




RESEARCH

Effect of Different Doses of Calcium and Boron Pre-harvest Spray on Post-harvest Quality of Acid Lime (*Citrus aurantifolia* var. SunKagati-1)

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LICENCE



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Abstract

Acid Lime (*Citrus aurantifolia* var. SunKagati-1) has been cultivated in 67 districts of Nepal. Its cultivation is becoming increasingly popular among farmers in Nepal. However, adequate post-harvest loss reduction measures are lacking. A field experiment was conducted in lime orchards in Chitwan during August-September 2019 and 2020. Pre-harvest spraying of different concentrations of calcium (0.1, 0.2, and 0.3%) and boron (0.2, 0.4, and 0.6%) in lime fruit trees was done before 45 and 30 days of fruit harvest. Fruits with the same maturity indices were harvested separately from the treated plants. The post-harvest study was conducted for 1 month under laboratory conditions. Different parameters like physiological loss of weight (PLW), decay loss, total soluble solids (TSS), titrable acidity (TA), ascorbic acid content (vitamin C), and freshness were recorded. Minimum PLW (18.2%) and decay loss (14.3%) were observed in boron (@0.6%) treated fruits. At the end of the experiment, minimum TA (1.79 and 2.12%) was recorded from boron (@0.6%) treated fruits in both years. Calcium (@0.3 %) and boron (@0.4 %) treated fruits expressed the maximum values for TSS in 2019 (9.67°brix) and 2020 (7.73 °brix), respectively. Fruit harvested from fruit trees sprayed with boron (@0.6%) showed the highest ascorbic acid content (55.47 and 49.61 mg/100g) and better lime freshness (2.67 and 3.0) in 2019 and 2020, respectively. This study concluded that the use of boron @0.6% as a pre-harvest spray can prolong the storage life of sour lime and maintain the fruit quality under environmental conditions of a mid-hill situation.

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Statement of Sustainability: This research on pre-harvest spraying of micronutrients on the post-harvest life of acid lime contributes to the Sustainable Development Goals (SDGs). Firstly, by reducing the post-harvest loss of acid lime, we support SDG 2 (Zero hunger) by minimizing the loss of agricultural production after harvest to ensure its long-term availability. Secondly, the research contributes to SDG 8 (Decent work and economic growth) by using the micronutrients as a pre-harvest spray that ensures quality production by increasing yield, thus attracting farmers to adopt this technology.

1. Introduction

Acid lime (*Citrus aurantifolia* var. SunKagati-1), belonging to the citrus group, comes third after mandarin and sweet orange in terms of area and production (MoALD, 2022). Recently, it has become more popular among the fruit growers of Nepal. The total area, productive area, total production, and productivity of sour cherry in Nepal were 8838 ha, 5715 ha, 36449 tons, and 6.38 t/ha respectively (MoALD, 2022). In the mid-hill region of Nepal, a few acid lime has been planted in kitchen garden since ancient times. Now with the development of varieties, its cultivation has spread commercially in terai, inner terai, and even in mid-hills (Paudyal et al., 2016). In Nepal, acid lime is produced from June to August in the terai and mid-hills, but the production is still unable to meet the demand. In Nepal, large quantities of acid lime are imported every year and the price of imported lime during the off-season (January to June) is very high. In

addition to low production, a significant proportion of fruits and vegetables suffer post-harvest losses, which is a major economic concern in Nepalese agriculture. Post-harvest loss could be rightly described as the qualitative and quantitative loss of horticultural produce at any time along the post-harvest chain, which includes the change in edibility and wholesomeness, i.e., quality of the produce, which ultimately prevents its consumption (Adeoye et al., 2009; Buyukbay et al., 2011). Turan (2008) reported that improper harvesting practices and improper post-harvest practices result in loss of produce before reaching the market along with loss of quality of produce such as deterioration in appearance, taste, and nutritional value. Various studies have been conducted in Nepal, where postharvest losses of fruits and vegetables range from 20 to 50 percent (Gautam and Bhattarai, 2012). Proper postharvest handling is an additional or complementary way of solving food needs (Bhattarai, 2018).

Nutrients are usually more readily available to plants as foliar sprays than soil applications. A foliar spray of micronutrients is 7-21 times more effective than soil application (Zaman et al., 2019). Different studies have recommended that calcium and boron have significant effects on fruit quality parameters like fruit cracking, fruit color, brix, and other standard characteristics of tomatoes (Heckman, 2009). Preharvest spraying of micronutrients reduces fruit decay during transportation and storage by strengthening the skin and has positive effects on root strength and fruit firmness (Wojcik and Lewandowski, 2003). Calcium spray reduces fruit decay, weight loss, and reduces the rate of fruit softening in apples (Saure, 2005). Boron (B) deficiency is also commonly observed in citrus orchards and results in premature fruit drop (Yang et al., 2021). The quality of limes cannot be improved but can only be maintained once they are detached from the tree. This highlights the importance of fruit quality at harvest, which largely determines the post-harvest life of limes (Samaradiwakara et al., 2019). Both pre-and postharvest management technologies need to be optimized to produce quality fruit and maintain quality during storage.

Increasing the availability of off-season fruits and maintaining their quality is the most important need at present. Therefore, the government at the same level has given special emphasis on promoting sour cherry cultivation. Although sour cherry cultivation is becoming popular among the farmers in Nepal, no attention has been paid to the post-harvest loss reduction activities and there are not enough studies conducted in this regard. As the area of sour lime is expanding, it is necessary to conduct various research to reduce its harvest and post-harvest losses and to extend its shelf life. However, in recent years the post-harvest handling of horticultural crops has become an area of research and there is some considerable work done in this area on different crops. In addition, the adoption of appropriate pre-harvest management technologies is still lacking. Both pre-harvest and post-harvest handling practices are required for better protection and shelf-life improvement of fruits and vegetables. Because of this, the present study aimed to determine the effect of pre-harvest micronutrient spraying on post-harvest quality of lime under ambient storage conditions.

2. Materials and Methods

2.1. Experimental Site

A field experiment was conducted in the acid lime (*Citrus aurantifolia* var. SunKagati-1) orchard of Mr. Gyan Hari Kandel at Bharatpur-6, Chitwan from August to September 2019 and 2020. Pre-harvest spraying of different concentrations of calcium and boron in lime orchards was done before 45 and 30 days of fruit harvest. After fruit harvest (from September to November 2019 and 2020), a laboratory experiment was set up with fruits harvested from treated plants to study their post-harvest quality parameters.

2.2. Experimental Setup and Crop Management

An experiment was conducted on the Sunkagati-1 variety. The experimental design consisted of seven treatments with different concentrations of calcium and boron (calcium @ 0.1%, calcium @ 0.2%, calcium @ 0.3%, boron @ 0.2%, boron @ 0.4%, boron @ 0.6%, and control) sprayed twice 45 and 30 days before fruit harvest (Figure 1 and Table 1). These treatments were arranged in a randomized complete block design with three replications. A single plant was considered as an experimental unit. Three replications were maintained. A total of 21 plants were selected for the experiment. Calcium nitrate and borax were used to provide different concentrations of calcium and boron. Fruits with the same maturity index were harvested separately from each treated plant. The harvested fruits from each plant were separately packed in a bamboo basket lined with sterilized paddy straw and transported to DoAR, Lumle. The harvested fruits were not treated again and the changes in their characteristics were studied by keeping them in an ambient

condition. The fruits packed in the bamboo basket were separately transferred to the plastic tray and the post-harvest study was conducted for 1 month under laboratory conditions ($20.4 \pm 3^\circ\text{C}$, and $86.2 \pm 6\%$ RH).



Figure 1. Photos exhibiting foliar spray of calcium and boron in acid lime orchard, its harvesting, and laboratory setup.

Table 1. Treatment details of the experiment.

Treatment Symbol	Treatment Details
T1	Calcium @ 0.1 %
T2	Calcium @ 0.2 %
T3	Calcium @ 0.3 %
T4	Boron @ 0.2 %
T5	Boron @ 0.4 %
T6	Boron @ 0.6 %
T7	Control (No application of calcium and boron)

2.3. Parameters Analyzed

After keeping the fruits in ambient condition, changes in the post-harvest quality of acid lime were observed and their data were recorded accordingly in one-week intervals. A total of 50 uniform fruits (in terms of shape, size, and color) were kept in each experimental unit. Different qualitative parameters of fruits: physiological loss of weight (PLW), decay loss, total soluble solids (TSS), titrable acidity (TA), amount of ascorbic acid content (vitamin C), and freshness were recorded. On the day of observation, two fruits from each experimental unit were used as destructive samples for TSS, TA, and ascorbic acid determination.

- **Physiological Loss in Weight (PLW):** For PLW, 5 fruits from each treatment were separated and marked to observe their weight changes and recorded accordingly. PLW was calculated based on the weight change (difference between the initial and final weight of the fruit) divided by the initial weight and expressed as a percentage.

$$PLW(\%) = \frac{\text{Initial weight of fruit (g)} - \text{Final weight of fruits (g)}}{\text{Initial weight of fruit (g)}} \times 100$$

- **Decay Loss Percent:** The fruit was visually inspected for symptoms of decay throughout the storage period. Samples with decayed and diseased symptoms were counted and discarded at the end of each storage interval. Decay loss was calculated as the number of discarded fruits (decayed and diseased) divided by the total number of fruits, expressed as a percentage.

$$\text{Decay loss (\%)} = \frac{\text{Number of discarded fruit}}{\text{Total fruit number}} \times 100$$

- **Total Soluble Solid (TSS):** For TSS determination in °Brix, the handheld refractometer (model: Atago, Japan, N-1 Brix 0-32%) was used by placing two to three drops of clear juice on the prism surface.
- **Titration Acidity (TA):** The determination of TA was performed using the acid-base titration method (Tyl and Sadler, 2017). Five ml of fresh lime juice was titrated against 0.1 N NaOH (sodium hydroxide). The amount of NaOH consumed to the endpoint was represented by the appearance of pink coloration, and this volume of NaOH was noted. TA was calculated using the following formula:

$$TA (\%) = \frac{0.1 \text{ N (NaOH)} \times V1 (\text{mL}) \times 0.064 \frac{\text{g}}{\text{mol}}}{V2 (\text{mL})} \times 100$$

Where, V1 is the volume of NaOH consumed during titration of the sample (mL), V2 is the volume of the sample (mL), and 0.064 g/mol is an acid milli-equivalent conversion factor.

- **Amount of Ascorbic Acid Content (Vitamin C):** The ascorbic acid content of ripe fruits was measured by the volumetric method according to Sadasivsm and Manickam (1991). Five mL of working standard solution was pipetted into a 100 ml conical flask. Then 10 mL of 4% oxalic acid was added and titrated against the dye (V1 mL). Two ml of sample was pipetted out, 10 ml of 4% oxalic acid was added and made up to a known volume (12 mL) and centrifuged for 10 minutes. 5 ml of this supernatant was pipetted out, 10 mL of 4% oxalic acid was added and titration was performed against the dye (V2 mL). The amount of dye consumed until the endpoint was the appearance of a pink color that persisted for a few minutes.

$$\text{Ascorbic acid} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{0.5 \text{ mg} \times V2 \text{ mL} \times 12 \text{ mL} \times 100}{V1 \text{ mL} \times 5 \text{ mL} \times \text{weight of sample (g)}} \times 100$$

Where, V1 is the amount of dye consumed during the titration of the working standard solution and V2 is the amount of dye consumed during the titration of the supernatant solution (sample).

- **Fruit Freshness:** Fruit freshness was measured using a 1-5 hedonic scale. Scoring was done by observing the freshness of the fruit on the last day of storage under ambient conditions. On visual observation, the freshest or least shriveled fruit was scored 1 and the least fresh or most shriveled fruit was scored 5.

2.4. Data Analysis

The data collected during the study period were entered in Microsoft Excel 2010 (Microsoft Corp., USA). The analysis of variance was performed using the Statistical Tool for Agricultural Research (STAR) version 2.0.1 (IRRI, Philippines) and significance was determined using Fisher's least significant difference at $p < 0.05$ (Gomez and Gomez, 1984).

3. Results and Discussion

3.1. Physiological Loss in Weight (PLW %) and Decay loss (%)

In 2019, preharvest spraying of different concentrations of calcium and boron differed significantly in PLW ($p < 0.01$) and percentage of decay loss ($p < 0.01$) of lime fruits (Table 2). The lowest PLW (27.51%) was recorded with boron-sprayed fruits at 0.2%, followed by calcium-sprayed fruits at 0.2% (28.39%) and boron-sprayed fruits at 0.6% (28.46%). Decay loss was lowest (1.33%) in boron @ 0.6% treatment and highest (30.93%) in calcium @ 0.3% pre-harvest spray of lime plants (Table 2). In the second year of the experiment, no significant differences were observed in PLW ($p = 0.13$) of lime, but the decay loss percentage differed significantly ($p < 0.01$). Minimum PLW (6.18%) was recorded from pre-

harvest spray of boron @ 0.4%. Minimum decay loss (19.17%) was recorded in calcium @ 0.2% followed by boron @ 0.4% spray (23.33%) (Table 3).

Table 2. The pre-harvest spray of different concentrations of calcium and boron on lime plants and their post-harvest effects on physiological loss in weight and decay loss of lime fruits under ambient conditions in 2019.

Treatments	PLW (%)	Decay Loss (%)
Calcium @ 0.1 %	31.86	18.00
Calcium @ 0.2 %	28.39	12.00
Calcium @ 0.3 %	36.10	30.93
Boron @ 0.2 %	27.51	28.73
Boron @ 0.4 %	31.66	14.67
Boron @ 0.6 %	28.46	1.33
Control (Water Spray)	29.97	10.67
Mean	30.56	16.61
CV (%)	8.31	21.83
P-value	0.01	0.01
LSD (0.05)	4.51	4.86

Table 3. The pre-harvest spray of different concentrations of calcium and boron on lime plants and their post-harvest effects on physiological loss in weight and decay loss of lime fruits under ambient conditions in 2020.

Treatments	PLW (%)	Decay Loss (%)
Calcium @ 0.1 %	7.05	41.33
Calcium @ 0.2 %	9.57	19.17
Calcium @ 0.3 %	9.29	48.67
Boron @ 0.2 %	11.73	37.33
Boron @ 0.4 %	6.16	23.33
Boron @ 0.6 %	7.93	27.33
Control (Water Spray)	9.87	26.00
Mean	8.80	31.88
CV (%)	26.02	27.23
P-value	0.13	0.01
LSD (0.05)	Ns	15.44

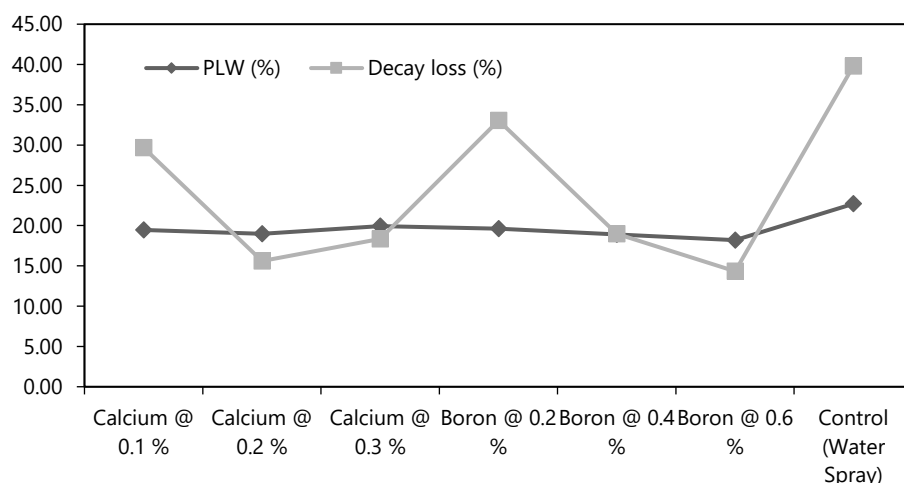


Figure 1. Average PLW and Decay loss of acid lime in 2019 and 2020.

In both years, pre-harvest spraying of different concentrations of calcium and boron resulted in a significant difference in PLW and decay loss of lime fruit (Figure 1). Combined analysis of results from both years showed that minimum PLW (18.2%) was in boron @ 0.6%, followed by boron @ 0.4% (18.91%) and calcium @ 0.3% (18.98%). Decay loss was minimum (14.33%) in boron @ 0.6% and calcium @ 0.3% (15.58%). Fruit without any treatment (control) showed the highest PLW (22.7%) with a maximum decay loss of (39.8%). Similar results in Asian pear were also reported by Khalaj et al. (2016). Pilch and Wojcik (2001) also reported lower decay loss with the application of calcium and boron spray. Calcium is the major component of the cell wall and plays an important role in maintaining fruit quality, preserving fruit

firmness, minimizing weight loss, and reducing fruit decay in storage (Guardiola and Garcia, 2000). Pre-harvest spraying of calcium delays senescence and increases consumer acceptance with fewer adverse effects in fruit storage (Lester and Grusak, 2004). Preharvest application of calcium nitrate @ 0.3% improves the storage life and quality of sour lime fruit at ambient temperature and relative humidity (Maida et al., 2018). Calcium applications in fruit maintain membrane permeability, reduce ripening process, and weight loss during storage (Aguayo et al., 2008).

3.2. Titrable Acidity (TA)

Changes in the TA of acid lime during postharvest storage conditions were recorded in both years and are presented in Figure 2. In both years, TA decreased gradually each week. In each treatment, the TA of the fruit was recorded maximum during harvest and after the 4th week, its value was decreasing. At the time of harvest, the TA was maximum (7.68 and 7.28%) on fruit sprayed with calcium at 0.2% in 2019 and 2020, respectively. After the fourth week, TA was minimum (1.31 %) in boron @ 0.4 % in the first year, while in the second-year boron @ 0.4 % had maximum TA (3.22 %). Fruit sprayed with boron @ 0.6% had 1.79% and 2.12% TA in the first and second years, respectively. Similar results were reported by Wójcik and Lewandowski (2003) with foliar spraying of calcium and boron on strawberries. The fruit may use the acids during storage to reduce the amount of acid present in the fruit throughout the storage period. The metabolic processes of living tissues that result in the depletion of organic acids were the primary cause of the change in total titrable acids during storage (Bhattarai and Gautam, 2006). Boron is a critical component for maintaining conductive tissues and controls other elements with changes in the TA of fruits (Dixit et al., 2013).

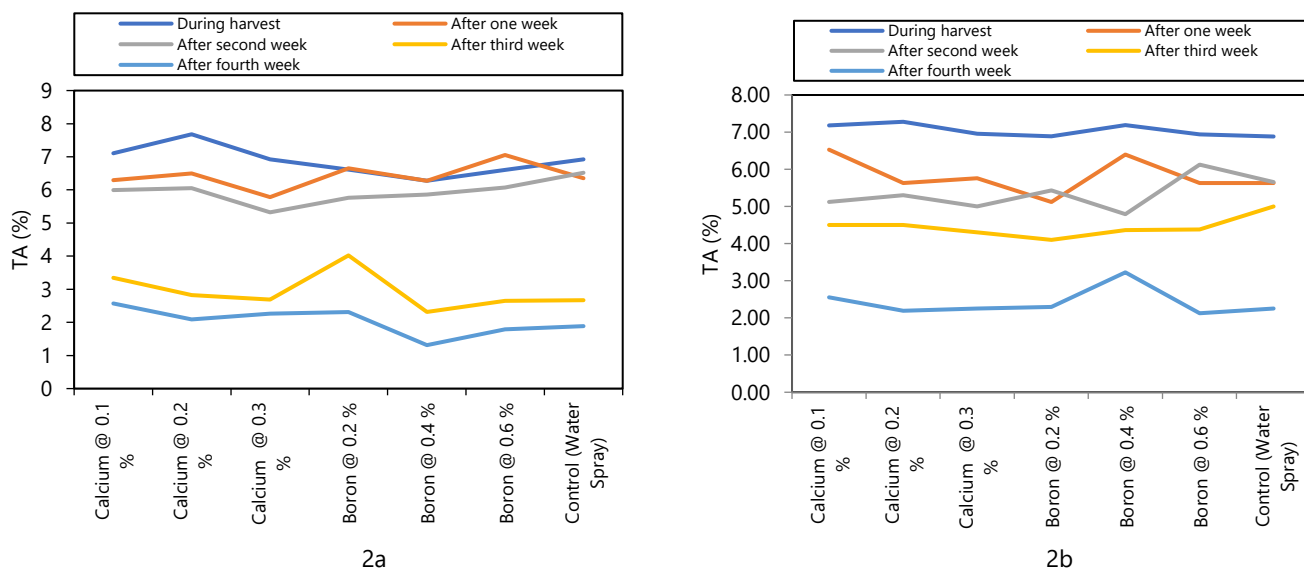


Figure 2. Effect of calcium and boron pre-harvest spray on TA of acid lime during postharvest storage in different periods (a) Change in TA during the first year of the experiment (b) Change in TA during the second year of the experiment.

3.3. Total Soluble Solid (TSS)

Changes in TSS of acid lime during postharvest storage conditions were recorded in both years and are presented in Figure 3. In the first year, a significant difference was recorded in the TSS of acid lime after the fourth week, while no differences were observed in the second year. Calcium @ 0.3% expressed the maximum values for total soluble solids (9.67 °brix) followed by boron @ 0.6% (9.40 °brix) in the fourth week of the first year. In the second year, boron @ 0.4% recorded the maximum TSS (7.73 °brix). Xu et al. (2021) also reported that boron-treated plants had a higher percentage of soluble solids in fruit. The result is consistent with the findings of Chauhan et al. (2015) and Dutta (2004). Boron, an important micronutrient involved in cell division and glucose metabolism, affects the relationship between plants and water and is involved in the transport of sugar in plants (Dixit et al., 2013). The increase in sugars may be caused by hydrolytic enzymes that break down complex polymers into simpler compounds. It has also been noted that borate and sugar react to form a sugar-borate complex, and borate has been suggested to enhance the movement of sugar throughout the plant (Gauch and Dugger, 1953). The foliar application of zinc and boron significantly increased the fruit juice content and TSS of sweet orange (Sajid et al., 2012).

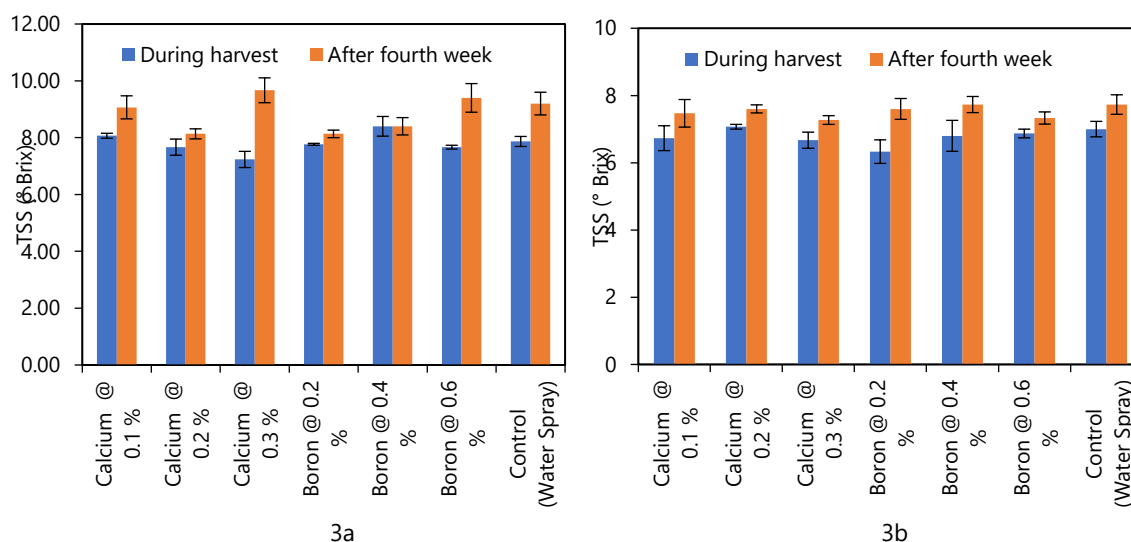


Figure 3. Effect of calcium and boron pre-harvest spray on total soluble solids (TSS) of acid lime during postharvest storage in different periods (a) Change in TSS during the first year of the experiment (b) Change in TSS during the second year of the experiment.

3.4. Amount of Ascorbic Acid

The amounts of ascorbic acid content in acid lime from treated trees during post-harvest are shown in Figure 4. At the time of harvest, the maximum ascorbic acid content of 66.27 and 60.92 mg/100g was observed from trees sprayed with boron @ 0.6% in 2019 and 2020, respectively. After one month of storage, fruits from trees treated with boron @ 0.6% recorded the maximum ascorbic acid content (55.47 and 49.61 mg/100g) in both years, while the minimum was recorded in the control (40.60 and 39.72 mg/100g). The result is in agreement with the findings of Chauhan et al. (2015) with the maximum ascorbic acid of mango at the end of the storage period. Boron most likely increases the ascorbic acid content in the fruit by improving membrane integrity (Milagres et al., 2019). The present study is supported by the findings of Venu et al. (2016) in acid lime. Boron slowed down the oxidation process, which in turn delayed the conversion rate of L-ascorbic acid. Since ascorbic acid is produced from sugar, the application of boron resulted in a higher level of ascorbic acid. Similar results were reported by Ali et al. (1991) in guava and Al-Obeed et al. (2017) in tangerine.

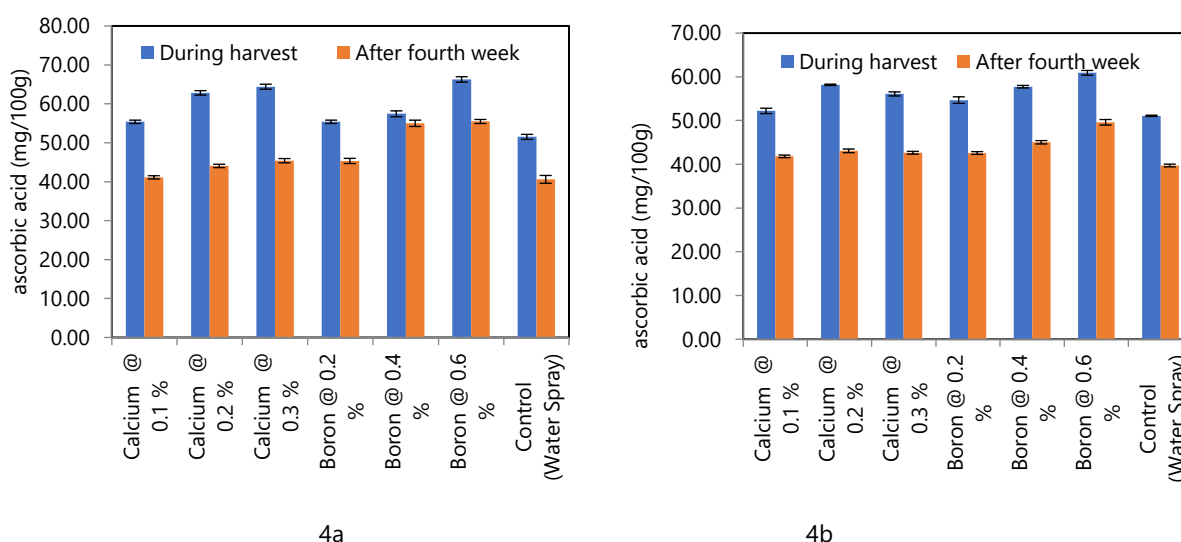


Figure 4. Effect of calcium and boron pre-harvest spray on ascorbic acid content of acid lime during postharvest storage in different periods (a) Change in ascorbic acid during the first year of the experiment (b) Change in ascorbic acid during the second year of the experiment.

3.5. Freshness of Fruit

After one month of study, the fruit harvested from boron-sprayed fruits @ 0.6% had the most preferred freshness of lime in 2019 and 2020 (2.67 and 3.0, respectively) followed by boron 0.4% (3.0 and 3.5). The use of boron @ 0.6 % and 0.4 % was more effective in maintaining the freshness of fruits than other treatments. Meena et al. (2017) also reported that Mandarin with the pre-harvest treatment of (Ca 3.0% + B 0.6% + Zn 0.6%) had the longest shelf life with the lowest PLW, decay percentage, and better freshness till the end of storage period. Similar results were reported in bananas (Raja et al., 2022). Davis et al. (2003) also reported that foliar application of boron-maintained fruit freshness increased the shelf life of tomatoes.

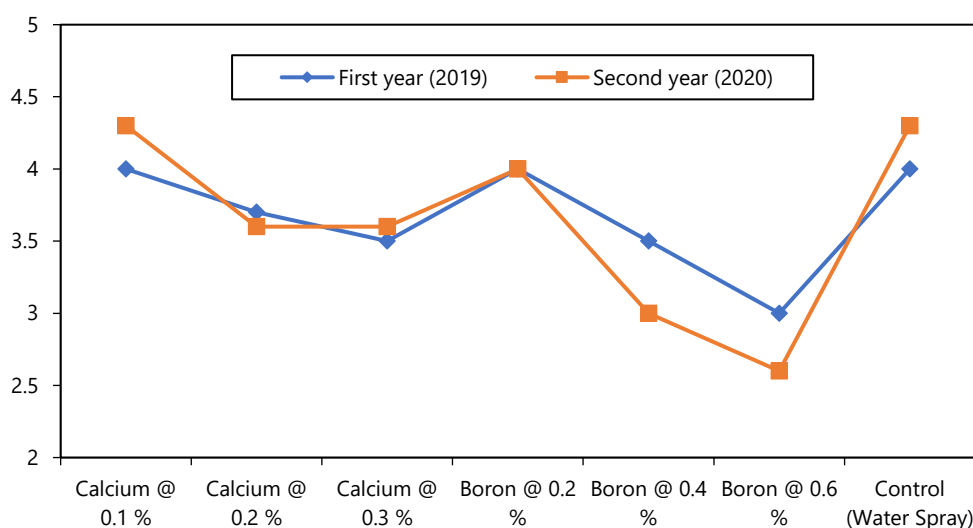


Figure 5. The freshness of acid lime after 1 month storage period in 2019 and 2020 (1: excellent, 2: good, 3: average, 4: poor, and 5: very poor).

4. Conclusion

Better pre-harvest and post-harvest management techniques are the key to minimizing post-harvest losses of horticultural commodities. Pre-harvest spraying of micronutrients in fruit trees is a promising technology to improve the post-harvest life of acid lime. Pre-harvest spraying of boron @ 0.6% showed a positive effect in terms of good post-harvest life of acid lime by maintaining both external and internal quality of fruits. These micronutrients help in improving the quality of fruit, which is easily available, economical to use, and safe for consumer's health. Thus, we concluded here that pre-harvest spraying of boron @ 0.6% two times before fruit harvest (i.e., 45 days before harvest and 30 days before harvest) can be used as a suitable technology to increase the shelf life of acid lime fruits.

Author Contributions: Conceptualization, Asmita Khanal; Data curation, Asmita Khanal and Sandip Timilsina; Funding acquisition, Purushottam Prasad Khatriwada; Investigation, Asmita Khanal, Sandip Timilsina and Susmita Khanal; Methodology, Asmita Khanal and Sandip Timilsina; Resources, Asmita Khanal and Sandip Timilsina; Software, Asmita Khanal and Susmita Khanal; Supervision, Purushottam Prasad Khatriwada; Validation, Asmita Khanal; Visualization, Sandip Timilsina; Writing – original draft, Asmita Khanal and Susmita Khanal; Writing – review & editing, Sunil Aryal. All authors have read and agreed to the published version of the manuscript.

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