

Calcium influences biochemical and antioxidant enzymatic activities in tomato fruits during storage

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ABSTRACT

Postharvest quality maintenance of tomatoes is very crucial because of its perishable nature. Calcium, directly involved in the physiological process and enzymatic activities, thereby helps in maintaining the postharvest quality. Increasing calcium concentration reduces respiration and ethylene production in succession; it increases the shelf life and quality of tomatoes. Hence, a field experiment was conducted with different sources and levels of Ca, namely, calcium sulfate, calcium nitrate, calcium silicate, poultry manure, and press mud applied at 0, 20, 40, 60, and 80 kg Ca ha⁻¹. Ripened tomatoes were harvested from each plot. Eight fruits were selected randomly from every treatment and divided into destructive and nondestructive samples, arranged in a factorial completely randomized block design for conducting a storage study. Shelf life, ascorbic acid, lycopene, beta-carotene, and polygalacturonase were analyzed at a 5-day interval up to 15 days. The results revealed that the effect of calcium sources had significant ($p \leq 0.05$) impacts on fruit quality. The tomatoes harvested from poultry manure retained the highest shelf life (15 days) at 80 kg Ca ha⁻¹ and the highest ascorbic acid content (37.5 mg 100 g⁻¹) at 60 kg Ca ha⁻¹. However, the lowest lycopene (2.86 mg 100 g⁻¹), beta-carotene (1.81 mg 100 g⁻¹), and lesser polygalacturonase activity (1.99 kJ m⁻²) were obtained with poultry manure at 80 kg Ca ha⁻¹. From the results procured in this experiment, it was inferred that the application of poultry manure at 80 kg Ca ha⁻¹ was found to be effective in improving the quality attributes of hybrid tomato as it imparts multiple essential nutrients along with calcium.

1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is a perennial plant grown in temperate climates and belongs to the nightshade family. It is botanically a fruit but considered a vegetable for a culinary purpose [1]. It is the world's third most important vegetable crop widely grown in various soil and climatic conditions [2]. The area and production of tomatoes in India (second advance estimate of 2019–20) were 812 ha and 20,573 metric tonnes, respectively. It functions as an important source of nutrients that rich in vitamins, minerals, and dietary fiber, including antioxidants like lycopene and beta-carotene, which serve as an anticarcinogen [3,4]. It is considered as a cash crop for small- and medium-scale farmers providing employment opportunities

in production and processing industries [5,6]. Farmers are interested more in tomato production for its multiple harvests, high profitability, and its ability to improve farm income and human nutrition [6,7]. The fleshy fruits are highly perishable climacteric horticulture produce, vulnerable to decay and quality loss due to cell wall degrading enzymes, high respiration, and ethylene production after harvest. This increase in postharvest losses makes its production unprofitable [8–10]. The postharvest loss is around 20% to 50% of the harvested produce which is lost during harvesting, transportation, and storage [11].

Calcium (Ca) has the potential to decrease fruit softening and postharvest decay, delay senescence, and retain fruit quality during storage. It also maintains tissue firmness, membrane integrity, and cell turgor, which helps in delaying membrane lipid catabolism and extends the storage life of tomato fruits. Calcium ions (Ca²⁺) are also involved in many physiological processes in vegetables, thus playing a vital role in maintaining their quality [12].

Calcium is an important secondary element that maintains plant cell wall rigidity, membrane stability which is associated with

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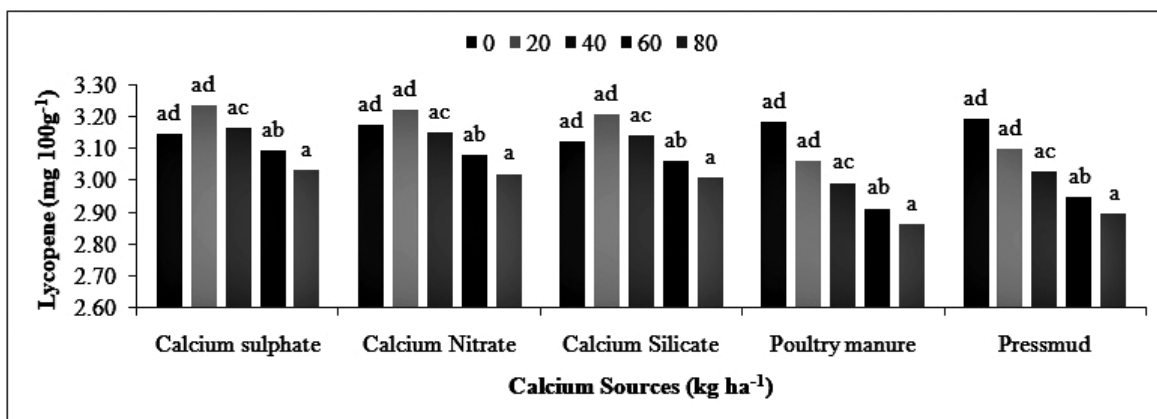


Figure 1: Effect of various calcium sources and levels on lycopene content of hybrid tomatoes.

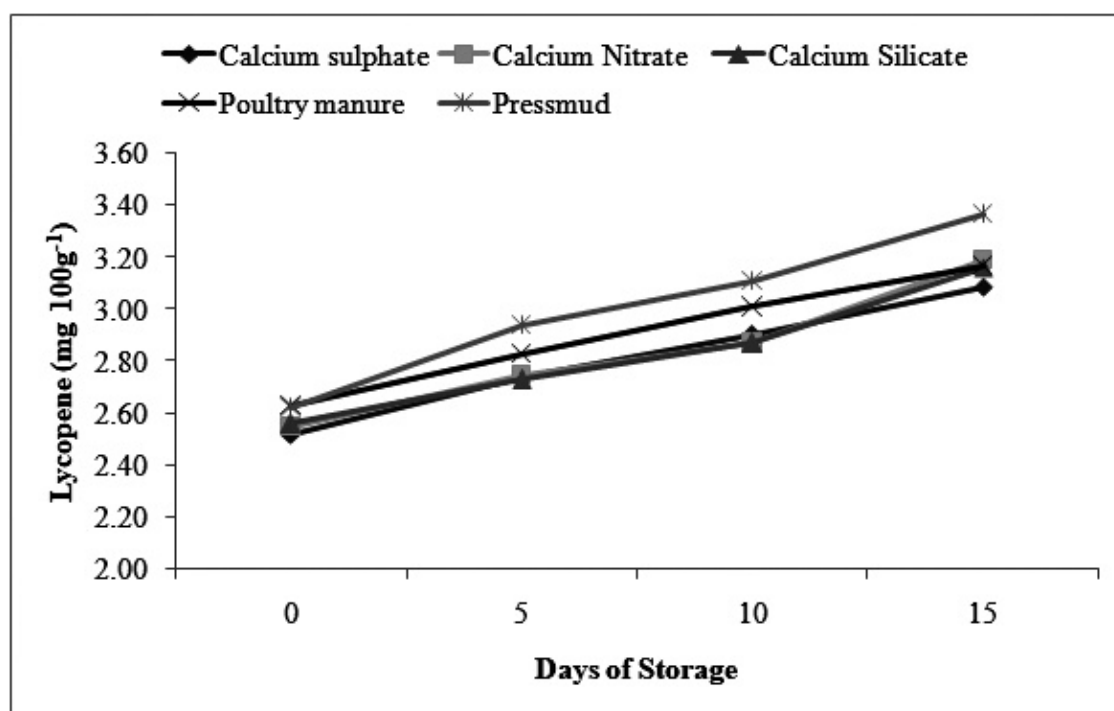


Figure 2: Effect of various calcium sources and levels on lycopene content of hybrid tomatoes.

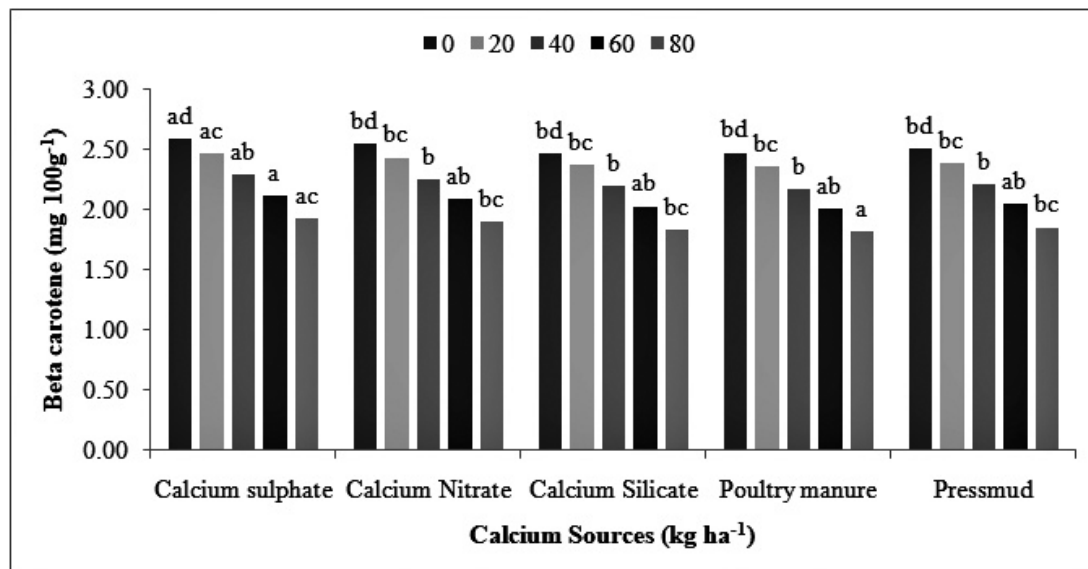


Figure 3: Effect of various calcium sources and levels on beta-carotene content of hybrid tomatoes.

postharvest life, and ripening of tomato fruits which lowers the incidence of many physiological disorders and extends the postharvest life of tomato fruits [13,14]. Soil calcium not only enhances the growth and yield of the tomato crop in field but also plays a major role in postharvest quality benefits, which increases ascorbic acid (vitamin C) content and firmness, decreases respiration rate and ethylene production, delays ripening, and extends the storage life of tomato fruits. Apart from that, calcium also plays a major role in the fruit physiology of tomatoes by taking part in signaling pathways of hormones such as ethylene, auxin, and abscisic acid [15].

Therefore, keeping these points in view, this work was carried out to examine the role of calcium in regulating biochemical constituents and enzymatic activity, which in succession enhances the storage life of hybrid tomatoes fruits.

2. MATERIALS AND METHODS

2.1. Treatments and Experimental Design

The field experiment was conducted on Palaviduthi soil series in the farmer's field belonging to Devarayapuram, Coimbatore (N 11°00.218, E 076°48.094), using hybrid tomato Shivam with six calcium sources, namely, calcium sulfate (CaSO_4), calcium nitrate ($\text{Ca}(\text{NO}_3)_2$), calcium silicate (Ca_2SiO_4), poultry manure, and pressmud applied at five levels of calcium (0, 20, 40, 60, and 80 kg Ca ha^{-1}) for improving the quality of hybrid tomato. The experiment was laid out in 5 x 5 factorial arrangements in a randomized block design (RBD) with three replications. The tomato hybrid Shivam, which is considered as a ruling tomato hybrid, and the 25-day-old seedlings of tomato hybrid were transplanted in the laid-out field with the spacing

of 45 x 30 cm. The major nutrients such as nitrogen, phosphorus, and potassium were applied in the form of urea, di-ammonium phosphate, and muriate of potash, respectively, as per soil test crop response-integrated plant nutrient supply fertilizer prescription to achieve a targeted yield of 70 tonnes ha^{-1} . All agronomic practices such as weeding, cultivation, and irrigation were carried as per recommendations carried out and the crop was grown to maturity.

2.2. Sample Collection, Processing, and Analysis

From every treatment (25), the tomatoes were harvested at the red ripen stage separately at each replication, and 8 fruits were selected randomly for conducting the experiment. The selected tomato fruit samples were placed on tables in a factorial completely randomized design for storage study and analyzed for quality attributes. The tomatoes were analyzed for ascorbic acid, lycopene, beta-carotene, and polygalacturonase activity at a 5-day interval (0, 5, 10, and 15 days after harvest), and the shelf life of hybrid tomato was recorded.

2.3. Fruit Quality Attributes

2.3.1. Ascorbic acid

The ascorbic acid content of the fruit samples was determined by the 2,6-dichlorophenol indophenol titration method. Ten gram of fresh fruit sample was macerated with 4% oxalic acid and made up to 100 ml using 4% oxalic acid. 10 ml of the extract was titrated against the standardized dye, and the appearance of pale pink color was considered as an endpoint [16]. The ascorbic acid content was determined using the titer value and dye factor values (0.1 l^{-1}) and expressed as mg 100 g^{-1} .

Table 1: Effect of various calcium sources and levels on ascorbic acid content in the fruits of hybrid tomato.

Sources	Levels of calcium (kg ha^{-1})					Mean
	0	20	40	60	80	
Calcium sulfate	30.7	32.1	33.0	34.1	33.4	32.7
Calcium nitrate	31.5	32.1	33.8	34.8	34.0	33.3
Calcium silicate	31.4	32.7	33.4	36.3	33.9	33.5
Poultry manure	31.2	33.6	34.0	37.5	34.3	34.1
Press mud	30.8	32.4	33.5	34.8	33.7	33.0
Mean	31.1	32.6	33.5	35.5	33.9	33.3
	<i>S</i>		<i>L</i>		<i>SL</i>	
SEd	0.10		0.20		0.22	
CD ($p = 0.05$)	0.20		0.18		0.44	

Table 2: Effect of various calcium sources and levels of calcium on ascorbic acid content of hybrid tomatoes during the storage period.

Sources	Time (days)				Mean
	0	5	10	15	
Calcium sulfate	32.0	33.4	34.1	34.6	33.5
Calcium nitrate	31.6	32.5	33.1	33.4	32.6
Calcium silicate	31.5	32.4	33.0	33.8	32.7
Poultry manure	32.1	33.3	34.9	36.1	34.1
Press mud	31.1	32.6	33.7	34.7	33.0
Mean	31.8	33.2	34.1	35.1	33.6
	<i>S</i>	<i>T</i>		<i>ST</i>	
SEd	0.10	0.08		0.20	
CD (<i>p</i> = 0.05)	0.20	0.16		0.39	

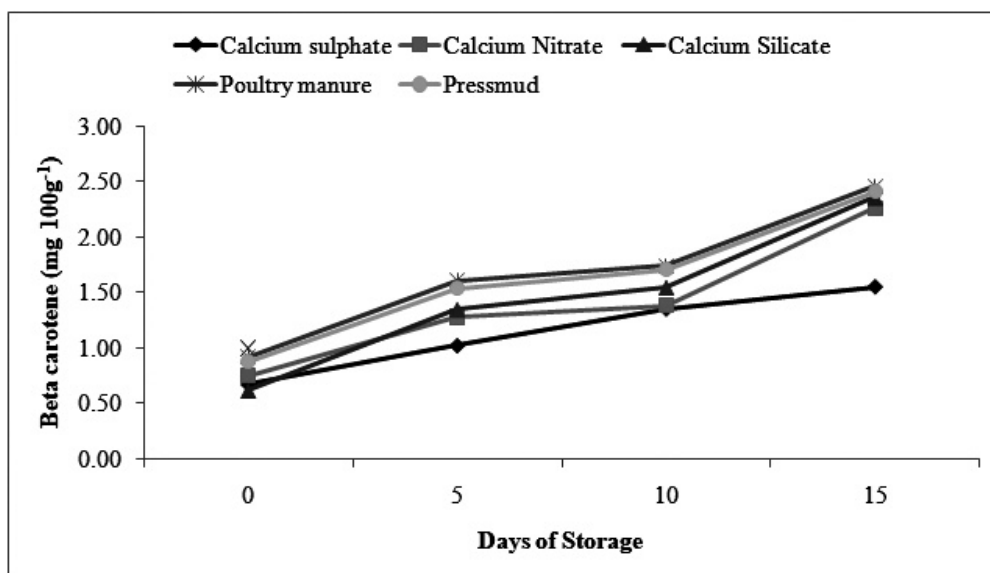


Figure 4: Effect of various calcium sources and levels on beta-carotene content of hybrid tomatoes during the storage period.

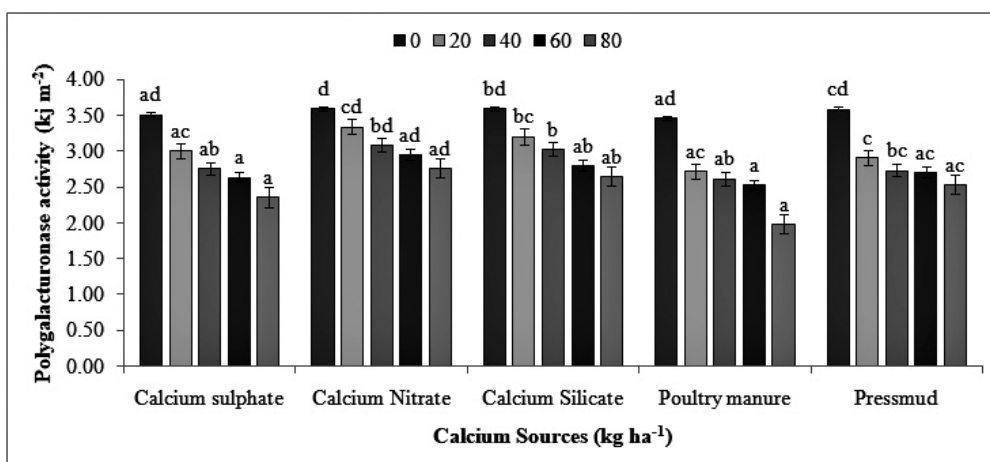


Figure 5: Effect of various calcium sources and levels on polygalacturonase activity of hybrid tomatoes.

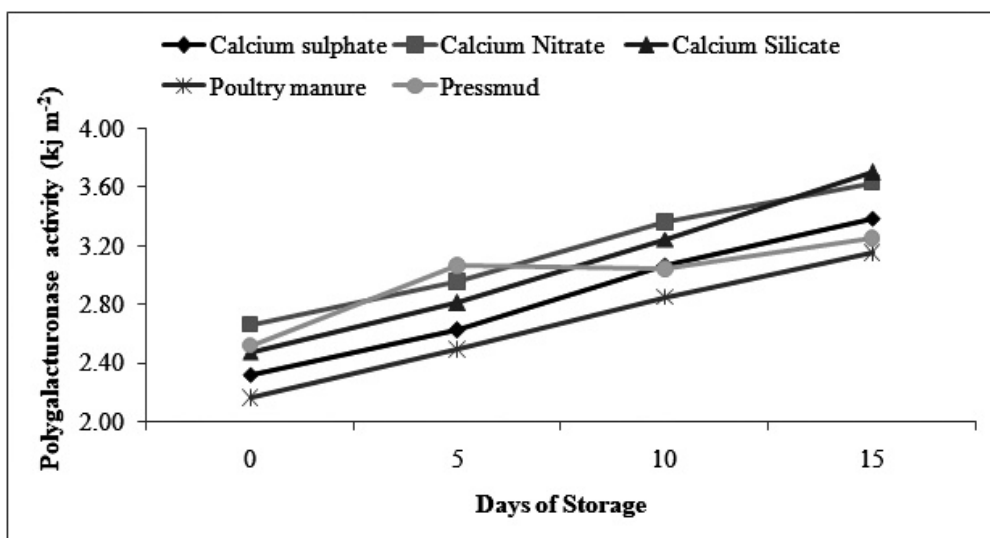


Figure 6: Effect of various calcium sources and levels on polygalacturonase activity of hybrid during the storage period.

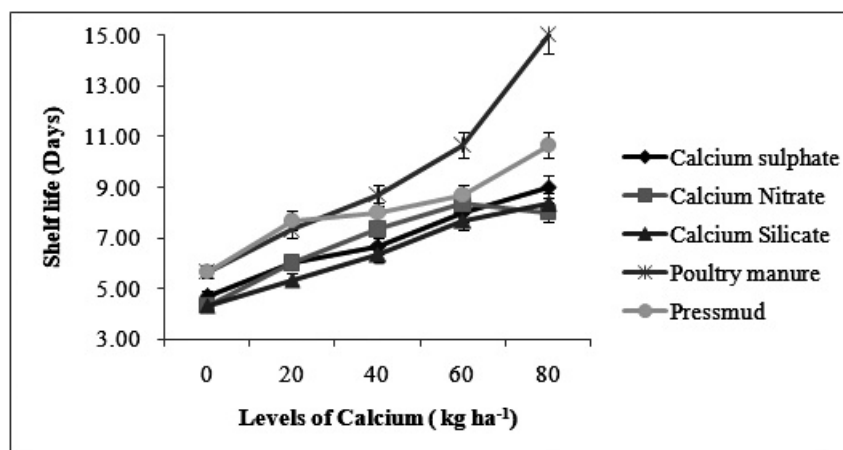


Figure 7: Effect of various calcium sources and levels on shelf life activity of hybrid during the storage period.

2.3.2. Lycopene and beta-carotene content

To determine the lycopene and beta-carotene content in the fruit sample, 1 g of samples was crushed thoroughly in a pestle and mortar. The lycopene pigment was extracted by dissolving in 10 ml of acetone, and the mixture was transferred to a separating funnel containing 20 ml of petroleum and 20 ml of 5% sodium sulfate was added. The extract was decanted, and the absorbance was measured for lycopene content at 503 nm and beta-carotene at 453 nm [17].

2.3.3. Polygalacturonase

The homogenized (10 g) fruit sample was mixed with 13 ml of Tris-HCl buffer and centrifuged at 15,000 rpm for 1.5 minutes. Then, 5% trichloroacetic acid was added and centrifuged at 2,000 rpm for 30 minutes.

2.3.4. Shelf life of fruits

The shelf life of fruits was determined by counting the number of days up to which the fruits remain acceptable for marketing. It was decided based on the appearance and spoilage of tomato samples [18].

2.4. Statistical Analysis

The data obtained from this study were subjected to the analysis of variance to find out the significance [19]. The mean separation was determined by the least significant difference and was indicated by the symbol (*, ** for 5% and 1%) wherever treatment differences were confirmed to be significantly different [critical differences (CD)] at the 5% level of significance. Nonsignificant comparisons were indicated as NS.

3. RESULTS AND DISCUSSION

3.1. Ascorbic Acid

Ascorbic acid in tomato is known for its antioxidant properties, plays a major role in the regulation of cell division and expansion, and also serves as a cofactor for various enzymes. The different

calcium sources and levels had a significant effect on ascorbic acid content in the tomato fruits during the storage period, which varies from 32.7 to 34.1 mg 100 g⁻¹. Initially, lesser ascorbic acid content was noted in control (30.7 mg 100 g⁻¹) and on increasing calcium concentration; the ascorbic acid content got increased until 60 kg Ca ha⁻¹ and then declined at 80 kg Ca ha⁻¹. The mean ascorbic acid content was higher (37.5 mg 100 g⁻¹) when calcium was applied at poultry manure at 60 kg ha⁻¹ (Tables 1 and 2). The gradual rise in the ascorbic acid content of fruit was noted on the ripening of tomatoes. This change could be due to oxidation, where cellular respiration rate increases with the onset of the ripening process, a characteristic of climacteric fruits [19,20]. The ascorbic acid content consistently increased with increasing calcium concentration, as it reduces loss of ascorbic acid in the storage due to oxidative regulation processes in the cytosol, where calcium delays rapid oxidation of ascorbic acid [12]. It also might be due to the action of calcium on counteracting the degradation of ascorbic acid content due to excessive light exposure and increase in fruit temperature [21].

3.2. Lycopene and Beta-Carotene Content

The ripening of tomato fruits includes chlorophyll degradation, synthesis, and storage of carotenoids in which lycopene is a powerful natural antioxidant, which increases the nutritional value and health properties of tomatoes [22]. The impact of various sources and levels of calcium had no significant effect on lycopene but showed a significant influence on beta-carotene content in tomatoes. The highest lycopene (3.23 mg 100 g⁻¹) and beta-carotene (2.58 mg 100 g⁻¹) content was found in the fruits harvested from calcium sulfate treated plot at 20 kg Ca ha⁻¹ and the lowest lycopene (2.86 mg 100 g⁻¹, Figs. 1 and 2) and beta-carotene (1.81 mg 100 g⁻¹, Figs. 3 and 4) content was recorded in poultry manure applied at 80 kg Ca ha⁻¹. On ripening, the lycopene and beta-carotene content increases; perhaps with increasing calcium concentration, both lycopene and beta-carotene in the fruit decrease. The increase in lycopene and beta-carotene on ripening could be attributed to the reason that lycopene and beta-carotene are good indicators of maturation, a huge accumulation of lycopene caused by rapid chlorophyll degradation upon storage [23,24]. This may possibly be due to a progressive activation of

the molecular mechanisms involved in carotenogenesis regulation during the transition between the breaker and red ripening stages [22]. This might also be due to increased activity of phytoene synthase I and accumulation as precursors of lycopene and also by means of agronomic and environmental factors [25]. However, lycopene and beta-carotene decrease with increasing calcium concentrations, as they delay the ripening process that in turn delays the degradation of chlorophylls and also the development of carotenoids [26,27]. The deficiency of calcium in tomato fruits may stimulate ethylene production and biosynthesis of carotenoids; perhaps in the soil may counteract potassium absorption due to competition. Thus, the effect of potassium on carotenoid synthesis is disturbed and results in reduced production of lycopene and beta-carotene.

3.3. Polygalacturonase Activity

Polygalacturonase, part of a multigenic family with hydrolytic function, catalyzes the link α 1–4, which increases soluble pectins and enhances softening of fruits, as it is involved in the degradation of the cell wall [28]. The results revealed that different calcium sources and levels had a significant impact on the polygalacturonase activity of tomato fruits on ripening. The enzyme polygalacturonase activity varies from 1.99 to 3.6 kJ m^{-2} . On increasing calcium concentration, the polygalacturonase activity got reduced; however, it got increased during the storage period (Figs. 5 and 6). The lowest polygalacturonase activity (1.99 kJ m^{-2}) was recorded in the fruits harvested from plots treated with higher poultry manure at 80 kg Ca ha^{-1} , and the highest polygalacturonase activity (3.60 kJ m^{-2}) was recorded in the fruits supplied with calcium sulfate applied at 20 kg Ca ha^{-1} . On ripening, the activity of the polygalacturonase enzyme increases rapidly; nevertheless, on increasing calcium concentration, the polygalacturonase enzyme activity gets decreased. Ripening significantly degrades the cell wall components by means of enzymatic activity such as polygalacturonase and pectin methylesterase, which leads to softening of fruit tissue [29,30]. However, calcium in fruits tissues has an inhibitory effect on the activity of polygalacturonase, which retards the ripening process [31,32].

3.4. Shelf Life

The influence of various calcium sources and levels on the shelf life of tomato fruits during ripening is significantly different from the no calcium applied control plot. The minimum shelf life was recorded in control of 6 days, followed by calcium sulfate applied at 20 kg Ca ha^{-1} (6 days) and calcium nitrate at 20 $\text{kg calcium ha}^{-1}$ (6 days). On the other hand, the maximum shelf life period (15 days) was recorded in poultry manure applied at 80 kg Ca ha^{-1} , as shown in Figure 7. The shelf life was reduced by increased ethylene production, higher respiration, and transpiration, which leads to shriveling of fruit. Increasing calcium concentration significantly increases the firmness of tomatoes irrespective of different sources, which might be due to the accumulation of pectic polymers, and increases the cell wall strength and cohesion [33,34].

4. CONCLUSION

The experiment concluded that the application of different calcium sources at various levels had greatly influenced the quality and shelf life of the hybrid tomato fruits. Among all calcium sources and levels, poultry manure applied at 80 kg Ca ha^{-1} was found very effective in improving the quality parameters, such as ascorbic acid, polygalacturonase activity, lycopene, beta-carotene, and shelf life of hybrid tomatoes.

The quality attributes of hybrid tomatoes were positively influenced by poultry manure applied at 80 kg Ca ha^{-1} . In tomato production, calcium nutrition is more vital as it directly influences the growth, yield, and quality of tomatoes. Therefore, an adequate supply of calcium fertilizer is needed to improve the yield and quality of tomato, and the use of organic manure, namely, poultry manure, pressmud as calcium sources, was limited and it requires additional studies.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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