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Exogenous Application of Gibberellic Acid, Salicylic Acid, and Calcium Chloride on Physical Properties and Shelf Life of Tomato

DHITAL, Priya*, RAUNIYAR, Krish & MAHAT, Samikshya
Himalayan College of Agricultural Sciences and Technology (HICAST), Kathmandu, Nepal
*Corresponding author: priyadhital.agri@gmail.com

ABSTRACT

The study was conducted from May 2nd to June 19th of 2024 at HICAST, Kirtipur. A Completely Randomized Design (CRD) comprising ten treatments with three replications was executed to evaluate the physical characteristics and shelf life of tomato fruits of the 'Srijana' variety treated with GA_3(O.1%, 0.2%, and 0.3%), $CaCl_2$ (0.5%, 1%, and 1.5%), and SA (0.1mM, 0.2mM and 0.3mM). The statistical analysis of data was completed using GenStat and Microsoft Excel. The quality parameters were evaluated after 15 days of storage and 25 days of storage. All the tested treatments were stored at an average room temperature of 26.9±2 °C (dry) and 20.5±2 °C (wet). They showed a significant delay in weight loss and spoilage percentage in treated tomato fruits compared to the control set. GA_3 treatments were the most effective, with 0.1% of GA_3 (T1) resulting in the least spoilage (34.1%), reduced weight loss (17.85%), the highest firmness (1.5 kg/cm^2), and the longest possible extension of the average shelf life to 29 days. With the increasing storage period, the ripening progressed, marked by the declining values of firmness on the 25th day. A particularly noteworthy observation is that the higher concentrations of $CaCl_2$ (1.5%) provided significantly better results than lower concentrations (0.5% and 1%). 0.3 mM of SA was more effective than lower concentrations, though higher concentrations might yield even better outcomes.

Keywords: Concentrations, exogenous application, physical characteristics, quality, yield

INTRODUCTION

Nepal is the land of wonder with agro-climatic variability (NHS, 2016) and unique agro-ecological zones favored by varying altitudes, topography, and aspects offering vast opportunities for the production of different types of horticultural crops *i.e.*, fruits, vegetables, flowers, spices, and other plantation crops (Thapa and Dhimal, 2017). Owing to this, agriculture plays a dominant role in the Nepalese economy, contributing about 23.95% to the nation's GDP in the fiscal year 2022/2023. Whereas within horticultural crops; vegetables have contributed 14.46% to the AGDP of Nepal (MoALD, 2023).

Tomato, the poor man's apple, is one of the major fresh vegetables grown in Nepal belonging to the Solanaceae family and ranks 3rd position in terms of area (Ha), production (Mt), and yield (Mt/Ha) following the Cole crops (MoALD, 2023). Even though it is perennial in its native habitat, it is often grown as an annual crop. Botanically it is a berry fruit, however, it is categorized under vegetables for culinary purposes (MoEF&CC / IIVR, 2016). Tomatoes are consumed in different forms such as raw, cooked, sauces, salads, and even drinks. It is both a nutritionally and medicinally important crop, rich in powerful antioxidants such as lycopene, carotenoids, and flavonoids. These compounds are not only beneficial for health but also play a crucial role in preventing cancer, cirrhosis, and acidosis and neutralizing everyday toxins (Mallick, 2021). Despite the increasing production and domestic and international demand for tomatoes, Nepal still imports most of the tomatoes from India, China, Bangladesh, the UAE, and Qatar valued at over NPR 499,530,810 (TEPC, 2022). With issues such as subsistence farming, linkage to the international market, transportation facilities (CASA, 2020), lack of technical know-how, and traders' monopoly in price fixation (Kafle and Shrestha, 2017), the major factor contributing to the lack of supply of tomatoes is its "Perishable nature" and consequently prone to price fluctuations. The solution to the perishability of tomatoes lies both in the pre-harvest and post-harvest factors that can extend the shelf life and maintain the quality through the application of optimal technological levels (Mir and Beaudry, 2002). Among several post-harvest treatments such as refrigeration, freezing, use of high temperatures, and modified atmosphere packages (Mir and Beaudry, 2002), treatment with phytohormones, growth regulators and various chemical calcium compounds are alternative technologies to maintain and improve fruit quality (Aguilar-Ayala and Herrera-Rojas, 2023). During storage, exogenous application of certain chemicals such as Calcium chloride and PGRs such as Gibberellic acid and Salicylic acid which retard the ripening process can prove to be extremely valuable.

Gibberellins (GAs) are phytohormones that regulate plant growth and development.Its chemical formula is $C_{19}H_{22}O_6$. The effects of exogenous GA_3

treatment have been studied in different crops which showed that it could improve nutritional traits, retard decrease in ascorbic acid concentration, increase total phenolic content in tomatoes (Demes *et al*., 2021), inhibit the expression of fruit ripening regulators e.g., Ripening inhibitor (RIN) (Zhang *et al*., 2023) and prolong fruit ripening time. Calcium is an essential plant nutrient for plant growth and development and is present in the cell wall as a binding agent (Bhattarai and Gautam, 2006). Researchers have reported that calcium treatment contributes to cell-wall integrity, maintenance of firmness, delays softening and decay (Pila *et al*., 2010), inhibits ethylene production, and lowers respiration rates (Mansourbahmani *et al*., 2017). $CaCl_2$ is affordable and relatively easy to prepare therefore its adoption is feasible for marginal farmers in developing countries to reduce postharvest losses in tomatoes (Arah *et al.*, 2016). According to Bhattarai and Gautam (2006), it is not toxic even in higher concentrations making it safer to use. Salicylic acid ($C_6H_4(OH)CO_2H$) is an endogenous plant growth regulator and a natural phenolic acid. Exogenous application of SA treatment benefits postharvest storability in horticultural commodities by delaying fruit ripening, reducing cell wall degrading enzyme activity, maintaining cell membrane properties, improving fruit taste, retaining nutrient content, and enhancing antioxidant activity hence extending and improving the shelf life (Wang *et al*., 2022). Salicylic acid is considered a safe substance that can be put on vegetables and fruits in lower concentrations (Nicktam*et al*., 2023).

MATERIALS AND METHODS

Procurement of tomatoes

The “Srijana” variety of tomatoes was procured from farmers in Shankharapur municipality, Sankhu, Kathmandu. Fresh healthy tomatoes harvested in the turning and pink /breaker stage, evenly proportioned, unbruised, and with no injury and signs of disease, were selected and collected.

Experimental site

The experiment was conducted in the Horticulture laboratory of the Himalayan College of Agricultural Sciences and Technology. The research was performed in ambient storage conditions. A thermometer was used to measure the dry and wet temperatures in the research space. The observation room's temperature was recorded daily.

Experimental design and treatment details

The Completely Randomized Design (CRD) was used with 10 treatments and three replications of each treatment.

Table 1. Treatment Details

SN	Treatment	Treatment details
1	T1	0.1% Gibberellic Acid (Dipped in 0.1% GA_3 for 20 mins)
2	T2	0.2% Gibberellic Acid (Dipped in 0.2% GA_3 for 20 mins)
3	T3	0.3% Gibberellic Acid (Dipped in 0.3% GA_3 for 20 mins)
4	T4	0.5% Calcium chloride (Dipped in 0.5% $CaCl_2$ for 20 mins)
5	T5	1% Calcium chloride (Dipped in 1% $CaCl_2$ for 20 mins)
6	T6	1.5% Calcium chloride (Dipped in 1.5% $CaCl_2$ for 20 mins)
7	T7	0.1 mM Salicylic Acid (Dipped in 0.1 mM SA for 20 mins)
8	T8	0.2 mM Salicylic Acid (Dipped in 0.2 mM SA for 20 mins)
9	T9	0.3 mM Salicylic Acid (Dipped in 0.3 mM SA for 20 mins)
10	T10	Control (Dipped in distilled water for 20 mins)

The tomatoes were cleaned, washed, sterilized using sodium hypochlorite (500ppm for 10 min), and air dried before dipping in respective chemical solutions. A sample size of 10 tomatoes was allocated for each treatment. After treatment, the fruits were kept in a makeshift aluminum bowl. Data were taken in alternate day intervals until signs of decay or spoilage were observed and then, the physical parameters were analyzed after 15 days of storage and on the 25th day of storage until commercial condition. The ambient temperature of the storage room was noted.

Parameters observed

Physiological weight loss (PLW %)

To calculate the PLW% of the tomato, the initial weight was recorded before the application of the treatments and then at alternate day intervals during storage using a digital sensitive balance. Finally, the following formula was used to calculate the PLW %:

$$\text{PLW \%} = \frac{\text{wt. of sample at first interval-wt. of sample at second interval}}{\text{wt. of sample at first interval}} \times 100$$

Fruit length and diameter (cm)

A vernier caliper was used to measure both the fruit length and diameter of the tomato in cm. The fruit length was measured longitudinally while the diameter was measured transversely.

Firmness (Kg/cm^2)

The firmness of the fruits was determined using a penetrometer having varying pressure tester knobs. After the selection of the appropriate pressure tester knob for tomato fruits, the plunger was held against the surface of the fruit and forced into it with a steady pressure. This action provided the force necessary for breaking the flesh in Kg/cm^2 on the pressure tester which was recorded as the firmness of the given fruit.

Spoilage percentage

Spoilage % was determined by the visual observation. The fruits exhibiting signs of rotting such as the development of spots on the skin of fruits, and softening were considered as spoiled. The spoilage % was then calculated by dividing the number of spoiled fruits by the initial number of all fruits times 100.

Color

Color was evaluated using a standardized color chart by the Royal Horticulture Society (2019). The color of the fruit was matched to the closest on the color chart to determine the color intensity or shade of it to the maximum acceptable stage of tomato.

Shelf life

Tomato's shelf life was determined by calculating the number of days required to attain the last stage of ripening and then at the point where the fruit could still be sold *i.e.*, it remained acceptable for marketing.

Marketable fruit (%)

Marketable fruit was determined by using the following formula.

$$\text{Marketable fruit} = \frac{\text{initial weight} - \text{damage weight}}{\text{initial weight}} \times 100$$

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Statistical analysis

Data was systematically arranged using observed parameters, and analysis of variance was performed using EXCEL and GENSTAT. The available literature was used to assist in the analysis of the data and the discussion of the findings.

RESULTS AND DISCUSSION

Physiological loss in weight

After harvest, tomato fruits transform from maturity to senescence, leading to gradual weight loss and increased spoilage. The data presented in Figure 3 illustrates the physiological weight loss (PLW) in tomatoes during the storage period of 15 days. Results indicated that the PLW of the control group (T10) was the highest at 34.09% and the lowest in tomatoes treated with 1.5 % $CaCl_2$ (16.81%). Treatments, T1 (GA_3-0.1%), T5 ($CaCl_2$-1%), and T9 (SA-0.3mM) were found to be significantly (p<.001) effective in reducing weight loss in tomato fruits.

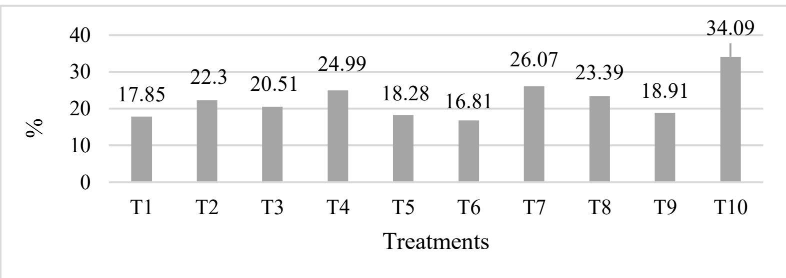

Figure 1. Effect of different treatments on physiological loss in weight

Weight loss in fresh tomatoes is primarily attributed to respiration and transpiration (Demes *et al*., 2021). Thus, reducing the rate of water loss and enzyme activities would lead to reduced weight loss. The results in the present study reveal that the higher concentrations of calcium chloride (1% and 1.5%) were the most effective in reducing weight loss, aligning with the results reported by Demes *et al*. (2021). Calcium forms a network with pectin in the fruit cell wall that restricts moisture loss, leading to reduced weight loss (Genanew, 2013). Similarly, gibberellic acid is a phytohormone that reduces fruit loss by decreasing respiration and delaying senescence (Aguilar-Ayala and Herrera-Rojas, 2023). Singh and Patel (2014) also reported that the GA_3 treatment in tomatoes decreased the tissue permeability, thereby reducing the rate of water loss and delaying ripening. Similar findings were demonstrated by Chen *et al.* (2020), who noted that the exogenous application of GA_3 delayed the ripening time through the regulation of transcript levels of ethylene-related genes. With the delayed aging of fruit, the loss of water, flavor, and nutrition is also significantly reduced (Zhang *et al.*, 2023).

Chavan and Shakhale (2020) found that salicylic acid caused a very gradual increase in weight loss in tomatoes during storage. This is further supported by Ünal *et al.* (2021) who reported the lowest weight loss in tomatoes treated with 1mM and 0.5mM SA stored at ambient temperature. SA has been reported to cause the closing of stomata in fruits which resulted in suppressed respiration rate and minimized weight loss (Tareen *et al.*, 2012).

Fruit length and diameter

The initial average mean length and diameter were recorded at 4.36 cm and 4.19 cm, respectively (Figure 4). After the 10th day of storage, the results indicated that none of the treatments maintained the initial average fruit length and diameter. With lengths 4.32 cm and 4.29 cm, it is evident that treatments T1 (0.1% GA3) and T7 (SA 0.1 mM) were effective in preserving fruit length,*i.e.*, closest to the initial average. Similarly, treatments T7 (SA 0.1 mM) and T8 (SA 0.2 mM) were the most effective in maintaining a diameter of 4.15 cm and 4.16 cm, respectively, *i.e.*, close to the initial average diameter of 4.19 cm. However, these differences were not statistically significant.

Firmness (kg/cm^2)

Firmness is closely associated with the ripening stage and is an important judgment factor, after visual appearance, for consumers. Table 2 represents the firmness of tomatoes during 15 days of storage at ambient temperature. Maximum firmness was recorded in treatment T1 (0.1% GA_3) valued at 1.5 kg/cm^2, followed by T6 ($CaCl_2$ 1.5%), measuring 1.42 kg/cm^2. On the contrary, the lowest firmness was observed in treatment T8 (0.2 mM SA) followed by T7 (0.1 mM) and the control. The firmness continued declining with the storage period and during the 25th day of storage, among the remaining treatments (T1, T2, T3, T4, T5, and T6) it was observed that the firmness declined by 23.95% in T4 (the least) followed by T2 (25.38%) while others have firmness declined by not more than 50%, which indicates the effectiveness of GA_3 and $CaCl_2$.

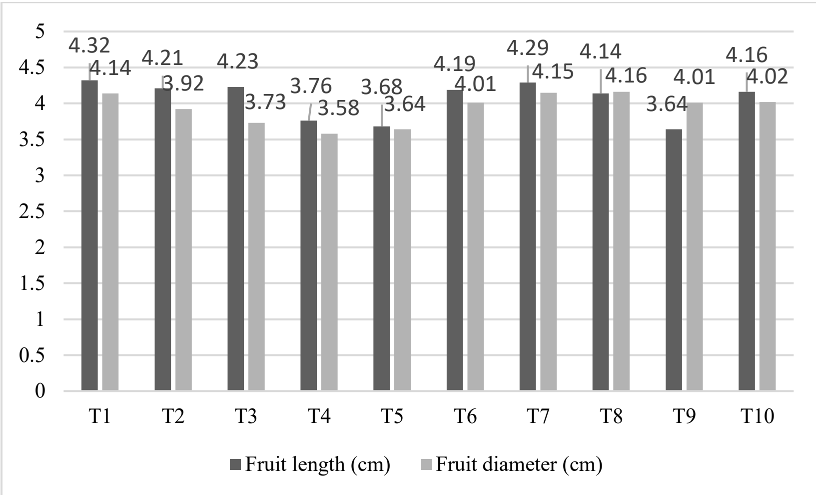

Figure 2. Effect of different treatments on mean length and diameter

These findings are supported by the research of Demes *et al.* (2021) who demonstrated that the same concentrations of $CaCl_2$ and GA_3*i.e.*, 1.5% and 0.1% were the most effective in delaying the fruit softening in tomatoes. It may be due to the inhibiting action of GA_3 that inhibits ethylene biosynthesis and activation of ripening regulator genes as per the study of Li *et al.* (2019). Likewise, higher concentrations of $CaCl_2$ 1% and 1.5% were effective in maintaining the firmness in tomatoes which is per the findings of Gharezi *et al.* (2012) who treated cherry tomatoes with varying concentrations of $CaCl_2$ and concluded that calcium dipping retarded the metabolism, slowed respiration rate and hence improved the firmness of tomatoes. An extension of this idea can be found in the study of Li *et al.* (2012), that the addition of calcium improves cell wall rigidity and inhibits the activity of enzymes like Polygalacturonase, which causes softening of fruits (Gautam and Bhattarai, 2009). The results of the present study revealed significant retention of firmness through the application of SA as reported earlier by Chavan and Shakhale (2020) in tomatoes. Like GA_3 and $CaCl_2$, SA also causes inhibition of cell wall and membrane degrading enzymes such as polygalacturonase, lipoxygenase, cellulase, and pectin methyl esterase and therefore, a lower rate of ethylene production (Nicktam *et al.*, 2023).

None of the treatments was significant in maintaining the length and diameter, representing the inevitable physiological changes and shrinkage that occurs in fruit after harvest. Ghareziet *al*. (2012) also noticed a similar result in cherry tomatoes treated with calcium chloride where all the tomatoes shrunk in size regardless of the treatment.

Table 2. Effects of different treatments on Firmness (kg/cm^2)

Treatment code	**Treatments**	**Firmness (kg/cm^2)**
T1	0.1% GA_3	1.5
T2	0.2% GA_3	1.23
T3	0.3% GA_3	1.32
T4	0.5% $CaCl_2$	1.05
T5	1% $CaCl_2$	1.1
T6	1.5% $CaCl_2$	1.42
T7	0.1mM SA	0.91
T8	0.2mM SA	0.88
T9	0.3mM SA	1
T10	Control	0.95
	SE±M	**0.08**
	LSD at 5%	**0.18**
	CV%	**9.1**

SE±M = Standard error of differences of mean, LSD= Least Significant Difference, CV = Coefficient of Variation

Spoilage %

The treatment T1 (0.1% GA_3) was found to have significantly lowest spoilage % *i.e.*, 34.81%, followed by T6 (1.5% $CaCl_2$) with 35.07% and T9 (0.3 Mm SA) with 35.26% during the storage period, as shown in Table 3. Whereas, the highest spoilage was recorded in the treatment T10 or the control (64.63%). Comparing the LSD value at 5% with the mean differences of the treatment, it is found that the treatments T1, T5 (1% $CaCl_2$), T6, and T9 (0.3 mM SA) are significantly different than the control, indicating their effectiveness in reducing the spoilage percentage. In like manner, Singh and Patel (2014) also reported the maximum decrease in decay loss in tomatoes treated with GA_3 on the 12th day of storage and suggested it was due to a decrease in the ripening process during the storage period.

Spoilage % increases with the increased rate of ripening during which the cell wall breaks down due to the action of hydrolases leading to fruit softening (Gautam and Bhattarai, 2006). This cell wall carbohydrate metabolism and the consequent loss of firmness have been associated with increased susceptibility to fungal infections (Conway *et al*., 1987). In the present study, the majority of the spoiled tomato fruits exhibited a distinctive woolly mold appearance and emitted a foul odor which can be a sign of fungal infection.

Color of the tomato

Initially the fresh, visually appealing and pink stage tomato was selected and grouped into treatment which was compared. On the initial observation day, color closely matched 55A on the color chart. The treatments maintained consistent color change from pink stage (55A) to red stage (44B) throughout the ambient storage conditions aligning with Yang *et al.* (2022) findings.

Shelf life days and marketable fruit%

Shelf life and marketable fruit percentage during postharvest treatments on tomatoes are summarized in Figure 5. The data represented shows shelf life and marketable fruit% % were significantly ($p < 0.05$) affected by GA_3, $CaCl_2$, and SA during the storage period. The fruits treated with T1, T6, and T9 extended the average shelf life to almost twice that of the control (15 days). Fruits treated with T1 (0.1% GA_3) had the longest possible average shelf life of 29 days followed by those treated with T6 ($CaCl_2$ 1.5%) at 27 days and T9 (0.3 mM SA) at 24 days. In the same fashion, the results for marketable fruit% follow almost the exact pattern where T1, T6, and T9 have twice the amount of marketable fruit than in control (35.57%) *i.e.*, 65.2%, 64.94%, and 64.74%, respectively.

These results also support the view of Pila *et al*. (2010) who reported that the fruits treated with 0.1% GA_3, 1.5% $CaCl_2$, and 0.4mM SA had the most significant impact on the shelf life than other concentrations and control, and caused the extension of storage life by 18, 17 and 15 days respectively. GA_3 and $CaCl_2$ were found effective over other treatments extending shelf life up to 21 days (Dhami *et al*., 2023) which can be attributed to the negative roles of GA_3 towards the ripening of tomatoes as demonstrated by Li *et al*. (2019). Similarly, Bhattarai and Gautam (2009) reported that the higher the concentration of $CaCl_2$ (1% and 1.5%), the longer the shelf life of tomatoes was than the lower concentrations. Moreover, fruits treated with SA did exhibit longer shelf life days than the control, however, it wasn't as significant as other treatments. This discrepancy might be due to the concentrations of SA used to treat fruits and storage temperature, contrary to Mandal *et al*. (2016) who compared lower concentrations of SA (0.2, 0.4, 0.6, and 0.8 mM) with higher concentrations of SA (1 and 1.2mM) at refrigerated conditions and found that higher concentrations of SA were significantly effective in extending the shelf life of tomatoes up to 32.75 days.

Table 3. Effects of different treatments on Spoilage%

Treatment code	Treatments	Spoilage %
T1	0.1% GA_3	34.81
T2	0.2% GA_3	41.31
T3	0.3% GA_3	39.21
T4	0.5% $CaCl_2$	44.45
T5	1% $CaCl_2$	36.02
T6	1.5% $CaCl_2$	35.07
T7	0.1mM SA	48.61
T8	0.2mM SA	54.25
T9	0.3mM SA	35.26
T10	Control	64.63
	SE±M	**3.62**
	LSD at 5%	**7.61**
	CV%	**20.4**

SE±M = Standard error of differences of mean, LSD= Least Significant Difference, CV = Coefficient of Variation

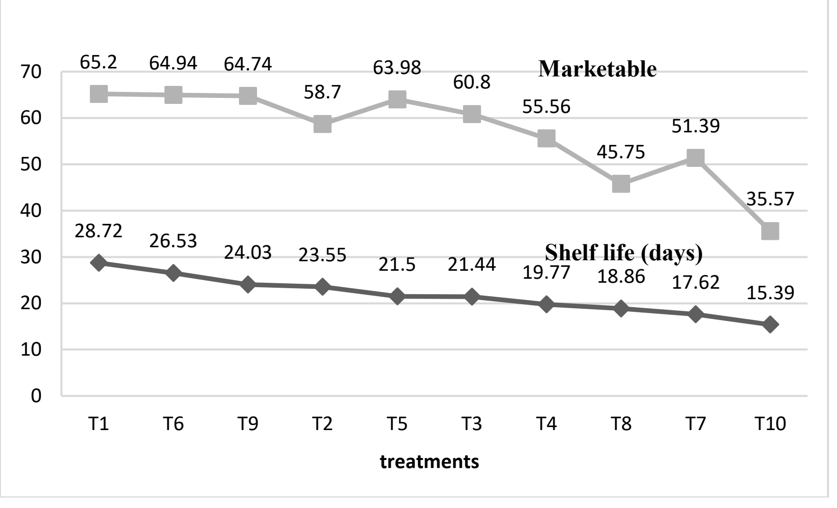

Figure 3. Effect of different treatments on shelf life and marketable fruit, %

Chavan and Sakhale (2020) also treated tomatoes with a higher concentration of SA *i.e.*, 200 ppm SA, and reported those fruits had a storage life of 32 days compared to the control that lasted only for 12 days stored at 24ºC. Likewise, the treatment of tomatoes with 0.75 mM salicylic acid prolonged the shelf life by 7 days along with a lower weight loss percentage and was proved to be more effective than oxalic acid (Kant *et al*., 2013). Similar results were observed by Tareen *et al*. (2012) in peach fruits treated with 2 mmol L^{-1} extending its shelf life up to five weeks without any spoilage stored at 0ºC.

CONCLUSION

Plant growth regulators gibberellic acid (GA_3) and salicylic acid (SA), along with chemical calcium chloride ($CaCl_2$), have shown great potential to positively impact and maintain the physical properties of tomatoes over an extended period.

Overall, the findings indicate that the exogenous application of gibberellic acid, calcium chloride, and salicylic acid successfully extends the shelf life of tomatoes up to 29 days under ambient conditions through the maintenance of physical characteristics, while in some cases even improving them. These treatments offer a safe, eco-friendly, economical, and sustainable alternative to high-tech equipment for effectively reducing post-harvest loss.

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