Research Article

Effect of Nitrogen Doses and Timing on Fruit Quality of Guava in Winter

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Abstract | The guava fruit plants produce heavy fruit yield during summer season but these plants then produce very little fruit yield during winter season due to low carbohydrates. To increase guava fruit production during winter season, this experiment 'effect of nitrogen doses (0, 75, 150 and 225 kg ha^{-1}) and timing (September, October and November) on fruit quality of guava in winter' was conducted at farmer field of village, Gher Khan, District, Haripur during 2017-2018 with the objectives to optimize the appropriate dose of nitrogen and timing of application for growth and fruit quality of guava crop in winter. The experiment was laid out in RCBD with split plot arrangement and three replications. The experimental results revealed that highest ascorbic acid (207.59 mg $100g^{-1}$) was recorded in guava fruit tree treated with nitrogen in September. Trees treated with nitrogen in November produced fruits with maximum fruit firmness (1.90 kg cm^{-2}) and titratable acidity (0.35%). Regarding nitrogen doses, maximum TSS-acid ratio (39.72%) and ascorbic acid (212.42 mg $100g^{-1}$) were observed in trees treated with 225 kg N ha^{-1}. Maximum ascorbic acid (217.76 mg $100g^{-1}$) and reducing sugar (5.85%) were recorded in trees fertilized with 150 kg N ha^{-1} while maximum non-reducing sugar (4.80%) and TSS (10.55 °Brix) were observed in trees treated with 75 kg N ha^{-1}. Interaction between nitrogen doses and its time of application significantly affected fruit firmness of guava fruit while TSS, ascorbic acid, TSS-acid ratio, reducing and non-reducing sugar was found non-significant. It is therefore concluded from results that optimum dose of urea as a source of nitrogen should be applied during the month of September at the rate of 150 kg ha^{-1} to guava fruit trees for better quality fruit production of winter season guava under the climatic condition of Haripur, Khyber Pakhtunhwa, Pakistan.

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Introduction

Guava (*Psidium guajava* L.) is an important crop of the Myrtaceae family. Guava trees grow well in subtropical and tropical climate. It contains high amount of ascorbic acid (McCook *et al*., 2012). It is the richest after orange in terms of vitamin C content (Muhammad *et al*., 2014). It is an important export fruit due to its nutritional value. The top exporting countries are Mexico, Thailand, India, Brazil and the Philippines, while the United States, China and the United Kingdom are the largest importers of guavas

(Comtrade, 2018). Guava cultivation in Pakistan is mostly in Punjab and Sindh provinces (Khushk *et al.*, 2009) and the punjab accounts for about 80.7% of the total production of guava across the country (Jain *et al.*, 2001).

Pakistan climate is arid and semi-arid and soil is low in organic matter as well as alkaline calcareous. Therefore, nutrients have a great role in increasing crop production. Similarly, deficiency of nitrogen badly affect crop production in the country (Rashid and Ryan, 2004; Ibrahim *et al.*, 2019). Nutrients have role in human health so nutrients deficiency should be take in to consideration (Welch and Graham, 2004). Nitrogen deficiency or toxicity caused different physiological malfunction and their symptoms appeared on leaves fruit and shoots. Deficiency of nitrogen in plant caused yellowing of leaves, stunted growth and thin stem (Agfact, 2002). Deficient amount of nitrogen application resulted decline growth and photosynthesis which limit fruit yield and quality (Marschner, 1995). The deficiency of nitrogen to plant is due to lake of N availability in soil. Nitrogen leaching occurred maximum during heavy summer rain fall or excess irrigation and maximum porous soil. Due to the nitrification which may cause a short term nitrogen deficiency in soil which can be relieved by dry weather condition. Rate of nutrients, time of application and its placement are very much important to increase nutrient uptake efficiency and decrease nutrient losses. Frequent nitrogen application to week or older trees may lead to nitrogen availability and decrease nitrogen deficiency (Zekri and Obereza, 2003).

Various nitrogen doses (0, 300 g and 600 g N tree^{-1}) were applied to guava tree. It was observed that application N tree^{-1} at rate of 60 gram significantly improved yield and quality attributes (TSS, ascorbic acid, reducing sugar) while fruit acidity was recorded decline by highest nitrogen application (Lal and Sen, 2001; Muhammad *et al.*, 2019). Soil application of 400 g N caused the maximum cumulative growth of the plant, maximum fruit number and highest fruit weight. Application of nitrogen improved fruit quality also increased fruit juice, TSS, acidity, total sugar (reducing and non-reducing) and ascorbic acid (Ghosh and Das, 2000). Among management factors, application of balanced fertilizer is key component that increases vegetative as well as reproductive growth leading to high productivity and standard of the produce (Jilani *et al.*, 2021). Different levels (300 g, 600 g and 900 g N tree^{-1}) of nitrogen was applied to guava trees. All levels significantly affected all parameters as compare to control plants. Significant effect of different nitrogen levels (0.0, 0.90, 1.36 and 1.82 kg plant^{-1}) on fruit size and fruit weight of guava were recorded which increased with increase in nitrogen content (Singh and Singh, 1970). Nitrogen at 600 g plant^{-1} gave high values for weight of fruit, yield, TSS, ascorbic acid and acidity (Bhatia *et al.*, 2001). Spray application of 15 % urea significantly increased terminal shoot length, fruit yield, diameter and length of fruit, weight of fruits, TSS, total sugar (reducing and non-reducing sugar), vitamin C content and reduced fruit acidity of guava (Dubey *et al.*, 2001). Whereas, application of 2% urea spray significantly affected fruit yield tree^{-1}, size of fruit, weight of fruit, total soluble solids and ascorbic acid, reduced fruit drop and decreased the acidity of ber fruits (Chauhan and Gupta, 1985). Banana plants treated with 200 g N plant^{-1} recorded highest plant height, pseudostem girth, number of functional leaves at shooting and leaf nitrogen content at harvest while 160 g N produced highest yield (Khan *et al.*, 2015). However, phalsa plant treated with nitrogen at rate of 100 g revealed maximum sprout shoot^{-1}, yield bush^{-1} and yield ha^{-1} (Kandolia and Bhuva, 1996). Peach plant treated with 450 g N tree resulted maximum plant height, fruit weight and yield (Arora *et al.*, 1999). Urea spray at 1 or 2% increased vitamin C and TSS content of the guava fruit (Shanmugavelu, 1987). Looking the importance of nitrogen; the present research experiment was design to know the "Effect of nitrogen doses and timing on fruit quality of guava in winter" with the objectives to optimize the appropriate dose of nitrogen and timing of application for growth and fruit quality of guava crop in winter.

Materials and Methods

The research experiment was conducted at village, Gher Khan, District, Haripur during 2017-2018. The research was designed in Randomized Complete Block Design (RCBD) with split plot arrangement. The experiment consist of two factor with application time having main plot and nitrogen levels was kept with sub plot. Total 12 treatments were used which were replicated thrice. There were total 36 treatments in experiment. Guava trees cv. 'Allahabad Safeda' of eight-year-old were selected for experiment. Except control all trees were applied with nitrogen during growing season to fulfill the nutrient requirement of winter season guava fruit. Cultural practices (weeding, hoeing etc) were regularly performed during experiment. Nitrogen doses were applied to guava trees in two split doses, the first dose at start of the month and second at dose mid-month. Urea was used a source of nitrogen while recommended doses of phosphorus (SSP) and potash (SOP) were also applied to all trees. There were two factors i-e, Factor A. Time of Application, September (1st and 15th), October (1st and 15th), November (1st and 15th), Factor B. Nitrogen, N_0= 0 Kg ha^{-1}, $N_{1=}$ 75 Kg ha^{-1}, $N_{2=}$ 150 Kg ha^{-1} and $N_{3=}$ 225 Kg ha^{-1}).

The following quality parameters were examined during research.

Fruit firmness (kg cm^{-2}): Fruit firmness of guava fruits were calculated in randomly selected fruits through Penetrometer and average fruit firmness was recorded.

Total soluble solids (°Brix): The Total soluble solids were examined through Brix refractometer for each treatment of all replication. Drop was taken from juice and placed on clean slab of a °Brix refractometer which is equipped with (%) sugar scale and then readings for average total soluble solids were calculated.

Titrable acidity (%): The titrable acidity was determined by using standard procedure of association official analytical chemists (A.O.A.C), 1984.

Ascorbic acid ($C_6H_8O_6$): 1 ml sample of guava juice was taken and then diluted it in solution of 1 N oxalic acid ($C_2H_2O_4$). The sample was then titrated against standard dye solutions and ascorbic acid was measured for randomly selected fruits.

TSS-acid ratio: The following formula was used to determine TSS-acid ratio of the sample.
TSS-acid ratio = TSS/percent acidity

Percent reducing and non-reducing sugars
Reducing and non-reducing sugars were examined by using the method of Lane and Eynon, as reported in Iqtidar and Saleemullah (2004).

Statistical analysis
The recorded data of all the parameters were analyzed through statistical software "Statistix-8.1" to determine the significance of the differences obtained by the experimental treatments. The difference among treatments means were examined by least significant difference (LSD) test at 5% level of probability (Steel and Torrie, 1980).

Results and Discussion

Fruit firmness (kg cm^{-2})
Analysis of variance presented that time of application has non-significantly affected fruit firmness of guava while nitrogen doses and its interaction with time of application significantly ($P \leq 0.05$) affected guava fruit firmness. Early to delay in nitrogen application, significantly decreased fruit firmness where maximum fruit firmness (1.95 kg cm^{-2}) was recorded in guava fruit trees treated with nitrogen during September which was statistically similar to fruit firmness (1.91 kg cm^{-2}) recorded in guava trees treated with nitrogen during October. The minimum fruit firmness (1.76 kg cm^{-2}) of guava fruits were recorded in trees treated with nitrogen during November. As concerned nitrogen doses, fruit firmness of guava ranged from 1.52 to 2.00 kg cm^{-2}. The maximum fruit firmness (2.00 kg cm^{-2}) was recorded in control trees (0 kg N ha^{-1}) while minimum fruit firmness (1.52 kg cm^{-2}) of guava fruits were observed trees treated with 225 kg N ha^{-1}. With regard to interaction, highest guava fruit firmness (2.24 kg cm^{-2}) was observed in guava fruit trees treated with nitrogen in September while lowest fruit firmness (1.24 kg cm^{-2}) was observed in guava trees treated with 225 kg ha^{-1} in November (Figure 1).

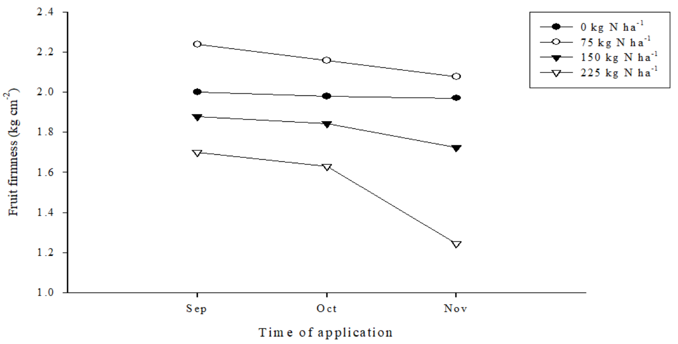

Figure 1: *Interaction of nitrogen doses and its time of application on fruit firmness of guava.*

It is thought that higher doses of nitrogen negatively affect fruit quality. The level of nitrogen fertilizer increase so fruit firmness is going to decline. High N doses generally reduced color development and flesh firmness in apple trees (Souza *et al*., 2013; Hoying *et al*., 2004; Nava *et al*., 2007). Nitrogen fertilization affect quality of various fruits (Locascio *et al*., 1984). High N supply typically induces higher growth rates, fruits are grown to larger size, but the supply of other constituents, particularly of phloem-immobile Ca, does not increase, resulting in dilution affects So, N in perennial fruit crops does not accumulate in fruits as nitrate, as it is not phloem-mobile. N is translocated as amino acids which brings us to the next aspect. It is argued that N tends to move the C skeletons away from structural metabolism to protein metabolism (Marschner, 1995). Fruit firmness in apple is closely related to cellulose which is why high N supply tends to reduce fruit firmness. The present results confirm the results of Drake *et al.* (2002) who recorded highest fruit firmness in apple by use of lowest nitrogen rate.

Total Soluble Solids (°Brix)

Total soluble solid of guava fruits were significantly affected by time of application and nitrogen doses. The interaction of time of application and nitrogen doses was found non-significant ($P \leq 0.05$) (Table 1). Mean table 1 presented that guava fruit trees fertilized during September produced highest TSS (10.32 °Brix) which was statistically similar to TSS (10.28 °Brix) of guava fruit trees fertilized during October. The guava fruit trees fertilized during November showed lowest TSS (10.10 °Brix). Furthermore, the means for nitrogen doses showed that highest TSS (10.55 °Brix) were recorded in guava fruit trees applied with 75 kg N ha^{-1} which was closely followed by TSS (10.42 °Brix) of guava fruit trees applied with nitrogen at 150 kg ha^{-1}. Moreover, the lowest TSS (9.85 °Brix) were produced in untreated trees (Control trees).

The increase in TSS of guava fruit trees treated with nutrients in September might be correlated with higher fruit weight which contain maximum TSS. Struik *et al.* (1989a) examined that optimum temperature increases photosynthesis that enhanced vegetative growth of plant which plays a vital role in better translocation of sugars into fruit tissues. Further these are similar to the the findings of Calderon *et al.* (2004) and Fidler (1973). The fruit color and TSS affected negatively by nitrogen application (Nava *et al.*, 2007). The present research indicated that higher rates of nitrogen beyond optimum level cause reduction in total soluble solids of guava. These finding are in line with those reported by other researchers working with orange, grape fruit and date palm (Dawoud, 1991; Smith, 1969). The content of TSS shows declined trend with application of nitrogen fertilizer. This might be due to high self-shading caused by excessive vegetative growth due to nitrogen application. The application of nitrogen fertilizer to apple tree resulted heavy vegetative growth and decrease light shedding on fruit surface which affect TSS content of apple fruit (Dris *et al.*, 1999; Drake *et al.*, 2002). However, the observed values of TSS contents were within the range with high nitrogen dose. The present findings proved the results of Paula *et al.* (1991) that higher doses of nitrogen reduce TSS of guava fruit.

Table 1: *Fruit firmness, TSS, titratable acidity and TSS-acid ratio of guava as affected by nitrogen doses and its time of application.*

Treatments	Fruit firmness (kg cm^{-2})	TSS (°Brix)	Titratable acidity (%)	TSS-acid ratio
Time of application				
September	1.95	10.32 a	0.29	30.89
October	1.91	10.28 a	0.33	35.46
November	1.76	10.10 b	0.35	37.37
LSD ($P \leq 0.05$)	NS	0.16	NS	NS
Nitrogen doses (kg ha^{-1})				
Control	2.00 b	9.851 c	0.41 a	27.17 c
75	2.16 a	10.55 a	0.34 b	33.30 b
150	1.81 c	10.42 a	0.28 c	38.09 a
225	1.52 d	10.12 b	0.26 c	39.72 a
LSD ($P \leq 0.05$)	0.032	0.25	0.03	3.47
Interaction N-doses × Time of application	Fig. 1	NS	NS	NS

Means followed by different letters are significantly different at 5% level of significance by using LSD test, NS= Non-significant

Titratable Acidity (%)

The data for titratable acidity revealed that nitrogen doses significantly ($P \leq 0.05$) affected titratble acidity of guava fruits whereas the alone affect of application time and its interaction with nitrogen doses were found non-significant. The mean table 1 revealed that guava fruit trees of control treatment (unfertilized) showed higher titratable acidity (0.41%) while increasing the rate of nitrogen dose (75 to 225 Kg ha^{-1}) significantly decreased titratable acidity from 0.34 to 0.26%.

The decrease TA might be due to the conversion of the organic acids into sugars during fruit ripening. Increasing nitrogen rates produced bigger size fruits but it adversely affects fruit quality like titrable acidity (Spironello *et al.*, 2004). The present findings are in agreement with the findings of Jones (1999) who investigated that fruit quality is negatively affected by nitrogen fertilizer and reduction in acidity with increased nitrogen doses in tomato. The present results are supported by Bashir and Abu-Goukh (2003) and Mitra and Bose (1985) that TTA decreases by application of nitrogen.

TSS-acid ratio

Table 1 shows that nitrogen doses significantly affected TSS-acid ratio of guava fruits ($P \leq 0.05$). Moreover, time of application and the interaction between time of application and nitrogen doses were non-significant for TSS-acid ratio (Table 1). Mean presented that increasing nitrogen dose (75 to 225 kg ha^{-1}) significantly increased TSS-acid ratio (33.30 to 39.72). The TSS-acid ratio of 225 kg N ha^{-1} was statistically similar with TSS-acid ratio (38.09) of guava fruits tree treated with 150 kg N ha^{-1} (Table 1). The guava fruits tree of control treatment (Untreated plants) had TSS-acid ratio of 27.17.

Nitrogen application affects the content of some sugars, amino acids, and ascorbic acid which can influence the taste and flavor of fruits (Lee and Kaddar, 2000; Alcobendas *et al*., 2013). The present results show significant increase of TSS-acid ratio over control are in accordance with the results of Lima *et al.* (2008) who reported that higher doses of N treatment showed the highest total soluble solids concentration and TSS-acid ratio but the lowest titratable acidity (acid content) at ripening, while control treatment resulted opposite trend. The increased TSS content might be due to further synthesis and accumulation of photosynthates in the fruits (Murata, 1977) and Color development is associated with a loss of texture, increasing sugar content and decreasing acidity (Rana, 2006). Present results are supported by Lal and Sen (2001) who reported that TSS-acid ratio is linearly increased by increasing nitrogen fertilizer.

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

The statistical analysis showed that application time (AT) and nitrogen doses (ND) significantly ($P \leq 0.05$) affected ascorbic acid (AA) of guava, whereas the interaction of application time (AT) and nitrogen doses (ND) were found non-significant for ascorbic acid (AA) of guava (Table 2). Guava fruit trees treated with nitrogen in September recorded maximum ascorbic acid (207.59 mg 100g^{-1}) content which was statistically similar with ascorbic acid (206.31 mg 100g^{-1}) of guava fruit trees treated in October. The minimum ascorbic acid (200.49 mg 100g^{-1}) content of guava fruit trees were recorded in trees treated in November. Revealing the means for nitrogen doses, increasing nitrogen doses from untreated 75 to 150 Kg ha^{-1} significantly increase ascorbic acid of guava from 203.46 mg 100g^{-1} to 217.76 mg 100g^{-1} respectively. The ascorbic acid observed at 150 kg N ha^{-1} was statistically similar with ascorbic acid (212.42 mg 100g^{-1}) observed in guava fruit trees applied with 225 Kg N ha^{-1}. The control plants (Untreated plants) were observed for minimum ascorbic acid (185.55 mg 100g^{-1}) content.

Ascorbic acid of guava fruits were found higher in the trees that were treated with nutrient in September could be related to better vegetative growth of the plant and its complete crop maturity due to appropriate temperature and photoperiod that boosted the photosynthesis of plant and produced maximum assimilates and translocation to fruit tissues. Similar findings were observed by Ali *et al.* (2003) in citrus and Burton *et al.* (1989) in their investigation. Moreover, Ascorbic acid content increases as soil content increases. The ripe guava fruit contain high ascorbic acid contents, which includes dehydroascorbic acid and L-ascorbic acid (Gomez and Lajolo, 2008). L-ascorbic acid (AsA) improve nutritional quality of the fruit as it works as powerful antioxidant and minimize oxidative stress (Gallie, 2013; Huang *et al*., 2014). Current study revealed that control trees are going through stress due to no fertilization and gives less ascorbic acid content while it is higher at optimum level but again decline due to high nitrogen fertilizer stress. These results also support the results of Shin *et al.* (2005) who investigated that hydrogen peroxide (H_2O_2) in guava was high when there was low level of nitrogen but the level of hydrogen peroxide was slowly decreased by increasing application of nitrogen up to optimum level. Possibly, the use of smallest nitrogen dose caused nutrient deficiency related to stress, like resulting high ROS level as observed for the H_2O_2. In addition, at higher N doses, the H_2O_2 content began to increase. The present results are in conformity with the findings of Marschner (2012), Zhao *et al.* (2015) and Ochoa-Velasco *et al.* (2016) that application of high nitrogen doses during cultivation tends to decrease the values of ascorbic acid contents in various fruits. Liang *et al.* (2013) also reported that Recycling enzymes of ascorbic acid, such as enzyme MDHAR, which may be up-regulated in response to Nitrogen deficiency as a mechanism for the removal of the ROS. The similar behavior was recorded herein for 'Paluma' guava, regarding the enzyme MDHAR activity. On another side, the application of nitrogen in highest amount possibly reduced the functions of MDHAR enzyme which could contribute in decreasing the amount of ascorbic acid content.

Table 2: *Ascorbic acid (mg 100g^{-1}), reducing sugar and non-reducing sugar of guava as affected by nitrogen doses and its time of application.*

Treatments	Ascorbic acid (mg 100g^{-1})	Reducing sugar (%)	Non-reducing sugar (%)
Time of application			
September	207.59 a	5.78	4.74
October	206.31 a	5.66	4.66
November	200.49 b	5.52	4.48
LSD ($P \leq 0.05$)	5.42	NS	NS
Nitrogen doses (kg ha^{-1})			
Control	185.55 c	5.35 c	4.30 b
75	203.46 b	5.63 b	4.80 a
150	217.76 a	5.85 a	4.77 a
225	212.42 ab	5.77 a	4.63 a
LSD ($P \leq 0.05$)	10.38	0.09	0.20
Interaction N-doses × Time of application	NS	NS	NS

Means followed by different letters are significantly different at 5% level of significance by using LSD test, NS= Non-significant

Reducing sugar (%)

Analysis of variance Table 2 presented a significant ($P \leq 0.05$) response of reducing sugar to nitrogen doses. However, the alone affect of application time and the interaction of nitrogen doses and application was recorded non-significant for reducing sugar of guava (Table 2). Maximum reducing sugar (5.85%) was noted in guava fruit trees fertilized with nitrogen at the rate of 150 kg N ha^{-1} which was statistically similar to reducing sugar (5.77%) noted in guava fruit trees fertilized with 225 kg N ha^{-1} nitrogen. Control trees where nitrogen doses were not applied gave reducing sugar of 5.35%.

Reducing sugars which is consist of more than 65% of the TSS are important for tree growth and regulate tree movements by providing energy to the tree (Wang and Tang, 2014). Maximum reducing sugar by nitrogen application could be attributed to the role of nitrogen in different energy sources such as amino acid and amino sugar (Prasade and Mali, 2000). Decrease in reducing sugar at higher doses of nitrogen might be due to toxicity level, it decreases other nutrient molecule and enzymes which help in the synthesis of these quality parameters (Kaul and Bhatanagar, 2006). The current results are matched with the results of Bhatia *et al.* (2001) who revealed that nitrogen help in uptake of other nutrients which enhance the guava fruit quality. Kashyap *et al.* (2012) also support the current study as well.

Non reducing sugar (%)

It is evident from the Anova Table 2 that nitrogen doses (ND) significantly($P \leq 0.05$) affected ($P \leq 0.05$) non-reducing sugar of guava fruit. Moreover, the separate affect of application time (AT) and the interaction of ATxND was non-significant for non-reducing sugar of guava. The data for nitrogen doses showed that highest non-reducing sugar (4.80%) was determined in guava fruit trees supplied with 75 kg N ha^{-1} which was statistically at par to non reducing sugar (4.77 and 4.63%) of guava fruit trees supplied with 150 and 225 kg N ha^{-1}. The lowest non-reducing sugar (4.30%) was determined in unfertilized (Nitrogen) guava trees.

Maximum non reducing sugar was observed in guava fruit treated with nitrogen fertilizer. It might be due to nitrogen action which favored the soluble solids increase through sugar translocation from leaves to fruits to maximum amount of TSS available in fruit (Souza *et al.*, 2010). Non-reducing sugars significantly increased by increasing nitrogen dose. These findings are in agreement with the reports of Dudi *et al.* (2005) in Kinnow mandarin, Kashyap *et al.* (2012) and Ghosh *et al.* (2012) in pomegranate. Another possible reason of increased in non-reducing sugar could be the transformation of complex polysaccharide into simple sugar. The fruit content contents could easily be described by the fact that urea improve the process of photosynthesis which led to carbohydrate accumulation and helped to enhance sugar content of the fruit. Lal *et al.* (2000) findings in guava also in agreement with the present results.

Conclusions and Recommendations

It was concluded from the results of the current experiment; that guava trees cv. 'Allahabad Safeda' treated with nitrogen during the month of September showed better quality attributes than other treatments. Maximum reducing sugar and non-reducing sugars were noted in trees treated with nitrogen at 150 kg ha^{-1}. However, maximum TSS-acid ratio and ascorbic acid were observed in trees treated with nitrogen at 225 kg ha^{-1}. The maximum TSS and fruit firmness were recorded in trees treated with 75 kg N ha^{-1}, while maximum titratable acidity was observed in trees of control treatment. The interaction of nitrogen application at various timing significantly affected fruit firmness of guava while rest of parameters showed non-significant affect.

On the basis of above conclusions, it is recommended that the guava trees cv. 'Allahabad Safeda' should be treated with nitrogen during the month of September to produced quality guava fruit during winter season and nitrogen dose at the rate of 150 kg ha^{-1} should be applied to guava trees during early season (September) to obtain better fruit during winter season.

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Novelty Statement

Optimum application of nitrogen fertilizer to guava in winter season enhances the quality of guava fruits.

Author's Contribution

Junaid Khan: Principal author and PhD Scholar, who did research, analysis and wrote draft of this MS.
Sajid Khan: Major Supervisor, who provided all technical guidelines in the whole study program.

Conflict of interest
The authors have declared no conflict of interest.

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