

PRANAM-Ca: An Eggshell derived Calcium fertilizer and its impact on blossom end rot in Tomato cultivation

Abstract:

Eggshells, amounting to a staggering 76.7 million tons annually, are often inadequately disposed of in landfills, leading to significant nutrient loss for both plants and humans, while contributing to environmental pollution and emitting unpleasant odors. In response to these challenges, recent research has been dedicated to repurposing eggshells, exploring their applications in diverse fields such as biochemistry, biomedicine, environmental technology, and agriculture. Recognized as an exceptional source of calcium, eggshells offer notable benefits for calcium-demanding crops, including tomatoes, chilli and cotton. In a comprehensive study conducted, we investigated the application of organic foliar calcium derived from eggshells on tomato plants. The results demonstrated substantial improvements in addressing physiological disorders, promoting overall growth, and enhancing yield, signifies the importance of calcium fertilizer for growing healthy crop. With this background, Multiplex R&D team has made an effort to develop an in-house technology to derive the calcium from waste eggshell. Afterwards, how to scale-up and commercialise it as a sprayable form of Organic calcium fertilizer, PRANAM-CA. Present paper aims to describe the technique to derive calcium from eggshell and its impact on managing the blossom end rot in Tomato cultivation under protected glass-house condition.

Keywords: Egg shell derive Calcium (ESDC), Blossom end rot (BER), Tomato (**Add cultivation or foliar spray or treatment**)

Introduction:

Eggs are one of the most widely consumed foods worldwide, making the egg production business a critical component of the global food market (Anon, 2023). The volume of global egg production exceeded 86.3 million metric tons in 2021, down from about 87.07 million metric tons in 2020. Since 1990, the volume of global egg production has increased by over 100 per cent (Shahbandeh, 2023). Worldwide, China is the highest producer of eggs (462 billion eggs annually) followed by United states (99 billion eggs annually) and India (93 Billion

eggs) (Anon, 2023). An eggshell is the outer covering of a hard-shelled egg and of some forms of eggs with soft outer coats (Hunton, P, 2005). The composition of good quality eggshell contains approximately 2.2 grams of calcium in the form of calcium carbonate. About 95 per cent of the dry eggshell is calcium carbonate weighing 5.5 gram. Along with this, it also contains 0.3 per cent of phosphorus and magnesium. The traces of sodium, potassium, zinc, manganese, iron and copper are also present in egg shell (Butcher and miles, 2019). It is mostly discarded as a landfill or burned that contributes to different types of pollution like soil, water, air (Kattimani *et al.*, 2022) and also incur considerable disposal costs in the world (Verma *et al.*, 2012). The disposal of eggshells not only provide site for flies and abrasiveness, but also contributes to the loss of many materials (Hamester *et al.* 2012). Eggshell waste has been ranked as the 15th major food industry pollution problem by the Environmental Protection Agency (Ajala *et al.*, 2018; Faridi and Arabhosseini, 2018).

Hence eggshell waste from industrial processes can be used for agriculture, as an agent to increase soil pH (Vandeginste, 2021; Faridi and Arabhosseini, 2018), as a fertilizer and It can also be processed into more useful products such as animal feeds, medicines, and building materials (Ajala *et al.*, 2018). **(Check the English language used here. It might be interesting to also know that the eggshell has also been applied as source of green coagulant in water treatment. Conveying more importance of the application of egg shell? I recommend the following articles: <https://doi.org/10.1007/s11696-021-01703-x>)** The use of these shells as an alternative source of CaCO_3 (calcium carbonate) may reduce the impact on the natural reserves of limestone, a non-renewable natural source (Neves 1998; Boron 2004 cited in Faridi and Arabhosseini, 2018). **CaCO_3 ? It should be CaCO_3**

Ca deficiency in plants can be caused by insufficient level of available calcium in the growing medium (Khan *et al.*, 2017), which indirectly leads to a major physiological disorder like BER in tomatoes etc. **(What is the meaning of Ca at first mention?)**

Tomato (*Solanum lycopersicum* L.) is a key member of the Solanaceae family, renowned as one of the world's most extensively cultivated and economically significant vegetables. Calcium plays a crucial role in the normal growth and development of plants by maintaining membrane structures, enhancing nutrient uptake, and activating metabolic processes. (Tuna *et al.*, 2007; Sarwat *et al.*, 2013). In addition, the Ca is needed to maintain cell wall integrity and to ensure bindings between cells (Marschner, 1995). Calcium also reduces detrimental effects of stress by regulating antioxidant metabolism (Zorrig *et al.*, 2012; Ahmad *et al.*, 2015). Several factors are involved in the incidence of blossom-end rot (BER) in tomato

fruit, but the main one is insufficient Ca uptake and transport through the plant, resulting in Ca deficiency in the fruits. Sprays of Ca-containing products are considered to be a possible measure to overcome the local Ca deficiency in tomato fruit.

Blossom-end rot (BER) is a common nutritional disorder of tomato, pepper, eggplant, pumpkin, squash and watermelon that is caused by a shortage of calcium in enlarging fruits. This disorder typically occurs when plants are growing rapidly and the first fruits are developing. As fruit cells breakdown due to a lack of calcium, dark blemishes appear on the blossom-end of affected fruits. These may enlarge until the entire bottom of the fruit becomes dark, shrunken and leathery. Factors that encourage blossom-end rot include: low soil pH and low levels of calcium, inconsistent watering, shallow watering or droughty conditions, and excessive use of nitrogen fertilizers (Khan et al., 2017).

Many studies have demonstrated that Ca deficiency have initiated BER incidence in the tomato fruits (Shear 1975; Ho *et al.*, 2015; Taylor and Locascio 2004). Calcium is an important nutrient that plays a key role in the structure of cell walls and cell membranes, fruit growth and development, as well as general fruit quality (Usten *et al.*, 2006). Foliar application of fertilizer stands out as the most effective method for enhancing the nutritional status of plants (Shabbir *et al.*, 2015). The aim of this greenhouse study was to investigate the effects of organic foliar calcium and calcium chloride (CaCl_2), CaNO_3 2 (inorganic) form of application on yield and quality of tomato. **What benefit will it bring to scientific relevance to today's world? The CaNO_3 2 should be corrected. It should be $\text{Ca}(\text{NO}_3)_2$**

Material and Methods

A pot experiment was conducted in commercial greenhouse, during the season of 2021/2022 at Research & Development unit, Peenya, Bangalore, Karnataka. Greenhouse temperature was maintained at $26\text{ }^\circ\text{C} \pm 4$ with 80 per cent of moisture. Soil mixture was prepared by mixing 25 per cent compost in 75 per cent sandy loam soil and 8 kg of soil mixture at 18 per cent moisture was filled in each grow bags of size 12 x 12-inch. **(What is the nature of the compost?)**

The pot mixture of the experiment was sterilised before use for the study. The experiment was laid out in completely randomized design with four treatments and five replications. Treatment includes 0.5 per cent concentration of calcium chloride, calcium nitrate and egg shell derived calcium which were sprayed three times at an interval 20days. One-month old tomato (local cultivar) seedlings were transplanted to the pots. The concentrations of calcium chloride, calcium nitrate and egg shell derived calcium foliar solutions were fixed

based on their pH value in water, to avoid adverse effect of acidic pH on plants, 0.5 per cent was chosen. **(Add the percentage purity or concentrations of the reagents used? Can be added to table I)**

List 1: Experimental details (The table below should be Tagged Table 1: and should be renamed the experimental design matrix or details of preparation).

Sl.No.	Treatment details	Dosage (%)
1	T1- Calcium Chloride	0.5
2	T2- Calcium Nitrate	0.5
3	T3- Egg shell derived Calcium	0.5
4	T4-Control	-

Although the Experimental detail indicated in the table show the 4 treatments, it does not illustrate the five replicates mentioned in the methodology. Table should be revised to convey the information)

2.1 Ca Preparations and Plant Treatments:

To test the possibilities to increase Ca content in tomato fruits, treatments with 3 different sources of Ca along with **PRANAM Ca** (A commercially available Product by Multiplex, used for this study) were applied. The method of preparations, application rates and intervals were as follows. **(What is the specification of commercial PRANAM?)**

Preparation of PRANAM Ca:

The chicken and duck egg shells were selected due to their inherent variations in calcium concentrations. The shells, procured from diverse restaurants and hatcheries, underwent a systematic processing regimen. Initial cleaning involved washing the shells in water to eliminate impurities, followed by a meticulous drying process in a hot air oven. This step aimed to remove excess moisture and pre-emptively address concerns related to rotting and unpleasant odors associated with the yolky part. **(What is the drying temperature, and time for drying?), It might also be interesting to know the mass configuration of duck and egg shells used, since it's a mixture. Beside the duck shell composition was not mentioned in the entire manuscript. There are a lot of studies carried out in this area using eggshells. I suggest it's a blend of eggshell and duck shells. This fact should be clearly defined.**

Acid hydrolysis:

Given that calcium carbonate constitutes a substantial portion of egg shells and possesses a weak molecular bond, a strategic approach involving chemical treatment was employed. Nitric acid was introduced to react with the carbonate bond, facilitating the conversion of calcium carbonate into calcium nitrate, a water-soluble compound. The acid-induced reaction was allowed to transpire over a known duration, resulting in the generation of a dense, dark reddish-brown liquid. Subsequently, filtration using Watt Mann filter paper was employed to isolate the liquid from residual particulate matter. **What is the percentage purity or concentration of the acid used? A known duration, how much time please?**

To quantify the concentration of the water-soluble calcium solution, the specific gravity of the liquid was determined using the formula $\text{Density} = \text{Mass}/\text{Volume}$, yielding an observed value of 1.62 g/ml. Notably, the water-soluble calcium content in the liquid was established at 15 per cent. This scientifically rigorous methodology ensures the production of a potent and consistent source of calcium suitable for application in agricultural settings, particularly for augmenting the calcium uptake by tomato plants. The flow chart for the same is displayed below (Fig.1). **(Schematic representation of experimental methodology in Fig. 1 the word “pH Adjustment” should be aligned properly, word descriptions (labels) should be added to Fig.1 for purpose of clarity of the methodology. i.e preparation of samples, acidic treatment, pH adjustment etc) How did you adjust the pH of solution, did you prepare a buffer with the acid and base?)**

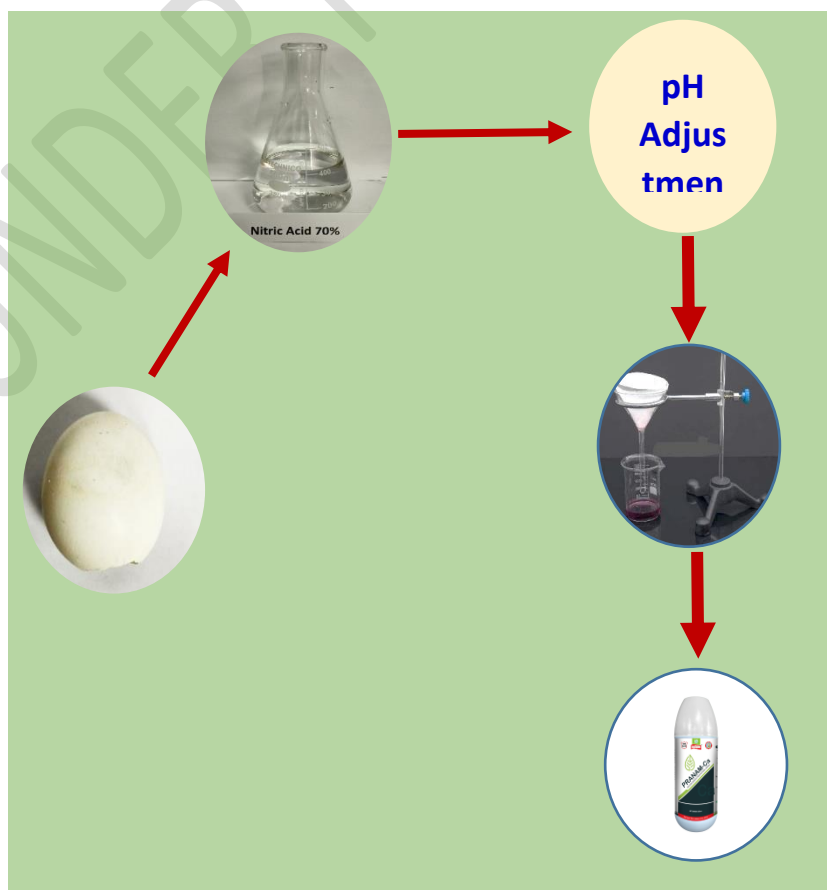


Fig.1: Preparation of PRANAM Ca

3. Results and Discussion:

The **foliar spray treatments** exhibited a positive impact on the fruit yield, height and physiological parameters of the tomato. **Analysis revealed a noticeable** increase in the overall mass of fruits, indicating a positive correlation between calcium supplementation and enhanced fruit development. The treatment T2- Calcium Nitrate 0.5% recorded maximum fruit yield (402 g/plant) followed by T3- Egg shell derived Calcium 0.5% (389 g/plant), whereas, the lowest fruit yield was observed in the plants which were left untreated (252.4 g/plant). **This results should be visualized using appropriate bar-charts or tables for of presentation to convey results (Please cite the Tables or Fig used to present theses findings).** The incidence of blossom end rot, a common symptom associated with calcium deficiency, showed a considerable reduction in plants treated with calcium foliar sprays. The treatment T3- Egg shell derived Calcium 0.5% showed less incidence of fruits infected with BER (1.2 fruits) and more incidence was observed in the plants which were left untreated (6.2). **Where is the visual plot of this results (No citation of Figures or Tables)?** This suggests a potential protective effect of the treatment against blossom end rot. Calcium is the one of the important nutrient that maintains the cell integrity and cell structure which enhanced the fruit quality by increasing the fruit firmness. Foliar application of calcium significantly reduced the blossom end rot. Peyvast *et al.* (2009), observed that calcium application affected yield and quality parameter, further calcium deficiency reduces yield while less fruit firmness, high fruit cracking and more blossom end rot was recorded in calcium deficient plants. **The results are also in close proximity with findings of Fernades *et al.* (1984)** that regular irrigations and calcium along with phosphorus fertilizer controlled the blossom end rot infestation in tomato. **The reference used in the comparison is outdated. Is there no other recent research in the last 10years to be used in the comparison?** Similarly, the number of fruits exhibiting cracking symptoms were lower in plants subjected to calcium foliar spray treatments. **Where is the source of this projectional statement? (No citation of Figures from which these results were obtained), are there personal finding to justify this claim?** This observation implies a role for calcium supplementation in mitigating fruit cracking issues and the occurrence of fruit dropping, a phenomenon often linked to calcium imbalance, was notably reduced in Ca treated plants suggesting the foliar spray treatments positively influenced fruit retention and the lowest number of fruit cracking was observed in the plants treated with T2- Calcium Nitrate 0.5%

(0.4), followed by T3- Egg shell derived Calcium 0.5% showed less incidence of fruit cracking (0.6) as against (4.2) in the control plants. **What Figure are you interpreting results here? For the purpose of clarity, these results findings can be visualized using appropriate tables or professionally drawn bar charts. These Tables or Figures must be cited for clarity.** Besides, increase in the biomass yield, the supplementation of Ca to the plants at the vegetative growth has also showed increased plant height (5.7-6.0 cm) when compared to untreated control (4.1 cm). **Where are the evidences in result presentation? The increasing tendency in tomato plant height was similar with the findings of (Chaudry *et al.*, 1999; Jaha and Krishi 2001) observed differences in plant height among cultivars of tomato pot under evaluation and screening trial. These references are quite outdated. Please, if possible, authors should use references since 2013 till date for projection of results.** Our findings were in accordance with Jadhav *et al.* (2020) who reported that, dry fruit yield of chilli was significantly influenced by foliar spray of $\text{Ca}(\text{NO}_3)_2$. Foliar spray of $\text{Ca}(\text{NO}_3)_2$ resulted in increased absorption of Ca and $\text{NO}_3\text{-N}$ by plant canopy, this $\text{NO}_3\text{-N}$ might have enhanced fruit yield by enhancing cell division, that led to increased flowering and fruiting synergized by Ca present in $\text{Ca}(\text{NO}_3)_2$ and also due to timely and spot availability of calcium and nitrogen in readily available forms to the chilli plants which resulted in better absorption of nutrients by plant leaves leading to more flowers and fruits. Similar observations on increased fruit yield in chilli crop due to foliar spray of mono potassium phosphate and KNO_3 were reported by Veerendra Patel (2014) and Somimol (2012). The results of the present investigation were in accordance with the findings of Sarkar *et al.* (1999) and Chaity and Sarkar (2009). **Please use the appropriate scientific representation of the $\text{Ca}(\text{NO}_3)_2$, and $\text{NO}_3\text{-N}$ etc.) The presentation of results can be broken into separate paragraphs, with each beginning with the citations of Tables or Fig from which the results were obtained.**

Additionally, it is worthy to note that the authors mentioned foliar treatment. What is the foliar spray treatment about? It wasn't mentioned in the methodology nor the introduction section. These track changes will improve the manuscript for clarity.

Conclusion

By utilizing eggshells in this innovative manner, we not only address the challenge of waste disposal but also harness a valuable resource to enhance agricultural productivity in an environmentally sustainable manner. These findings underscore the potential of integrating organic calcium derived from eggshells as a viable and effective solution in modern agricultural practices.

The conclusion section seem ambiguous, should be re-written to convey what was done in this research, the principal findings, suggestion or recommendation for further studies.

UNDER PEER REVIEW

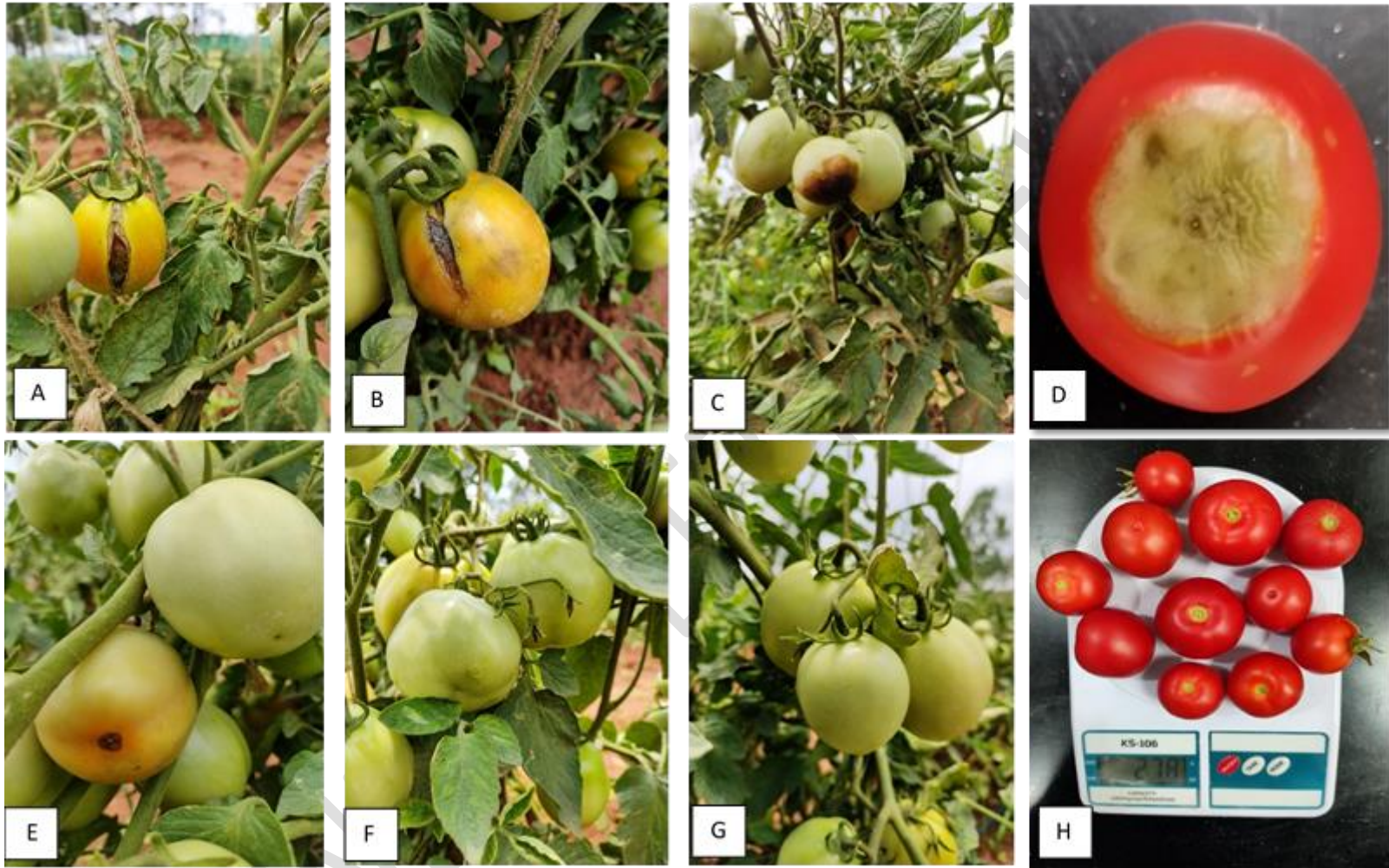


Fig.2: Physiological parameters of tomato fruits. A-B: Symptoms of fruit cracking; C-E: Symptoms of blossom end rot; F-G: PRANAM Ca sprayed healthy fruits; H: Biomass of the fruits

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Table 1: Effect of spraying different sources of Calcium on plant height and fruit physiological characters of tomato (local variety)

Treatment details	Height (cm)	Blossom end rot	Fruit Cracking	Fruit dropping
T1- Calcium Chloride 0.5%	5.70 ^a (39.02)	1.4 ^b (77.42)	0.8 ^b (80.95)	1 ^b (84.38)
T2- Calcium Nitrate 0.5%	6.00 ^a (46.34)	1.2 ^b (80.65)	0.4^b (90.48)	1.2 ^b (81.25)
T3- Egg shell derived Calcium 0.5%	5.84^a (42.44)	1.2^b (80.65)	0.6 ^b (85.71)	1.4 ^b (78.13)
T4-Control	4.1b (0.00)	6.2 ^a (0.00)	4.2 ^a (0.00)	6.4 ^a (0.00)
CD@1%	0.96	1.538	1.116	1.597

***Values are the mean of five replications**

Table 2: Effect of spraying different sources of Calcium on height and yield parameters of tomato (local variety)

Treatment details	Yield (g/plant)
T1- Calcium Chloride 0.5%	319 ^b (26.38)

T2- Calcium Nitrate 0.5%	402 ^a (59.27)
T3- Egg shell derived Calcium 0.5%	389^a(54.12)
T4-Control	252.4 ^c (0.00)
CD@1%	90.37

*Values are the mean of five replications

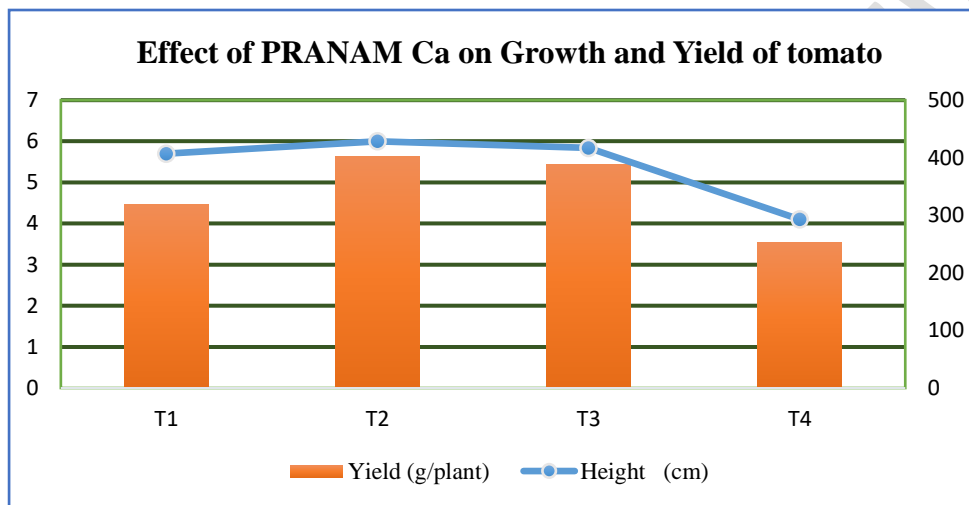


Fig.3: Effect of PRANAM Ca on Growth and Yield of tomato

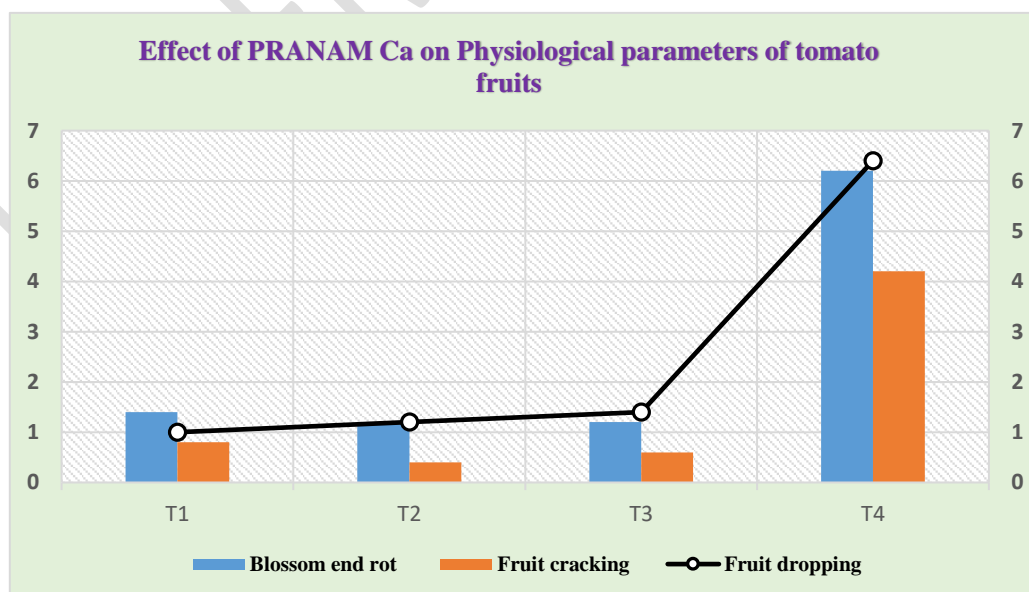


Fig.4: Effect of PRANAM Ca on Physiological parameters of tomato fruits