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EFFECTS OF NAPHTHALENE ACETIC ACID AND CALCIUM CHLORIDE APPLICATION ON NUTRIENT UPTAKE, GROWTH, YIELD AND POST HARVEST PERFORMANCE OF TOMATO FRUIT

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Abstract

Tomato plants were foliar sprayed with naphthalene acetic acid (0.02%) and calcium chloride (0.5%, 1%) individually as well as in combination to determine its effect on growth, nutrient uptake, incidence of blossom end rot, fruit yield, and enhancement of shelf life. The results showed increased absorption of calcium in tomato plants and fruits, which were treated with NAA in combination with $CaCl_2$. Higher level of $CACl_2$ (1%) with NAA (0.02%) increased plant growth and yield by improving mineral uptake of tomato plants. The improved calcium absorption also resulted in lowering occurrence of blossom end rot in tomato fruits. In addition, it was also observed that during storage at ambient conditions (20-25 °C) for sixteen days, tomato fruits maintained best quality for longer period of time when treated with calcium chloride (1%) along with naphthalene acetic acid (0.02%) as compared to other treatments. Although, fruit quality was lowered with passage of storage time but tomato fruits from treated plants maintained their quality for longer duration as compared to control.

Introduction

Tomato fruit quality is associated with changes in colour, texture, flavour, aroma and nutritive value while, it is affected by rate of fertilizer application, irrigation and soil and mineral composition (Mullins & Wolt, 1983). The foliar application of several compounds has been proven beneficial to improve the quality of fruits and resistance to physiological disorders (Alcaraz-Lopez *et al*., 2005). Calcium is vital for improvement in growth of plants. Its involvement is essential in plant cell division, elongation and permeability of cell membranes. Substantial amount of calcium is important for normal functioning of plant membranes. Formation of calcium pectate due to the binding of calcium with pectins has been found beneficial to increase the strength of cell wall and middle lamella (Carpita & McCann, 2000) which improve the fruit quality. Calcium also plays a very important role in improving the quality of tomato by increasing the firmness, reducing the physiological disorders, delaying ripening process and prolonging the shelf life of tomato (Sharma *et al.*, 1996).

Blossom end rot (BER) is key limiting factor in tomato crop production (Khan & Abbasi, 2011). Although numerous environmental factors involved in blossom end rot but inadequate Ca fertilization is thought to be the main cause of BER in tomato fruits especially in susceptible varieties (Saure, 2001). Low rate of mobility or translocation of calcium to fruit could be the basic mechanism behind occurrence of BER.

Calcium has been suggested to increase the yield of tomato (Peyvast *et al.*, 2009). However, some studies showed no positive results for Ca application in relation to yield and BER incidence (Saure, 2001). A number of factors could involve elucidating these distinctions, such as low rate of mobility or translocation of calcium. Poor absorption or translocation of calcium is due to osmotic stress and critical growth period (Adams & Ho, 1992). Occasionally, deficiency may also be caused due to environmental conditions such as high humidity and salinity (Adams & Ho, 1993).

Some plant hormones can regulate the Ca transportation and distribution within plants. Ca transportation from surface of apple to the pulp was promoted by IAA, GA and NAA while IAA improved movement of Ca from tree to the fruit (Zhou *et al*., 1999). NAA could activate membrane Ca^{2+}-ATPase activity for better Ca movement. Banuelos *et al.,* (1988) reported that transport of calcium in tomato fruit is concerned with the basipetal transport of auxins. Similarly application of $CaCl_2$ with NAA has been reported to increase the Ca concentration in apple fruits (Tomala & Dilley, 1990). Moreover, NAA has also played key role in transport of Ca in Chinese cabbage (Wen *et al.*, 1991). Dong *et al.* (2005) also suggested spraying $CaCl_2$ in combination with NAA to tomato plants for better Ca mobilization.

Keeping in view the above mentioned information from previous studies, present research was designed to assess the effects of NAA on the mobility of calcium in tomato variety "Sahil" and the role of calcium on plant growth, yield and postharvest performance of tomato.

Materials and Methods

Experimental methods: The experiment was carried out under high poly-tunnel at the Research Farm of Department of Horticulture, Pir Mehr Ali Shah-Arid Agriculture University, Rawalpindi (PMAS-AAUR) from October 2009- June 2010. Seeds of tomato (var. Sahil) were sown in sand. After germination seedlings of uniform size were transplanted in the tunnel with planting geometry 90cm X 60cm. The experiment consisted of six foliar spray treatments having three replications (5 plants/ replication) i.e., i) Water application (control), ii) NAA @ 0.02 %, iii) $CaCl_2$ @ 0.5%, iv) $CaCl_2$ @ 1.0%, v) NAA @ 0.02% + $CaCl_2$ @ 0.5% and vi) NAA @ 0.02% + $CaCl_2$ @ 1.0%. First foliar spray of treatments was done on the first inflorescence during anthesis and second spray was repeated after one week. Foliar sprays were done in the morning. The fruits were harvested at breaker stage and brought to the postharvest laboratory of the department for further studies by storing at ambient conditions (20-25°C).

Plant growth and yield response: Plant height, flower clusters and fruit set percentage were evaluated during plant growth. Plant height was recorded by using measuring tape while flower clusters were counted manually of every plant in each treatment. Results related to fruit set are depicted as fruit set percentage. Number of days to maturity was recorded from the time of anthesis up to the harvesting (breaker stage) of fruits of selected flower clusters on plants in each treatment. During fruit development stages any physiological disorder particularly blossom end rot (BER) were observed for every fruit cluster on each plant. Total yield was noted at the time of harvest by using weighing balance.

Leaf tissue analysis for N, P, K and Ca: Young but fully expanded leaves of selected plants were picked for leaf tissue analysis followed by dry ashing procedure (Chapman & Pratt, 1961). Calcium concentrations were determined by the procedure of atomic absorption spectroscopy (Berry & Johnson, 1966). Nitrogen was determined with digestion method (Buresh *et al.*, 1982), while phosphorous and potassium contents were measured by spectrophotometer and flame photometer, respectively.

Fruit tissue analysis for Ca determination: Three fruit slices from harvested fruits were taken for fruit tissue analysis followed by dry ashing procedure (Chapman & Pratt, 1961) and grinding subsequently. Calcium content was determined as described by Berry & Johnson (1966).

Postharvest Performance

Fruit quality evaluation: Quality of tomato was evaluated during storage. All fruit characteristics were measured with three replicates of four fruits and data was recorded on 0, 4, 8, 12 and 16 days of storage. Overripe tomatoes of different treatments with the passage of time during storage were excluded from the trial. Following fruit characteristics were measured during the course of study.

Weight loss and firmness: Tomato fruits (5 fruits per sample) were weighed initially at start of experiment and till the end of experiment with four day interval. The difference between initial and final fruit weight was considered as total weight loss during that storage interval and calculated as percentages on a fresh weight basis (Anon., 1990), While firmness was measured with Penetro meter.

Colour: Colour assessment for tomato fruit was done during storage with chromometer CR-400 (Konica Minolta Sensing, Inc. Osaka, Japan). The values were obtained for L*& h*scale for two opposite sides of each fruit.

Total soluble solids: Total soluble solids were measured during storage by using Digital Refractometer at ambient condition. Tomato fruit pieces were homogenised and filtered by using filter paper (Whatman No.1). A drop of filtered juice was placed on prism of refractometer and total soluble solids were recorded in °Brix (Anon., 1990). Titratable acidity was measured by titration method (Anon., 1990).

Ascorbic acid: Ascorbic acid was measured by the method according to Hans (1992). Tomato pulp (5g) was homogenised and blended with 0.1% hydrochloric acid (w/v) and was centrifuged at 10,000 rpm for 10 minutes to get the supernatant, absorbance of the supernatant was measured at 243nm by spectrophotometer.

Statistical analysis: The experiment was conducted according to the Randomized Complete Block Design (RCBD) by using six treatments, each comprising of three replications. Statistical analysis of the data was done by using Analysis of Variance (ANOVA) technique and difference among treatment means were compared by using Duncan's Multiple Range (DMR) test at 5 % level of probability (Steel *et al.*, 1997).

Results and Discussion

Plant growth and yield response

Plant height, flower cluster and fruit set percentage: Data showed significant difference among different treatments for plant height, flower cluster and fruit set percentage of tomato plants (Table 1). Maximum height was recorded in tomato plants treated with 0.5% $CaCl_2$ in comparison with minimum height in control plants. Sole application of $CaCl_2$ found better as compared to combination of calcium chloride and NAA. The results were also confirmed by Peyvast *et al.*, (2009) and Arreola *et al.*, (2008). It is also found that height of plants sprayed with calcium and NAA was low as compared to plants treated only with calcium. It might be due to decrease in nitrogen contents which resulted in decrease of plant height.

It is apparent from the results that flower cluster and fruit set percentage significantly increased (Table 1) with maximum number of flower clusters and highest fruit set percentage in the plants treated with calcium (1%) in combination with NAA (0.02%). Comparing treatments, it is clear that NAA treated plants have more number of clusters and fruit set percentage as compared to single $CaCl_2$ (0.5% and 1%) treatments. Hao & Papadopoulus (2003) and Tuna *et al.*, (2007) reported the same results. The increase in fruit set percentage is possibly due to increased resistance against flower drop in plants which further increase the number of fruits (Fletcher *et al.*, 2000). NAA also showed the positive effect on the number of flower clusters due to higher calcium contents in tomato plant. NAA reduces pre harvest fruit drop by reducing the effect of ethylene and resulted in the appearance of higher fruit set percentage (Alam & Khan, 2002; Yuan & Carbaugh, 2007).

Table 1. Effect of NAA and $CaCl_2$ application on growth and yield response of tomato.

	Plant height (cm)	Number of truss/plant	Fruit set (%)	Time to fruit maturity (days)	Physiological disorder analysis (%)	Yield (kg/plant)
Control	173.3 ± 1.7f	8.18 ± 0.24e	57.85 ± 1.02f	55.04 ± 0.93a	4.87 ± 0.06a	3.70 ± 0.06f
0.02% NAA	242.3 ± 1.4b	10.44 ± 0.06c	74.44 ± 0.22c	44.14 ± 0.015c	2.11 ± 0.008d	6.63 ± 0.06c
0.5% $CaCl_2$	261.0 ± 1.5a	9.17 ± 0.08d	69.57 ± 0.32e	46.15 ± 0.10b	3.05 ± 0.02b	5.09 ± 0.05e
1.0% $CaCl_2$	233.3 ± 1.7c	9.51 ± 0.26d	71.96 ± 0.04d	45.18 ± 0.18bc	2.32 ± 0.01c	5.94 ± 0.03d
0.02% NAA + 0.5% $CaCl_2$	206.0 ± 0.3d	11.22 ± 0.17b	77.77 ± 0.32b	41.78 ± 0.06d	1.95 ± 0.03e	7.68 ± 0.09b
0.02% NAA + 1.0% $CaCl_2$	192.3 ± 1.2e	12.00 ± 0.00a	82.32 ± 1.06a	40.85 ± 0.06d	1.65 ± 0.02f	8.62 ± 0.14a

Days to fruit maturity: In case of time taken for harvest maturity after fruit set, maximum days were recorded in untreated fruits and minimum days were taken by treated fruits (Table 1). Early maturity trend was found in fruit with the increase in concentration of calcium. Plants treated with foliar application of $CaCl_2$ and NAA in combination required shorter time to gain fruit maturity as compared to other treatments. The results are consistent with Schlegel and Schonnher (2002) who described that calcium application resulted in early fruit maturity of apples. The reason of early maturity is that calcium binds to pectin and form calcium pectate which is very helpful in increasing the rigidity of middle lamella and resistance against degrading enzymes like polygalacturanase (Grant *et al.*, 1973).

Physiological disorder analysis: Occurrence of blossom end rot (BER) was lowest (1.65%) in fruits treated with 0.02% NAA + 1% $CaCl_2$ in comparison with control, in which maximum occurrence of disorder (4.67%) was observed (Table 1). In this study, incidence of BER was lowered with increase of calcium content in tomato fruits. BER occurrence is directly related with deficiency of calcium (Centkowski & Tomala, 2000). NAA might be helpful in the proper development of xylem tissues which helps in uptake and translocation of calcium and thus reduction of physiological disorders. These findings were also in agreement with Lgbokwe *et al.*, (1987).

Total yield: Calcium affects the tomato production by increasing mineral contents, flower cluster, fruit set percentage and reducing physiological disorders leading to higher yield. The yield of treated plants was significantly higher as compared to control plants (Table 1). Maximum yield was recorded in plants treated with NAA in combination with calcium (0.02% NAA + 1% $CaCl_2$). These results are supported by Hao & Papadopoulus (2003) Calcium and NAA both not only increase yield of tomato by reducing the flower drop but also increase the fruit retention (Fletcher *et al.*, 2000 and Iqbal *et al.*, 2009).

Leaf tissue analysis for N, P, K and Ca contents: Results showed significant results for accumulation of all the nutrients in leaves of tomato plants (Fig. 1). It has been observed that calcium contents of leaves in treated plants significantly increased as compared to control plants. The higher calcium uptake was in plants treated with calcium and NAA both in combination as compared to individual application of calcium or untreated plants. Similarly, potassium contents also progressively increased with different treatments as compared to control plants. Calcium improves the nutrient status of plants by increasing the potassium and nitrogen content of plants. Although, nitrogen content of treated plants also improved but a mixed behaviour was observed for different treatments. The greatest increase in nitrogen was found in plants treated with 0.5% $CaCl_2$ but further increase in calcium led to decrease in nitrogen. On the other hand inverse correlation has been found between calcium and phosphorous (Fig. 1). Phosphorous contents of leaves decreased with the increase in calcium content.

The increase in calcium contents may be due to NAA because it increases cation exchange sites for calcium accumulation (Mengal & Kirkby, 2001). The results for potassium increase are supported by Tuna *et al.*, (2007) confirming that calcium role is significant in improving the status of potassium. Improved nitrogen contents in treated leaves might be due to pyruvate kinase (PK) enzyme which has a key role in the assimilation of nitrogen (Vanlerberghe *et al.*, 1990). The PK activity is dependent on adenosine diphosphate, phosphoenolpyruvic acid and cofactors K and Mg (Podesta & Plaxton, 1992). Thus increased potassium level accelerated PK activity and ultimately resulted increase in nitrogen content. Antagonistic effect between phosphorous and calcium could be the reason of low phosphorus contents in treated leaves compared to control (Paiva *et al.*, 1998).

Fruit tissue analysis for Ca (mg/g) determination: Highly significant results were observed for calcium contents in fruit tissues of tomato. Maximum calcium accumulation was observed in fruits of plants treated with 0.02 % NAA + 1 % $CaCl_2$ compared with minimum value of calcium in fruits of untreated plants. It was clearly observed that the fruits sprayed with NAA or in combination with calcium chloride accumulated more calcium as compared to fruits treated only with calcium (Fig. 2). Calcium is translocated by transpiration stream toward shoot apex due to this calcium become deficient in young leaves and fruits as compared to older leaves. IAA and NAA have the basipetal transport so auxins proton efflux can be encouraged during the growth due to which new cation exchange sites increased which are helpful in accumulation of calcium in fruits and young leaves (Mengal & Kirkby, 2001). Thus NAA plays a significant role in regulation and transportation of calcium.

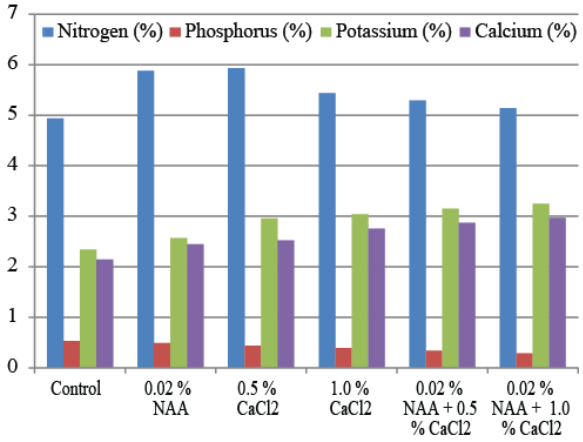

Fig. 1. Effect of NAA and $CaCl_2$ application on nutrient uptake by tomato leaves.

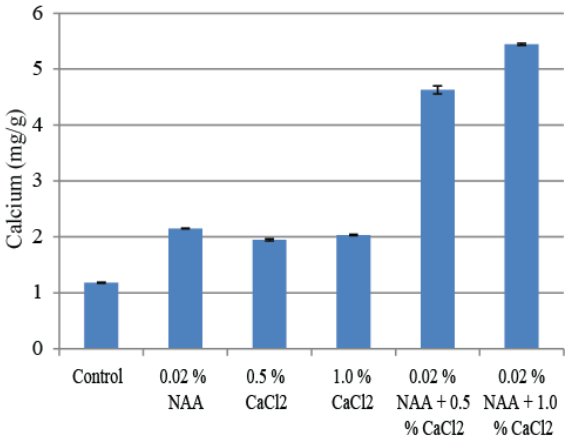

Fig. 2. Effect of NAA and $CaCl_2$ application on calcium contents in tomato fruit pericarp.

Postharvest analysis

Weight loss and firmness: It is evident that percentage of weight loss in tomato fruits increased significantly as ripening proceeded. The comparison between the treatments showed maximum weight loss in control fruits while minimum weight loss was recorded in fruits treated with 0.02 % NAA + 1 % $CaCl_2$ after 16 days of storage (Fig. 3). Reduction in weight loss is observed in all those fruits which are treated with calcium as compared to control fruits. Significantly higher fruit firmness was observed in fruits of plants treated with higher concentration of $CaCl_2$ (1%) in combination with NAA while control fruits retained minimum firmness and this pattern was continued in each sampling date till the end of storage. Overall, combined application of NAA and $CaCl_2$ showed best results in maintaining fruit firmness as compared to individual application of either both chemicals or control. This loss of firmness control might be due to rapid increase in ethylene production and respiration due to the climacteric nature of tomato which leads to increase in water transpires through the surface of the fruit causing shrivelling of fruit (Sabir *et al.*, 2004 & Bhattarai & Gautman, 2006). Higher firmness in calcium treated fruits could be due to its accumulation in the cell walls which facilitates the cross linking of the pectic polymers increasing wall strength and cell cohesion (White & Broadly, 2003). Akhtar *et al.*, (2010) and Shuiliang *et al.*, (2002) revealed that fruit treated with $CaCl_2$ maintained firmness and eating quality of loquat for longer time and their finding support results of present study.

Colour: During the colour change of fruit the values of L*(lightness) and h* (hue angle) were measured. The data showed highest value of L* in fruits treated with combined NAA and $CaCl_2$ while lowest value was recorded in control fruits (Fig. 4). Similarly, highest value of h* was also observed in control fruits as compared to the lowest value in fruits treated with 0.02 % NAA + 1 % $CaCl_2$.

Lowest change in fruit colour treated with Ca and NAA is due to the fact that calcium increases the resistance of cell wall and tissues. This resulted in control of ripening by maintaining the strength of tissues (Izumi & Watada, 1994; Hong and Lee, 1999). It might also be due to reduce in moisture loss. These findings are in agreement with the results of Abbasi *et al.*, (2005) and Delwiche & Baumgardner (1985). During the storage the chlorophyll content degrade gradually which appear in the form of carotenoids. It tends to changes the fruit colour toward red. It is evident from the results that calcium chloride delayed the ripening due to which colour development in calcium treated fruits is slow as compared to untreated fruits. In previous study the same results were also found that the calcium chloride slows down the ripening process (Stanly *et al.*, 1995).

Total soluble solids: During ripening TSS content of tomato fruit change. TSS contains sugars and other soluble salts. It is obvious from the data (Fig. 5) that control fruits showed highest value of total soluble solids as compared to treated fruits. The interaction of treatments with the storage time shows that total soluble solid increase with the increase in ripening during storage period. This change of soluble solids occurs due to break down of polysaccharides.

Calcium binds with pectin contents in vegetables and fruits by forming the salt bridge between Ca^{+2} and COO^{-} group (Stanly *et al.*, 1995). These pectic substances provide sites for the binding of calcium (Roy *et al.*, 1994). Due to this reason calcium pectate is formed thus helpful in reducing the degradation of cell wall and ultimately reduces the production of ethylene resulting in maintaining low TSS by slowing down the ripening process. In the previous study on tomato fruit the similar result was obtained by Bhattarai & Gautam, (2006) and Peyvast *et al.*, (2009).

Ascorbic acid: Tomato is a rich source of ascorbic acid. It functions as an antioxidant enzyme. The interactive effect among the treatments and storage showed that ascorbic acid decreases with the increase of storage time. It is observed from the data (Fig. 5) that ascorbic acid found maximum in the treated fruits and minimum was recorded in fruits with no treatment. During storage it is noticed that ascorbic acid increased first with the ripening stage from light pink stage to red stage of ripening after this it decreased with the increase in red colour and storage time.

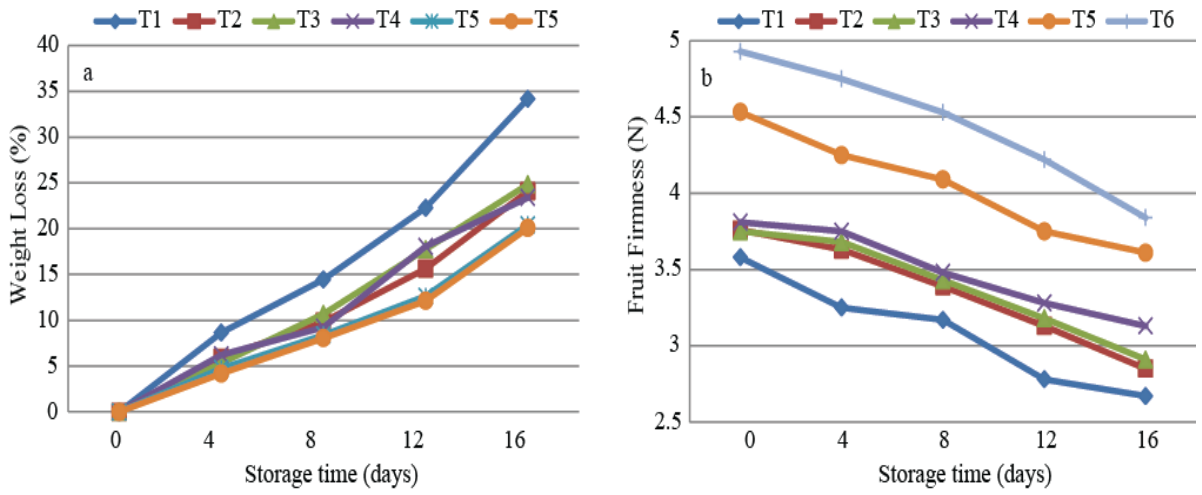

Fig. 3. Effect of NAA and $CaCl_2$ application on a) weight loss and b) fruit firmness of tomato fruit during storage.

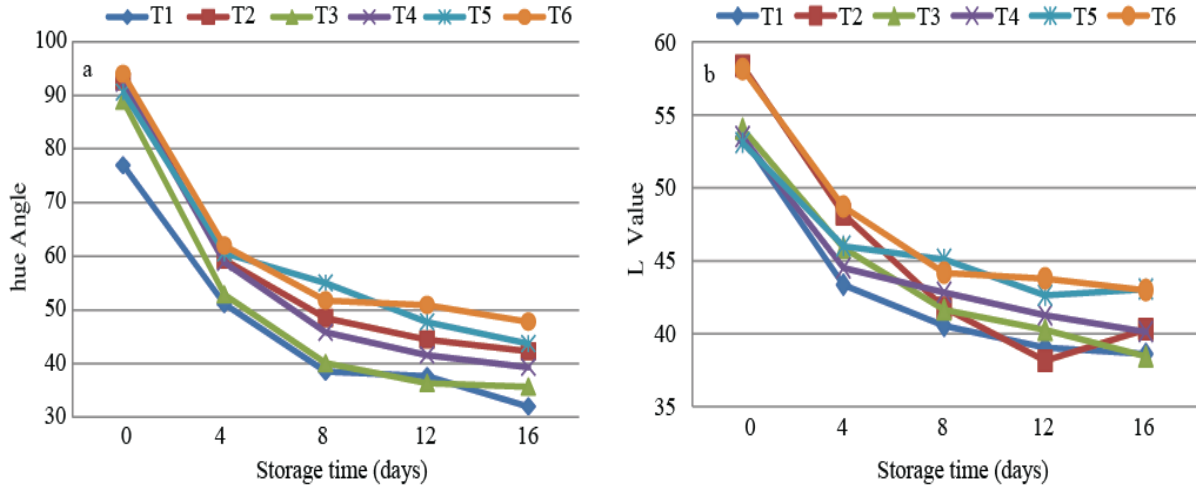

Fig. 4. Effect of NAA and $CaCl_2$ application on a) hue angle and b) L* value of tomato fruit during storage.

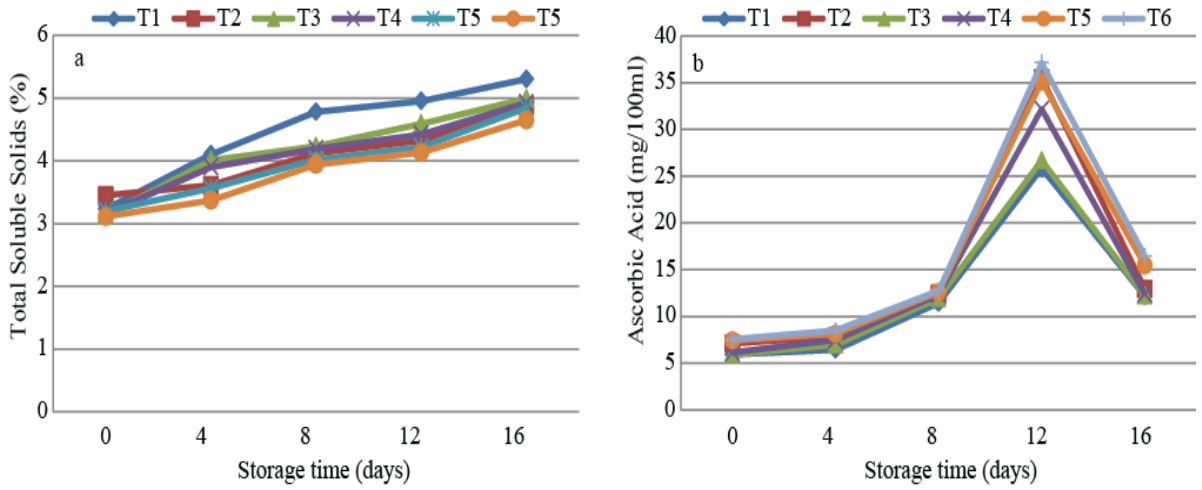

Fig. 5. Effect of NAA and $CaCl_2$ application on a) total soluble solids and b) fruit firmness of tomato fruit during storage.

where:
T_1 = Foliar spray of water
T_2 = Foliar spray of 0.02 % NAA
T_3 = Foliar spray of 0.5 % $CaCl_2$
T_4 = Foliar spray of 1.0 % $CaCl_2$
T_5 = Foliar spray of 0.02% NAA + 0.5% $CaCl_2$
T_6 = Foliar spray of 0.02% NAA + 1.0% $CaCl_2$

The same results were also found by Subbiah & Perumal (1990). The reason for high ascorbic acid in calcium treated fruits might be that metabolic activities were not fast as in untreated fruits. Therefore in untreated fruits the respiration rate and ethylene production were at higher rate due to which ascorbic acid constantly decreased rapidly as compared to Ca treated fruits.

Conclusion

Results showed that preharvest foliar spray of NAA and $CaCl_2$ at anthesis not only increased the absorption of calcium in leaves and fruits but also enhanced the yield and shelf life of tomato var. "Sahil". Due to augmented calcium level, plants were healthy with dwindle occurrence of blossom end rot and ultimately resulted improved growth, yield and shelf life of tomato fruit. It was also concluded that $CaCl_2$ (1%) in combination with NAA (0.02%) gave promising results. Further study is needed to evaluate the effect of NAA and $CaCl_2$ on tomato with different concentrations and application stages.

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