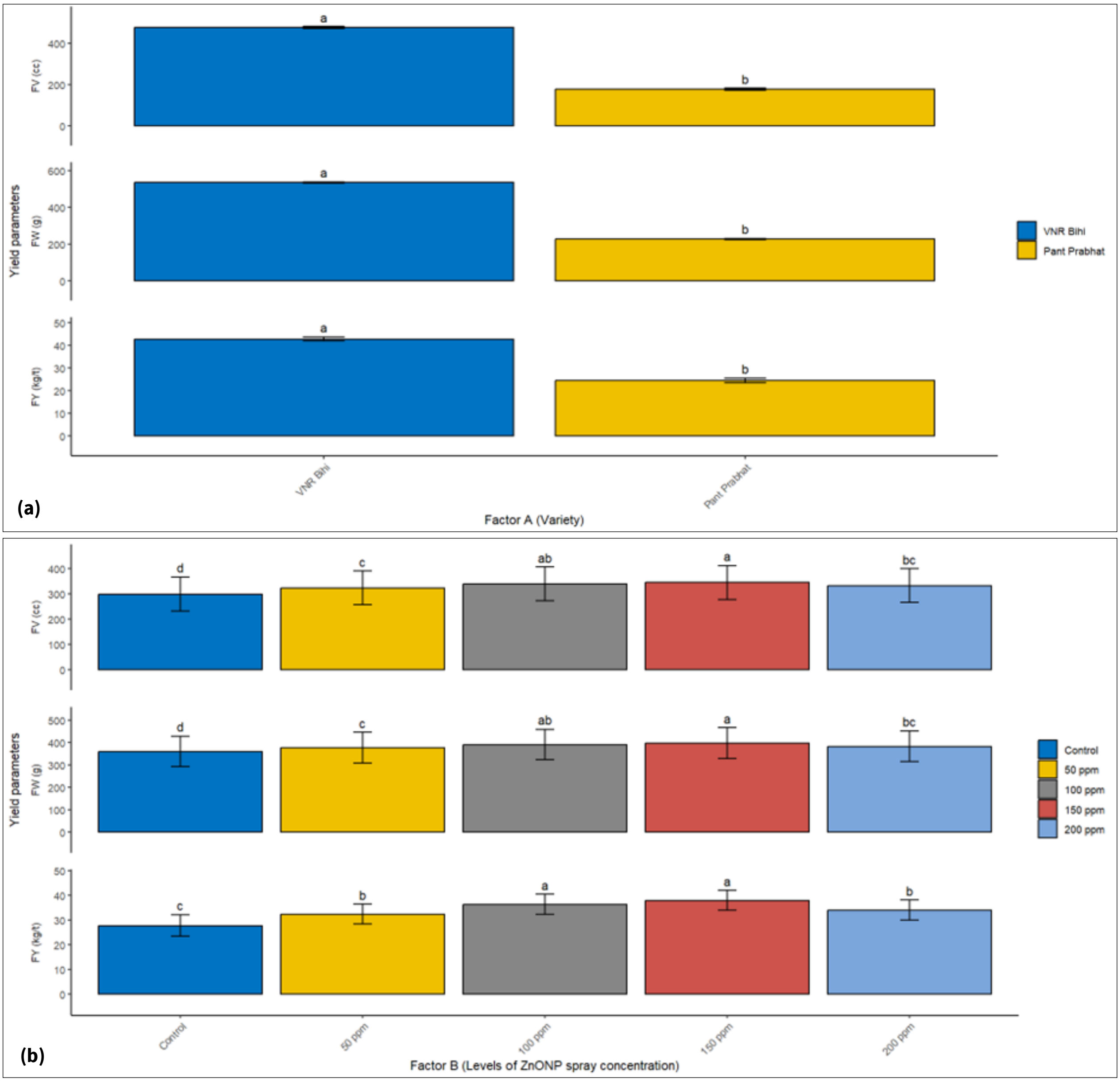


Fig. 5. Fruit volume, fruit weight and fruit yield as influenced by (a) different varieties; (b) different levels of ZnONP spray concentrations. Error bars represent the standard error of mean. FY (kg/t): Fruit yield (kg/ tree); FW(g): Fruit weight (grams); FV (cc): Fruit volume (cm^3).

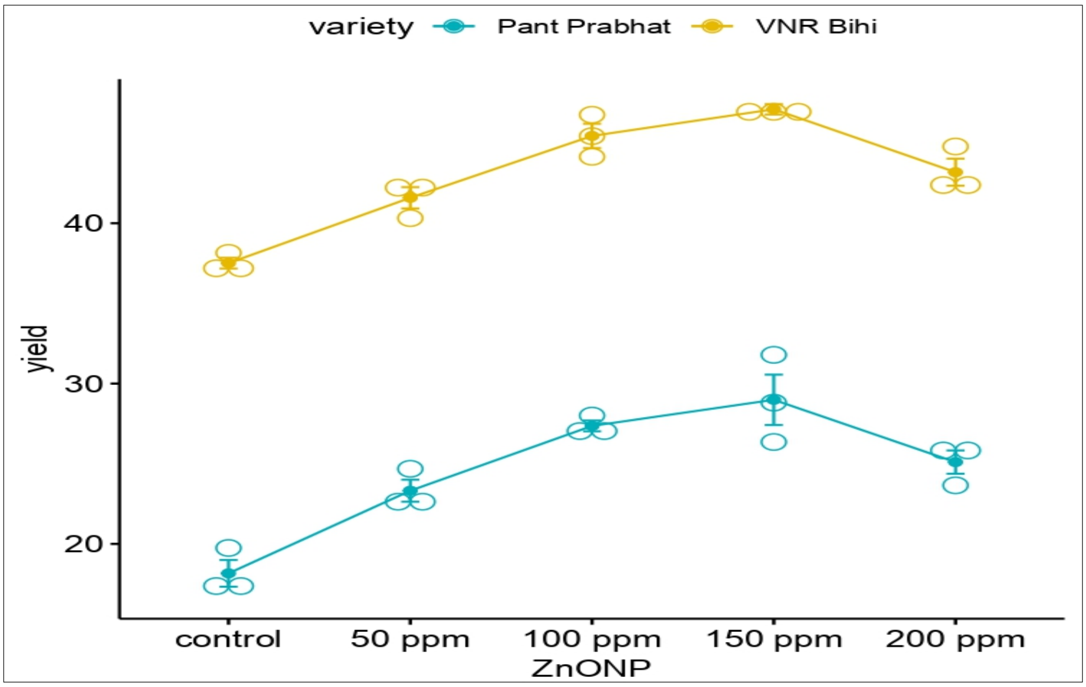


Fig. 6. Fruit yield (kg/ tree) as influenced by different combinations of variety and level of ZnONP spray concentration.

ppm and 200 ppm, respectively. The highest yield was recorded by the variety "VNR Bihi" with 150 ppm concentration of ZnONP spray (Fig. 6).

Fruit nutrient content

Fruit NPK and Zn content were significantly higher with Pant Prabhat as compared to that with VNR Bihi (Table 2). Increase in spray concentration of ZnONP increased N and K concentration till 150 ppm, recording 51.3 % and 14.3 % higher values, respectively, compared to control treatment. Different levels of ZnONP spray didn't have any marked effect on fruit P content, whereas, with each increased dosage there was significant increase in fruit Zn content, recording highest value with 200 ppm spray.

Fruit quality parameters

Between the two varieties selected for the study, significantly higher values of TSS, total sugar and ascorbic acid content were observed with Pant Prabhat compared to those with VNR Bihi (Fig. 7). The acidity content of the fruit recorded vice versa values for these two varieties. Increase in ZnONP spray dosage increased the TSS, total sugars and ascorbic acid contents, recording highest values with 150 ppm (13.1 ˚brix, 9.5 % and 181.6 mg $100g^{-1}$, respectively), remaining comparable to that with 100 ppm (12.8 ˚brix, 9.3 % and 179.3 mg 100 g^{-1}, respectively). The values declined with further increase in ZnONP spray dosage. Markedly lowest values of acidity were observed with 150 ppm (0.37 %), remained statistically at par with 100 ppm (0.38 %), whereas it was highest with control treatment (0.52 %).

Total antioxidant activity

The components of total antioxidant activity are total phenols, total flavonoids and antioxidant activity (Table 3). Pant Prabhat recorded 12.5 %, 8.6 % and 3.9 % higher activities of total phenols, total flavonoids and antioxidant activities compared to those with VNR Bihi.

Among different levels of ZnONP sprays, the total antioxidant activity markedly increased with each 50 ppm increase in spray concentration, except for a decrease observed with the increase from 150 ppm to 200 ppm. Though highest values were recorded with 150 ppm, these remained statistically at a par with 100 ppm spray. The increase in activities of total phenols, total flavonoids and antioxidant activity with 100 ppm spray were 17.9 %, 39.4 % and 15.5 %, respectively, higher than the control treatment (without ZnONP spray).

Economic analysis

Economic analysis of the two varieties and different levels of ZnONP is presented in Fig 8. Cost of cultivation increased with the increase in spray concentration of ZnONP following the trend as, control (USD 1668.9 ha^{-1}) < 50ppm (USD 1811.5 ha^{-1}) <100 ppm (USD 1930.6 ha^{-1}) < 150 ppm (USD 2049.6 ha^{-1}) < 200 ppm (USD 2168.7 ha^{-1}). Higher values of gross returns and net returns were recorded with VNR Bihi (USD 6138.1 $ha^{-1}$and USD 4212.2 ha^{-1}, respectively) compared to Pant Prabhat (USD 3512.2 $ha^{-1}$and USD 1586.3 ha^{-1}, respectively). B:C ratio recorded similar trend with VNR Bihi (2.20) recording 168.3 % higher value compared to Pant Prabhat (0.82).

There was marked influence of ZnONP spray on the gross returns and net returns of guava cultivation. Maximum values were recorded with 150 ppm spray (USD 5435.2 $ha^{-1}$and USD 3385.6 ha^{-1}, respectively), followed non-significantly by 100 ppm spray (USD 5200.4 $ha^{-1}$and USD 3269.8 ha^{-1}, respectively). Latter treatment (100 ppm spray) paid additional net income of USD 962 ha^{-1} compared to no spray of ZnONP (control), whereas, doubling the spray concentration (200 ppm) led to the loss of USD 561 ha^{-1} in net returns, compared to 100 ppm spray. ZnONP spray @ 100 ppm recorded highest value of B:C ratio i.e., 1.69, followed by 150 ppm (1.65), both remained statistically at par

Table 2. Effect of different varieties and different levels of Zn application on the fruit N, P, K and Zn content

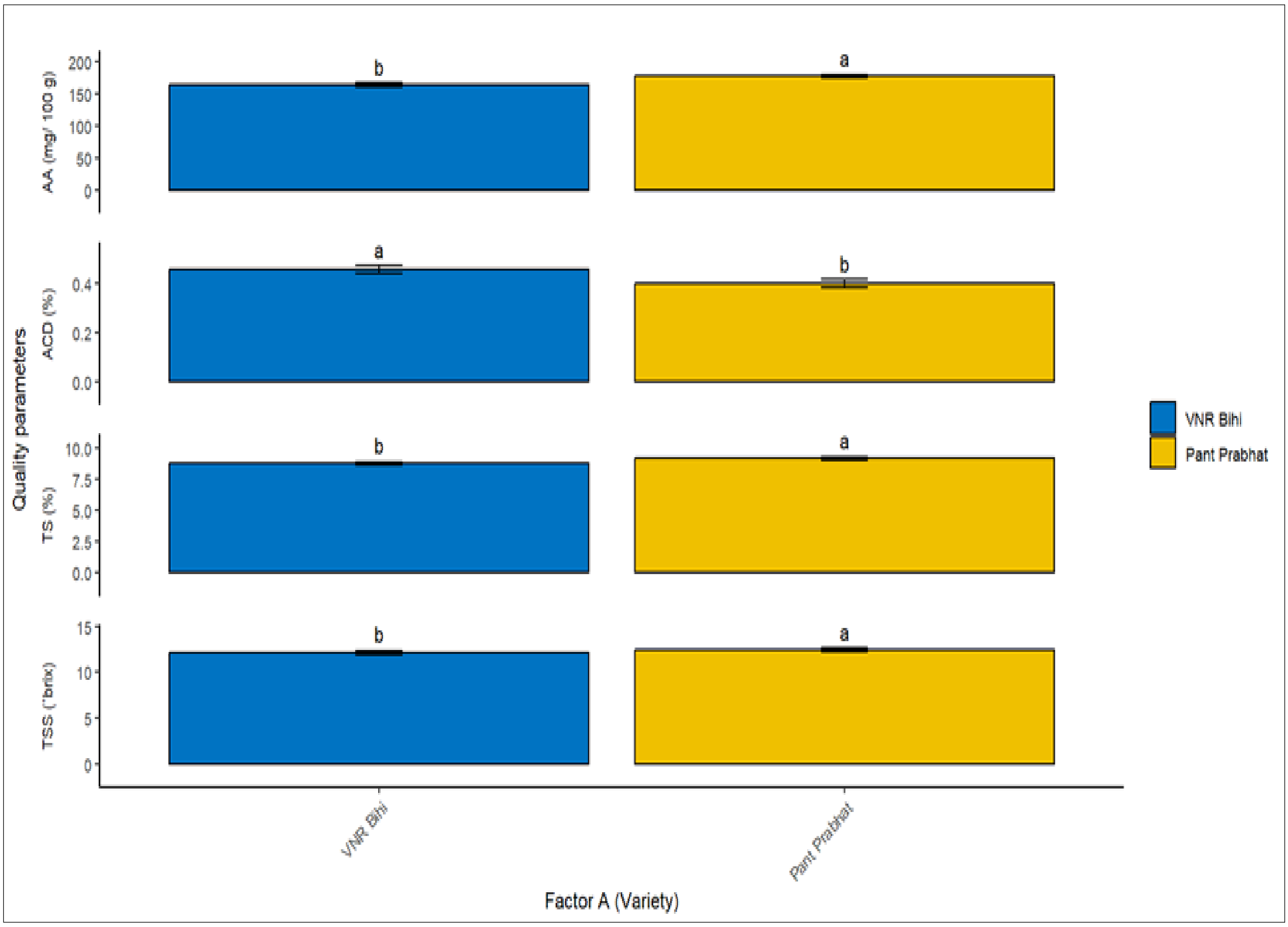
Treatments	Fruit N content (%)	Fruit P content (%)	Fruit K content (%)	Fruit Zn content (ppm)
Factor A (Varieties)				
Pant Prabhat	1.05^{a}	0.14^{a}	0.62^{a}	20.9^{a}
VNR Bihi	1.01^{b}	0.12^{b}	0.60^{b}	19.0^{b}
LSD (p= 0.05)	0.022	0.003	0.010	0.54
Factor B (Levels of Zn application)				
Control	0.78^{e}	0.13	0.56^{d}	14.2^{e}
50 ppm	0.97^{d}	0.13	0.59^{c}	17.9^{d}
100 ppm	1.14^{b}	0.13	0.63^{a}	20.2^{c}
150 ppm	1.18^{a}	0.13	0.64^{a}	22.7^{b}
200 ppm	1.07^{c}	0.13	0.62^{b}	24.8^{a}
LSD (p= 0.05)	0.036	NS	0.015	0.85

*Data is represented as mean. Different letters in a column represent significant difference (higher to lower as letters occur in alphabet a-z) among the treatments, at P < 0.05. N: Nitrogen; P: Phosphorus; K: Potassium; Zn: Zinc; ppm: parts per million

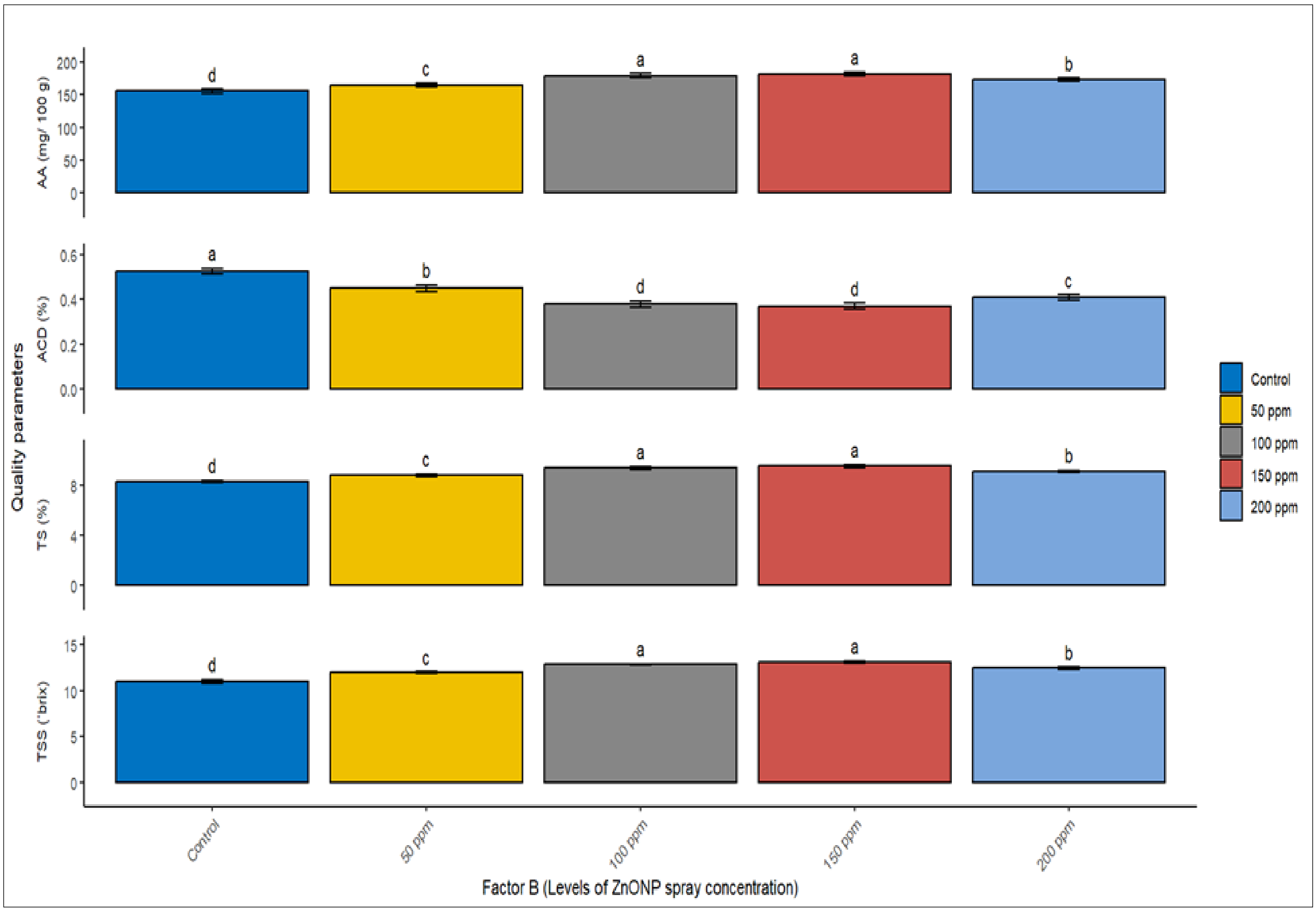
Table 3. Effect of different varieties and different levels of Zn application on the fruit antioxidant activity

Treatments	Total phenols (mg 100 g^{-1})	Total flavonoids (mg 100 g^{-1})	Antioxidant activity (%)
Factor A (Varieties)			
Pant Prabhat	175.8^{a}	22.6^{a}	97.7^{a}
VNR Bihi	156.2^{b}	20.8^{b}	94.0^{b}
LSD (p= 0.05)	2.7	0.4	1.0
Factor B (Levels of Zn application)			
Control	148.0^{d}	17.0^{d}	86.8^{d}
50 ppm	161.7^{c}	20.8^{c}	93.8^{c}
100 ppm	174.5^{a}	23.7^{a}	100.3^{a}
150 ppm	177.5^{a}	24.2^{a}	101.2^{a}
200 ppm	168.5^{b}	22.8^{b}	97.0^{b}
LSD (p= 0.05)	4.2	0.6	1.6

*Data is represented as mean. Different letters in a column represent significant difference (higher to lower as letters occur in alphabet a-z) among the treatments, at P < 0.05.

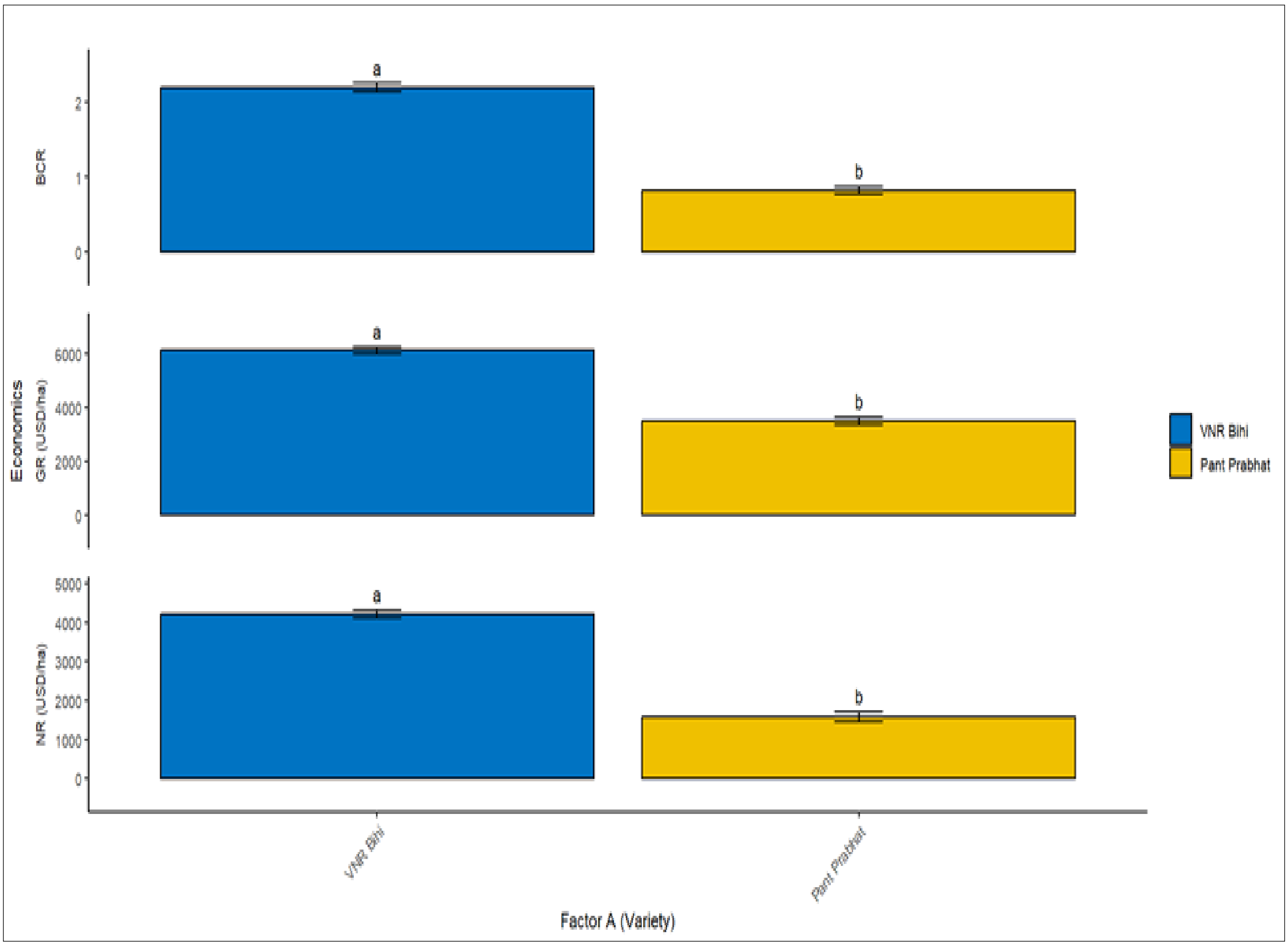


(a)

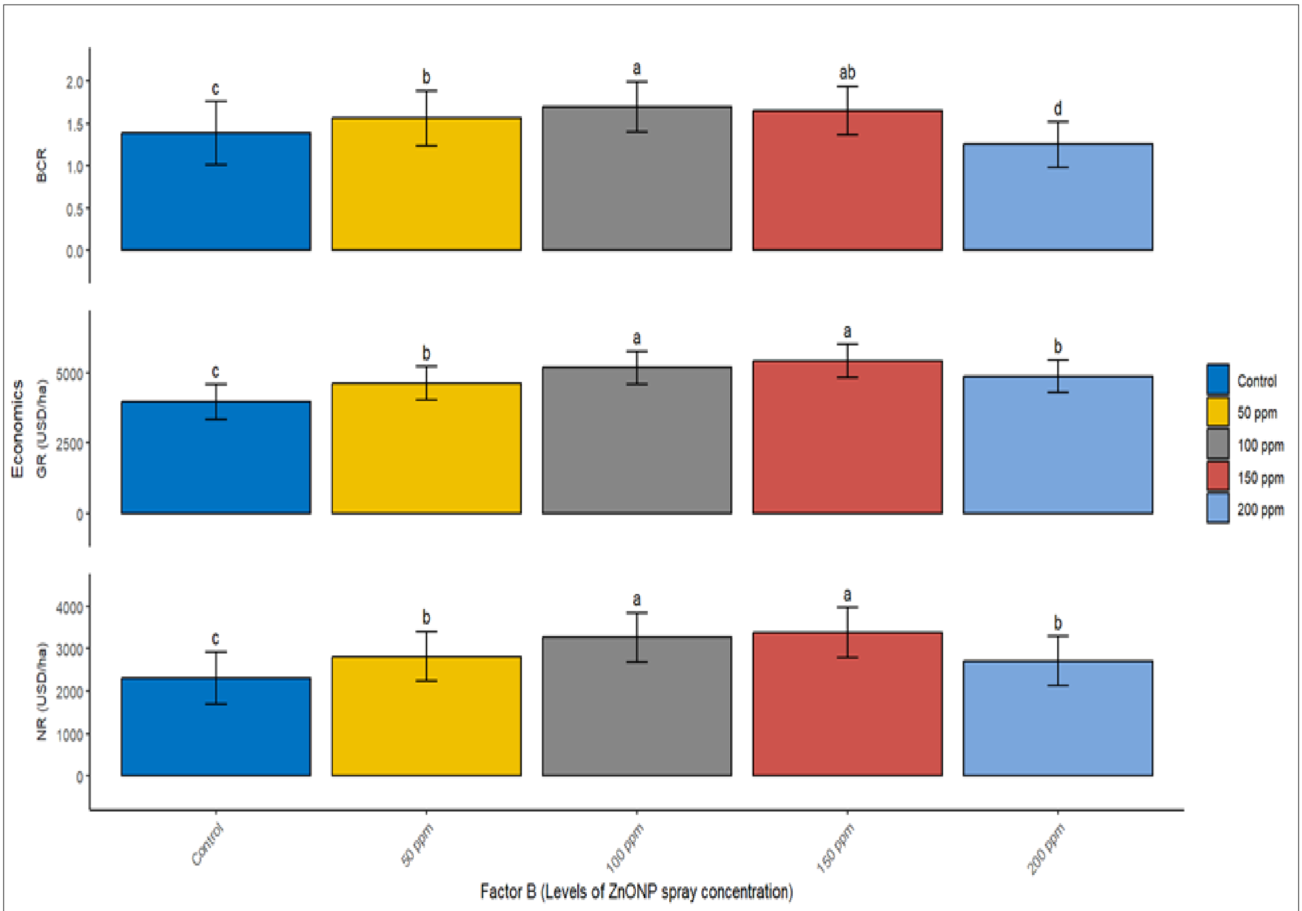


(b)

Fig. 7. Fruit quality parameters as influenced by (a) different varieties (b) different levels of ZnONP spray concentrations. Error bars represent standard error of mean. TSS: Total soluble solids; TS: Total sugar; ACD: acidity; AA: Ascorbic acid content.



(a)



(b)

Fig. 8. Economics as influenced by (a) different varieties; (b) different levels of ZnONP spray concentrations. Error bars represent standard error of mean. GR: Gross returns; NR: Net returns; BCR: BC ratio; USD: US dollars.

with each other and were 35.2 % and 32.0 % higher over 200 ppm spray of ZnONP. Partial budget analysis shows that all the levels of ZnONP spray concentrations are acceptable compared to control except that of 200 ppm, for both varieties (Table 4). However, among the acceptable treatments, the highest RR was recorded by 100 ppm in VNR Bihi whereas, it was for 50 ppm in Pant Prabhat closely followed by 100 ppm with a difference of only 5 %.

Discussion

Zn nutrition is a one of the major emerging challenges in the field of agriculture including horticulture. The current study focused on optimising the dose of ZnONP in two prominent varieties cultivated in India, in relation to its impact on fruit yield parameters, yield, nutrient uptake, quality parameters, enzyme activity and its economic analysis. VNR Bihi is characterized by the larger size fruit, the yield maximizing upto 25 t ha^{-1} (43). Comparatively, lesser sweetness of VNR Bihi attracts diverse consumers. On the other hand, Pant Prabhat is characterized by higher TSS level, with balanced acidity making it preferable for the dessert and fresh consumption (44). For the different treatments comprising ZnONP, the results revealed that increasing the Zn dosage in both varieties increased the number of fruits per tree, simultaneously reducing the fruit drop. Zn plays a vital role in the biosynthesis of IAA from tryptophan, as well as in increasing the concentration of endogenous gibberellins, which enhances fruit set and retention (47-51). Auxin balance also regulates the fruit drop as these accumulate at abscission zone (25, 52). Replacing regular fertilisers with their nanoformulations will be more efficient, as these are required in comparatively tiny amounts due to their entry through stomata or membrane transport proteins, thereby allowing for slow and sustained release (31). This may be the reason for the negative effect of the 200 ppm ZnONP spray on fruit set and fruit drop.

Zn ions are the integral part of Zn finger family of transcription factors which regulates the cell proliferation and differentiation as well as that of carbonic anhydrase which is responsible for the diffusion of carbondioxide(CO_2) to the site of carboxylation (47, 53). Sink strength increases with auxin, leading to the strong movement of metabolites towards it. Additionally, the stimulatory effect of Zn on metabolism increases fruit dimensions (50, 54). The application of Zn, especially in nano forms, has been shown to significantly enhance fruit number, size and quality by improving cell division, elongation and sugar translocation (55, 56). Our results were in line with those reported by (14, 23). The reduction in yield and other parameters with a 200 ppm ZnONP dosage may be attributed to a disruption in various mechanisms, including reduced stomatal and mesophyll conductance and inhibition of PSII activity (47). Similar yield reduction in guava with increase in Zinc sulfate dosage from 125 g/plant to 150 g/plant was reported by (57).

Zn application has a synergistic effect on the N and K uptake (58, 59). The increased concentration of ZnONP spray may have increased the growth, yield attributes and yield of the guava crop, which might have led to an increased nutrient content (60). It was also reported increase in N, K and Zn content whereas the influence was negligent on P uptake, even a decrease in P uptake with increased Zn levels was reported in previous studies (14, 59). Excess Zn reduces the phloem loading of P, whereas its deficiency increases root P permeability and transport inside the plant system (12). An increase in Zn content in fruit and leaves following Zn spray application has also been observed (61). Small concentration of foliar application of nano Zn (2 mL L^{-1}) had been reported to be more efficient in increasing NPK and Zn uptake than that with the application of $ZnSO_4$ @ 25 kg ha^{-1} (62). The application of nanofertilizers has tremendous potential to increase the nutritional value of fruits.

ZnONP plays a central role in redox reactions in sugar metabolism, thereby increasing the quantity of total sugars, reducing sugars and non-reducing sugars (31). TSS: acidity ratio reflects balance between sweetness and acidity, thus, serves as an important measure of the quality assessment of the fruit (63). The results were in conformity with (63, 14, 23). Zn is the only metal which is constituent of all the six classes of enzymes, TSS increase, sugar increase is resultant of the break down of complex polymers and starch degradation, respectively, whereas the decrease in acidity is due to conversion of acids into sugars and salts due to invertase activity (47, 64). In addition, Zn plays a role in auxin synthesis, leading to chlorophyll development, which in turn increases the synthesis of metabolites and the translocation of photosynthates to sinks, thereby enhancing TSS and total sugars (65, 66). Research indicates a decrease in TSS, total sugars and ascorbic acid content with an increase in Zn spray dosage from 150 ppm to 200 ppm. A decrease was observed when the spray dosage increased from 0.6 % to 0.7 % and a further decrease was observed at a 0.8 % concentration (67). The secondary metabolism activated by the application of ZnONP might be responsible for increasing the total phenols, flavonoids and antioxidant activity in the current study (54). Moreover, nanoparticle application promotes the biochemical compounds and active chemicals, as these have the ability to be absorbed, translocated and aggregated at specific locations in the cells, e.g., the plasma network (54).

The additional cost of ZnONP and associated labour cost for the spray led to the increase in the production cost for each treatment. Although gross and net returns increased up to 150 ppm spray, the most economically feasible dosage obtained in the current study is 100 ppm. This was also reflected in the B:C

Table 4. Partial Budget Analysis for different levels of ZnONP sprays compared to control for two different varieties

Treatments	TC (USD ha^{-1})	FC (USD ha^{-1})	VC (USD ha^{-1})	ΔVC (USD ha^{-1})	NR (USD ha^{-1})		ΔNR (USD ha^{-1})		RR		Decision	
					VNR Bihi	Pant Prabhat	VNR Bihi	Pant Prabhat	VNR Bihi	Pant Prabhat	VNR Bihi	Pant Prabhat
Control	1668.9	1668.9	-	-	3689.6	925.8	-	-	-	-	-	-
50 ppm	1811.5	1668.9	142.6	142.6	4129.4	1519.3	439.8	593.5	3.1	4.2	√	√
100 ppm	1930.6	1668.9	261.7	261.7	4561.7	1977.9	872.1	1052.1	3.3	4.0	√	√
150 ppm	2049.6	1668.9	380.7	380.7	4680.0	2091.2	990.4	1165.4	2.6	3.1	√	√
200 ppm	2168.7	1668.9	499.8	499.8	4000.3	1417.3	310.7	491.5	0.6	1.0	×	×

*TC: Total Costs; FC: Fixed Costs; VC: Variable Costs; ΔVC: Change in variable cost compared to control; GR: Gross Returns; NR: Net returns; ΔNR: Change in net returns compared to control; RR: Rate of returns. √: accepted; ×: rejected ratio. It is essential to optimise Zn usage in crops, as its nutritional value is correlated with its affordability among the population. Partial budget analysis is helpful in decision making as it is an important tool for determining the transfer and adoption of particular research (68). For both varieties selected for the current study, it has been shown that all levels of ZnONPs are suitable, except for 200 ppm. However, the 100 ppm dosage is best suited for adoption.

Conclusion

Zinc (Zn) is not only an essential nutrient for plant physiological processes but also important for the human system, including the functioning of the immune system, DNA maintenance, organ function and wound healing**.** Its deficiency is one of the reasons of the global health problems. Zinc is also required for brain development and fighting infections and plant-based sources in developing countries don't provide enough zinc for healthy development. Under such situations, the current study, considering the holistic parameters of guava fruit, becomes important. It revealed the importance of Zn nutrition in not only increasing the yield but also the quality parameters. Application of 100 ppm dosage of Zinc oxide nanoparticles (ZnONP) in addition to the current fertilization practice found to be optimum for enhancing the yield, nutrient concentration, quality parameters and biochemical parameters. In comparison to previous studies, it can also be inferred that nanofertilizers are more efficient and can reduce the amount of a particular nutrient needed for crop growth and development. Further exploration of the ZnONP can be done in other major horticultural crops for the agronomic biofortification and quality improvements. This study will pave the path for addressing the micronutrient requirement of the guava crop as well as for achieving the sustainable development goals of United Nations of zero hunger, good health and wellbeing.

Acknowledgements

The authors are grateful to the Horticulture Research Centre and Chemical Laboratory, Department of Agronomy, G. B. Pant University of Agriculture & Technology, for the support provided for conducting the experiment and laboratory analysis.

Authors' contributions

SS conceived the study, performed the experiments, analyzed the data and drafted the manuscript. AJ contributed to data analysis and manuscript writing. NS supervised the study and provided critical input during manuscript development. KS was involved in manuscript review and provided technical validation. PS contributed to data interpretation and critical review. KK participated in language editing and formatting. CJ assisted in literature review and reference management. PS supported the statistical review and contributed to the preparation of figures. US handled the final review and approval coordination. All authors read and agreed to the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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