

# Calcium silicate application as a salt stress mitigation strategy for vegetables in the salt-affected soils of sandy plains of Kerala, India

## Abstract

Soil salinity inhibits plant growth due to osmotic stress and reduced plant and water uptake. In the Onattukara sandy plains of Kerala, saltwater intrusion is a major problem in several panchayaths near the coastal area. In uplands also, cultivation is not possible due to the salt stress from saltwater intrusion. One of the strategies for removing the inhibitory effect of salt stress on plant growth is the application of beneficial nutrients to the plants. Therefore, a study was carried out to understand the effect of calcium silicate application on mitigating salt stress in vegetables and using tomato as a test crop. A detailed germination study was conducted in laboratory conditions prior to pot culture experiment to find out the critical level of salinity for tomato and two salinity levels 30 mM and 40 mM NaCl were selected for the pot culture experiment. For assessing the effect of the various levels of calcium silicate on reducing salt stress in tomato, a pot culture experiment was conducted in Onattukara region (AEU 3) in completely randomized design with ten treatments replicated thrice with varying levels of  $\text{CaSiO}_3$  from  $100 \text{ kg ha}^{-1}$  to  $150 \text{ kg ha}^{-1}$ . The treatments had a significant effect on all the biometric characteristics and yield attributes of the crop. The highest fruit weight, fruit per plant, and fruit yield were observed in treatment with soil test-based NPK recommendation and  $125 \text{ kg ha}^{-1}$  of  $\text{CaSiO}_3$  applied. It was also observed that the relative water content was higher and the water saturation deficit was lower in this treatment.  $\text{CaSiO}_3$  application @  $125 \text{ kg ha}^{-1}$  along with soil test-based recommendation of NPK can be recommended as a salt stress mitigation strategy for boosting vegetable production in the salt-affected soils of sandy plains of Kerala.

**Keywords:** Salinity, Onattukara sandy plain, Silicon, tomato, nutrient, yield

## 1. Introduction

“Saline soils contain various dissolved salts, including NaCl,  $\text{MgSO}_4$ ,  $\text{Na}_2\text{SO}_4$ , KCl,  $\text{MgCl}_2$ ,  $\text{CaSO}_4$ , and  $\text{Na}_2\text{CO}_3$ . Among soluble salts, sodium chloride (NaCl) is the dominant salt type with malignant effects on plant growth and fruit production” (Al-Saady *et al.*, 2012). “Kerala state has a coastal line of about 569.70 km long with nine districts adjoining the Arabian Sea, which account for 65 per cent of the total geographical area and 84 per cent of ground water resource of the state. The main salt distressed ecological units are Kuttanad, Orumundakan areas of Onattukara, Pokkali, Kaipad and Kole lands” (Swarajayalakshmi *et al.*, 2003). “With detrimental effects on germination, plant vigor and crop yield, salinity is one of the most serious factors limiting the productivity of crops. The big concerns affecting 15% of the total cultivated land in the world are salinity or sodicity. 20% of total cultivated and 33% of irrigated agricultural lands worldwide are afflicted by high salinity” (Shrivastava and Kumar, 2015). “The impact of salinity on crop yield accounted for about 30-50 per cent” (Joseph and Mohanan, 2013). “For all important crops, average yields in salt stressed environments are only a fraction, somewhere between 20% and 50% of record yields” (Shrivastava and Kumar, 2015).

“Exogenous application of silicon is a recent eco-friendly approach to enhance the salinity stress response in plants. Under salt stress, the application of Si may lead to an increase in  $K^+$  levels and a decrease in  $Na^+$  levels in the cytoplasm due to  $H^+$ -ATPase activity in the plasma membrane and tonoplasts” (Soleimannejad *et al.*, 2019). “Also externally supplied  $Ca^{2+}$  has been shown to ameliorate the adverse effects of salinity in plant metabolism, presumably by facilitating higher  $K^+/Na^+$  selectivity” (Hasegawa *et al.*, 2000). “Root applied Si is an important approach to decrease the undesirable effects of salt stress on plants. Adding calcium silicate in salinity stressed plants maintains membrane permeability, chlorophyll content, stomatal conductance, transpiration and net photosynthesis by diluting salts accumulated in saline environment” (Murillo-Amador, 2007). “Induction of salt tolerance due to calcium silicate application in tomato is due to accumulation of silicon in leaves limiting transpiration, reduction in sodium accumulation in leaves, protection of plasmatic membranes and chloroplasts ultrastructure, stimulation of  $H^+$ -ATPase activity, and protection of plant tissues from free radicals through an increase in antioxidative enzyme activities” (Wasti *et al.*, 2017). “ $Na_2SiO_3$  application in tomato seedlings reduced oxidative damage by decreasing reactive oxygen species, preserving the integrity of the root cell membrane and improving the absorption and distribution of Fe under salt stress conditions” (Ferrandez-Gomez *et al.*, 2024).

“Tomatoes are considered to be moderately sensitive to salt stress, and in soils with EC greater than  $2.5 \text{ dSm}^{-1}$ , tomato yields are estimated to decrease about 10% per each additional EC unit” (Suhandy, 2014). Tomato plants subjected to salt stress had reduced growth and development as a result of alterations in the  $Na^+/K^+$  ratio. Saltwater intrusion is a major problem in several panchayaths of Onattukara and it is reducing the productivity of vegetable cultivation in these areas. Therefore, there is a need to develop a strategy to improve the productivity of vegetables like tomato in salinity affected areas of Onattukara.

## 2. Materials & Methods

### 2.1. Germination test

To evaluate the effect of various levels of salinity on germination of tomato seeds a germination test was done in laboratory in a completely randomised design with five treatments and four replicates. Seeds of the tomato variety Vellayanivijai were used in the experiment. After five minutes of surface sterilization with a 0.1% mercuric chloride solution, seeds were cleaned with distilled water that had been sterilized. For every salinity level, 25 seeds of each genotype were planted in a Petri plate and each petri dish was covered with Whatman's No. 1 filter paper and then irrigated with 5 ml of test solution. Salinity levels was created by supplementing the Hoagland solution with NaCl (Singh *et al.*, 2011). Different levels of salinity *viz.*, (10mM, 20mM, 30mM and 40mM of NaCl) was evaluated along with control (only Hoagland's solution).

To finish the seedling growth, the Petri plates were maintained in a culture environment at  $20^\circ\text{C}$  with artificial light for nine hours per day. The entire treatments were replicated 4 times. On the fourteenth day of the experiment, measurements were made of the root and shoot's dry weight ratio,  $Na^+/K^+$  ratio, germination percentage, and germination speed in days. The concentration of  $Na^+$  and  $K^+$  was measured with a flame photometer. Based on the results of the germination test 30mM NaCl was found to be the critical level of salinity for tomato. Therefore

30mM NaCl and one higher level of salinity (40mM NaCl) were selected for the pot culture experiment.

### 2.1. Evaluating the effect of calcium silicate to mitigate salt stress in vegetables

To study the effect of different levels of calcium silicate application on salt stressed tomato plants, a pot culture experiment was conducted in AEU3 using completely randomized design with ten treatments and three replicates. The pot culture experiment was conducted at Onattukara Regional Agricultural Research Station, Kayamkulam, Alappuzha, Kerala. The field located in the AEU-3 (Onattukara Sandy Plain). The soil type is sandy loam soil and field experiment was conducted from January to April 2024. The soil of the experimental plot comes under the taxonomic family of Loamy skeletal kaolinitic isohyperthermic Ustic *Quartzipsammments*.

Treatments ( $T_1$  to  $T_5$ ) were tested at two levels of salt stress i.e., 30mM and 40 mM NaCl. Salinity levels were fixed based on the results of germination test. Salt stress was induced by irrigating with NaCl solutions corresponding to the required salinity levels. Salt stress was induced at 30 DAT.  $1/3^{\text{rd}}$  dose of each level of salinity was applied on first day (30DAT),  $2/3^{\text{rd}}$  dose was applied on 4<sup>th</sup> day and full dose was applied on 7<sup>th</sup> day.

Recommended dose of fertilizers and organic manures for tomato as per package of practices (KAU, 2016): FYM @ 20 t ha<sup>-1</sup> + 75:40:25 kg N: P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O ha<sup>-1</sup> was given in  $T_1$ . From the initial analysis of the soil samples the soil test based recommendation for the tomato plants was 117:11:136 of N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O kg ha<sup>-1</sup> as per NPK rating and recommendation suggested by the Kerala Agricultural University (2016). Lime was applied at a rate of 250 kg ha<sup>-1</sup>. Along with soil test based recommendation of primary nutrients, Calcium silicate was also applied as basal dose with three levels, 100 kg ha<sup>-1</sup>, 125 kg ha<sup>-1</sup> and 150 kg ha<sup>-1</sup>. Different biometric observations such as plant height at harvest, number of branches at harvest, fruit set percentage and yield attributes, and fruit quality parameters were recorded. Water relations such as relative water content and water saturation deficit were also noted.

## 3. Results and Discussion

### 3.1. Germination test

The data regarding effect of different levels of salinity on germination percentage, no. of days for germination, root shoot dry weight ratio and Na<sup>+</sup>/K<sup>+</sup> ratio of shoot and root of tomato seedlings are presented in Table 1 and 2. When salt concentration increased, germination of tomato seed was reduced and the time needed to complete germination lengthened, root/shoot dry weight ratio was higher and Na<sup>+</sup> content increased but K<sup>+</sup> content decreased.

The germination percentage of tomato seeds declined drastically from 96.52 % (control) to 30.87 % (at 40 mM NaCl). The effect of external salinity on seed germination may be partially osmotic or ion toxicity, which can alter physiological processes such as enzyme activities (Croseret *et al.*, 2001; Essa and Al-Ani, 2001). It is inferred that the speed of germination was reduced i.e. it took more days to complete the germination under salinity. The treatment  $T_5$  (Hoagland solution containing 40 mM NaCl) recorded the highest number of days for germination (12.15 days). The salinity notably affects germination in many species but also lengthens the time needed to complete germination (Amir *et al.*, 2011).

In spite of the negative effects of salt on roots, the root growth in tomato appears to be less affected whereas, shoot was affected drastically, so that, the dry weight ratio was higher in plant grown under salt stress than in control environment. The highest root/shoot dry weight ratio was observed at higher salinity, T<sub>5</sub> (Hoagland solution containing 40 mM NaCl). It may occur due to the mechanism of dry matter partitioning in stress conditions. Seedling send more assimilates to roots to improve uptake ability and so the seedling above ground growth may be declined. These results are in the line with the findings of Hamed *et al.* 2011.

Na<sup>+</sup>/K<sup>+</sup> ratio of both shoot and root of tomato seedlings increased with increasing salinity. It might be due to antagonistic effects of Na<sup>+</sup> and Ca<sup>2+</sup> on K<sup>+</sup> absorption and abnormal Na<sup>+</sup>/K<sup>+</sup> or Ca<sup>2+</sup>/K<sup>+</sup> ratio. Increased concentrations of Na<sup>+</sup> block channel protein used for the uptake of K<sup>+</sup>, and in this way reduce the uptake of K<sup>+</sup> (Qi and Spadling, 2004).

### **3.2. Evaluating the effect of calcium silicate to mitigate salt stress in vegetables**

#### **3.2.1. Biometric observations**

Biometric observations such as plant height at harvest, number of branches at harvest and fruit set percentage showed significant difference with the treatments applied (Table 3 & 4). Plant height was the highest in treatment T<sub>5</sub> (Soil test based POP + CaSiO<sub>3</sub> @ 150 kg ha<sup>-1</sup> @ 30 mM salt stress) at harvest. This may be attributed to the fact that silicon helps in increasing the erectness of leaves thereby enhancing photosynthetic capacity which results in higher plant height. Similar findings were reported by Fallah, 2012 in rice under hydroponic culture. Increase in plant height with Si application might be due to its hydrophilic nature, which helps plants to tolerate salt toxicity by improving the water economy of the plant.

The number of branches at harvest was the highest in T<sub>5</sub> (Soil test based POP + CaSiO<sub>3</sub> @ 150 kg ha<sup>-1</sup> @ 30 mM salt stress). Silicon addition helped plant growth, which might be due to the increased synthesis of photosynthates by the silicon addition and it was exerted in the increased number of branches. The increase in number of branches is due to higher nutrient use efficiency, physiological efficiency and photosynthetic rates (Ma *et al.*, 2001).

Application of treatment T<sub>4</sub> (Soil test based POP + CaSiO<sub>3</sub> @ 125 kg ha<sup>-1</sup> @ 30 mM salt stress) registered the highest value of fruit set percentage of 80.85%. The fruit set percentage may be high because application of silicon on the plants led to an improvement in leaf design with more erect leaves that intercept high solar light, boosting the photosynthetic efficiency and chlorophyll content. Application of silicol granules @ 10 mg kg<sup>-1</sup> recorded the highest fruit setting percentage of tomato in coastal saline soil (Elayaraja, 2020). These findings are also in conformity with those of Marodinet *et al.*, 2014.

#### **3.2.2. Yield attributes**

The results of the study on the effect of yield and yield attributes of salt stressed tomato showed

that different treatments had a substantial impact on the number of fruits per plant, fruit weight, and yield per plant (Table 5). The maximum number of fruits per plant, fruit weight and yield per plant were recorded in T<sub>4</sub>( Soil test based POP + CaSiO<sub>3</sub> @ 125 kg ha<sup>-1</sup> @ 30 mM salt stress). Increase in yield attributes of tomato due to silicon application might be due to higher photosynthetic activity of plant, more formation of carbohydrates and more uptakes of other nutrients. Similar results were also noticed by Gholami and Falah, 2013.

### 3.2.3. Fruit quality parameters

The highest TSS and ascorbic acid content were noticed in T<sub>4</sub>( Soil test based POP + CaSiO<sub>3</sub> @ 125 kg ha<sup>-1</sup> @ 30 mM salt stress) (Table 6) whereas highest lycopene content was noticed in both T<sub>4</sub> ( Soil test based POP + CaSiO<sub>3</sub> @ 125 kg ha<sup>-1</sup> @ 30 mM salt stress) and T<sub>5</sub> ( Soil test based POP + CaSiO<sub>3</sub> @ 150 kg ha<sup>-1</sup> @ 30 mM salt stress). Fruit quality might have been influenced by the calcium silicate application probably due to a suppression of respiration and a reduction in ethylene evolution. These findings were in accordance with Savvas, 2009 who reported that application of silicon under salinity stress conditions enhanced the fruit firmness, total soluble solids, β-carotene, lycopene and vitamin C in the tomato fruits. Stamatakis *et al.*, 2003 studied the effect of silicon and salinity on fruit yield and quality of tomato and reported that Total Soluble Solids (TSS), β-carotene, vitamin-C and lycopene contents of fruits were significantly increased by silicon.

### 3.2.4. Water relations

The highest Relative water content was observed in T<sub>4</sub>( Soil test based POP + CaSiO<sub>3</sub> @ 125 kg ha<sup>-1</sup> @ 30 mM salt stress) whereas T<sub>6</sub> (RDF and organic manure as per POP @ 40 mM salt stress ) recorded the highest value for water saturation deficit (Fig. 1.). The higher water status in salt-stressed plants in the presence of Si may be due to the reduction in excessive loss of water by transpiration. It was suggested that Si was deposited in the epidermal cells of aerial parts of plants, leaves and stems that decreased the water loss through the cuticle and the higher water content maintained by Si diluted the salts in plant body and consequently reduced deleterious effects of salinity on plant growth (Romero *et al.*, 2006). Si application maintains higher water status, photosynthetic activity and better plant defense system and membrane permeability/stability ( Aliet *al.*, 2011).

## CONCLUSION

The findings of this pot culture study suggest that the application of Calcium silicate @125 kg ha<sup>-1</sup> ameliorated the ill effects of salinity on crop growth and yield recorded the highest yield of 1.99kg/plant. The addition of calcium silicate at the rate of 125 kg ha<sup>-1</sup> was found to be best for biometric observations, fruit quality parameters, yield attributes and water relations. The lowest water saturation deficit was also recorded from the treatment applied with calcium silicate at the rate of 125 kg ha<sup>-1</sup>. The use of calcium silicate may contribute to reduce the negative effects of salinity and can be utilized as an effective tool for mitigating salt stress in vegetables.

**Table 1. Effect of different levels of salinity on Germination percentage, no. of days for germination and root shoot dry weight ratio**

<b>Treatments</b>	<b>Germination percentage (%)</b>	<b>Days for germination</b>	<b>Root shoot dry weight ratio</b>
T <sub>1</sub> – Absolute control (Hoagland solution containing 0 mM NaCl)	96.52	3.70	0.433
T <sub>2</sub> - Hoagland solution containing 10 mM NaCl	90.15	4.10	0.440
T <sub>3</sub> - Hoagland solution containing 20 mM NaCl	78.82	5.50	0.468
T <sub>4</sub> - Hoagland solution containing 30 mM NaCl	59.97	9.05	0.552
T <sub>5</sub> - Hoagland solution containing 40 mM NaCl	30.87	12.15	0.611
SE(m)	0.624	0.158	0.002
CD (5%)	1.882	0.478	0.005

**Table 2. Effect of different levels of salinity on Na<sup>+</sup>/ K<sup>+</sup> ratio of shoot and root of tomato seedlings**

<b>Treatments</b>	<b>Na<sup>+</sup>/ K<sup>+</sup> ratio of shoot</b>	<b>Na<sup>+</sup>/ K<sup>+</sup> ratio of root</b>
T <sub>1</sub> – Absolute control (Hoagland solution containing 0 mM NaCl)	2.230	1.910
T <sub>2</sub> - Hoagland solution containing 10 mM NaCl	2.460	2.087
T <sub>3</sub> - Hoagland solution containing 20 mM NaCl	2.885	2.197
T <sub>4</sub> - Hoagland solution containing 30 mM NaCl	3.638	2.900
T <sub>5</sub> - Hoagland solution containing 40 mM NaCl	4.210	3.365
SE(m)	0.030	0.027
CD (5%)	0.090	0.082

**Table 3. Effect of Calcium silicate on plant height(cm) and number of branches at harvest**

<b>Treatments</b>	<b>Plant height at harvest (cm)</b>	<b>No: of branches at harvest</b>
T <sub>1</sub> - Recommended dose of fertilizers and organic manures as per POP (30 mM salt stress)	35.05	3.20
T <sub>2</sub> - Soil test based POP (30 mM salt stress)	35.15	3.51
T <sub>3</sub> - Soil test based POP + Calcium silicate @100 kg ha <sup>-1</sup> (30 mM salt stress)	42.55	4.23
T <sub>4</sub> - Soil test based POP + Calcium silicate @125 kg ha <sup>-1</sup> (30 mM salt stress)	46.15	4.45
T <sub>5</sub> - Soil test based POP + Calcium silicate @150 kg ha <sup>-1</sup> (30 mM salt stress)	47.20	5.05
T <sub>6</sub> - Recommended dose of fertilizers and organic manures as per POP (40 mM salt stress)	30.75	3.05
T <sub>7</sub> - Soil test based POP (40 mM salt stress)	32.30	3.30
T <sub>8</sub> - Soil test based POP + Calcium silicate @100 kg ha <sup>-1</sup> (40 mM salt stress)	40.30	4.10
T <sub>9</sub> - Soil test based POP + Calcium silicate @125 kg ha <sup>-1</sup> (40 mM salt stress)	44.10	4.33
T <sub>10</sub> - Soil test based POP + Calcium silicate @150 kg ha <sup>-1</sup> (40 mM salt stress)	44.65	4.95
SE(m)	1.684	0.276
CD (0.05)	5.306	0.870

**Table 4. Effect of Calcium silicate on fruit set percentage (%)**

Treatments	Fruit set (%)
T <sub>1</sub> - Recommended dose of fertilizers and organic manures as per POP ( 30 mM salt stress)	55.50
T <sub>2</sub> - Soil test based POP ( 30 mM salt stress)	59.50
T <sub>3</sub> - Soil test based POP + Calcium silicate @100 kg ha <sup>-1</sup> ( 30 mM salt stress)	70.00
T <sub>4</sub> - Soil test based POP + Calcium silicate @125 kg ha <sup>-1</sup> ( 30 mM salt stress)	73.75
T <sub>5</sub> - Soil test based POP + Calcium silicate @150 kg ha <sup>-1</sup> ( 30 mM salt stress)	72.25
T <sub>6</sub> - Recommended dose of fertilizers and organic manures as per POP ( 40 mM salt stress)	54.65
T <sub>7</sub> - Soil test based POP ( 40 mM salt stress)	58.10
T <sub>8</sub> - Soil test based POP + Calcium silicate @100 kg ha <sup>-1</sup> ( 40 mM salt stress)	67.60
T <sub>9</sub> - Soil test based POP + Calcium silicate @125 kg ha <sup>-1</sup> ( 40 mM salt stress)	69.25
T <sub>10</sub> - Soil test based POP + Calcium silicate @150 kg ha <sup>-1</sup> ( 40 mM salt stress)	69.25
SE(m)	1.933
CD (0.05)	6.091

**Table 5. Effect of Calcium silicate on yield attributes of tomato plant**

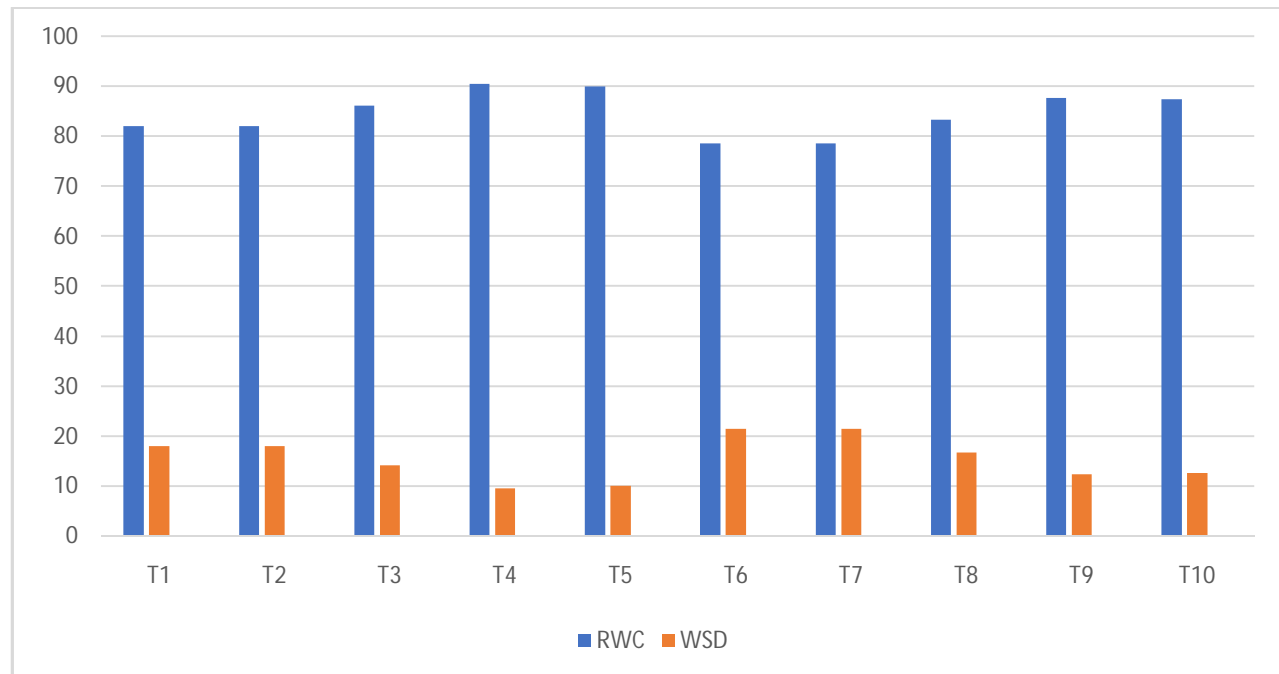
Treatments	Fruits per plant	Fruit weight( g)	Yield per plant (kg)
T <sub>1</sub> - Recommended dose of fertilizers and organic manures as per POP ( 30 mM salt stress)	40.10	21.05	0.61
T <sub>2</sub> - Soil test based POP ( 30 mM salt stress)	42.90	25.15	0.79
T <sub>3</sub> - Soil test based POP + Calcium silicate @100 kg ha <sup>-1</sup> ( 30 mM salt stress)	58.75	31.25	1.76
T <sub>4</sub> - Soil test based POP + Calcium silicate @125 kg ha <sup>-1</sup> ( 30 mM salt stress)	67.65	35.90	1.99
T <sub>5</sub> - Soil test based POP + Calcium silicate @150 kg ha <sup>-1</sup> ( 30 mM salt stress)	59.00	35.35	1.79
T <sub>6</sub> - Recommended dose of fertilizers and organic manures as per POP ( 40 mM salt stress)	38.90	19.50	0.42
T <sub>7</sub> - Soil test based POP ( 40 mM salt stress)	41.65	23.00	0.60

T <sub>8</sub> - Soil test based POP + Calcium silicate @100 kg ha <sup>-1</sup> ( 40 mM salt stress)	53.35	30.00	1.01
T <sub>9</sub> - Soil test based POP + Calcium silicate @125 kg ha <sup>-1</sup> ( 40 mM salt stress)	58.05	34.15	1.83
T <sub>10</sub> - Soil test based POP + Calcium silicate @150 kg ha <sup>-1</sup> ( 40 mM salt stress)	53.45	31.30	1.22
SE(m)	1.147	1.003	0.069
CD (0.05)	3.614	3.161	0.216

**Table 6. Effect of Calcium silicate on fruit quality parameters**

Treatments	TSS( <sup>o</sup> B)	Ascorbic acid (mg 100g <sup>-1</sup> )	Lycopene (µg cg <sup>-1</sup> )
T <sub>1</sub> - Recommended dose of fertilizers and organic manures as per POP ( 30 mM salt stress)	2.90	14.69	0.10
T <sub>2</sub> - Soil test based POP ( 30 mM salt stress)	3.08	14.86	0.10
T <sub>3</sub> - Soil test based POP + Calcium silicate @100 kg ha <sup>-1</sup> ( 30 mM salt stress)	3.38	19.65	0.11
T <sub>4</sub> - Soil test based POP + Calcium silicate @125 kg ha <sup>-1</sup> ( 30 mM salt stress)	4.06	23.27	0.12
T <sub>5</sub> - Soil test based POP + Calcium silicate @150 kg ha <sup>-1</sup> ( 30 mM salt stress)	4.03	22.93	0.12
T <sub>6</sub> - Recommended dose of fertilizers and organic manures as per POP ( 40 mM salt stress)	2.72	13.04	0.09
T <sub>7</sub> - Soil test based POP ( 40 mM salt stress)	2.88	13.16	0.10
T <sub>8</sub> - Soil test based POP + Calcium silicate @100 kg ha <sup>-1</sup> ( 40 mM salt stress)	3.09	17.96	0.10
T <sub>9</sub> - Soil test based POP + Calcium silicate @125 kg ha <sup>-1</sup> ( 40 mM salt stress)	3.89	22.20	0.11
T <sub>10</sub> - Soil test based POP + Calcium silicate @150 kg ha <sup>-1</sup> ( 40 mM salt stress)	3.86	21.91	0.11
SE(m)	0.04	0.285	0.002
CD (0.05)	0.126	0.898	0.007

Fig. 1. Effect of Calcium silicate on relative water content (RWC) and water saturation deficit (WSD)



Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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