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Edaphic silicon nutrition of tomato biostimulates their growth, yield and antioxidant composition under greenhouse conditions

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ABSTRACT

Silicon (Si) has multiple benefits in crops. Most of the studies on Si have been carried out by applying some type of stress. It has even been suggested that the positive response of Si is determined by the degree of stress in the plant, and there is little information on Si and its effect on the plant when there is no induced stress factor. The objective of the study was to determine the effect of edaphic Si on the growth, production and concentration of antioxidants in tomato under greenhouse conditions without induced stress. The treatments were three doses of Si (0.06, 0.12 and 0.18 g/plant) and a control (0.0 g/plant). The treatments were distributed in a completely randomized design with four repetitions. The addition of Si in tomato plants increased biomass production, the number of fruits and yield. In addition, in the treatments with the highest dose of Si, the concentration of antioxidants increased, as well as the total antioxidant capacity. It is suggested to include Si in tomato fertilization programs as a sustainable alternative to improve crop growth and productivity.

Keywords: *Solanum lycopersicum*, fertilization, non-stressors.

RESUMO

Nutrição edáfica do tomate com silício bioestimula seu crescimento, produção e composição antioxidante em casa de vegetação

O silício (Si) exerce múltiplos benefícios às culturas. A maioria dos estudos sobre Si foi realizada aplicando algum tipo de estresse. Sugeriu-se que a resposta positiva do Si é determinada pelo grau de estresse na planta. Há pouca informação sobre o efeito do silício na planta na ausência de fator de estresse induzido. O objetivo do estudo foi determinar o efeito do Si edáfico no crescimento, produção e concentração de antioxidantes em tomateiro em casa de vegetação sem estresse induzido. Os tratamentos foram três doses de Si (0,06, 0,12 e 0,18 g/planta) e uma testemunha (0,0 g/planta). Os tratamentos foram distribuídos em delineamento inteiramente casualizado com quatro repetições. A adição de Si nas plantas de tomate aumentou a produção de biomassa, o número de frutos e a produtividade. Além disso, nos tratamentos com maior dose de Si, a concentração de antioxidantes aumentou, assim como a capacidade antioxidante total. Sugere-se incluir Si nos programas de fertilização do tomate como uma alternativa sustentável para melhorar o crescimento e a produtividade das culturas.

Palavras-chave: *Solanum lycopersicum*, fertilização, não estressores.

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Tomato (*Solanum lycopersicum*) is the most cultivated vegetable in the world, due to its nutritional content and its demand in the daily diet (Domínguez *et al.*, 2020), with 37.3 million tons produced in 2022 (FAOSTAT, 2022). Tomato is an important source of carotenes, phenolic compounds, vitamins, and minerals (Ali *et al.*, 2020). After water availability, nutrition is the second most influential component in tomato management (Ullah *et al.*, 2021). Conventional tomato nutrition is based on chemical fertilization (Hasnain *et al.*, 2020). However, the use of these chemicals generates residues that are dispersed in the ecosystem, causing water, air and soil contamination, thus affecting living organisms and human health. (Rivas-Garcia *et al.*, 2022).

For the aforementioned reasons, the search for alternative treatments to conventional nutrition and the development of more sustainable practices as plant biostimulation is ongoing (Schjoerring *et al.*, 2019). Biostimulants are any natural source substances or microorganisms that are additive to fertilizers and pesticides to improve nutrient uptake, promote plant growth and increase tolerance to biotic or abiotic stress (Drobek *et al.*, 2019; Rivas-Garcia *et al.*, 2021).

Many studies have demonstrated the importance of silicon fertilization from various sources (i.e. calcium and potassium silicate, and silicic acid) for increased agricultural production, lower pest and disease incidence, and increased nutritional quality of fruits (Al-Murad *et al.*, 2020). Furthermore, Si treatment improves biotic and abiotic resistance, photosynthetic processes, nutrition, production, and quality in a variety of crops (Huang *et al.*, 2021; Hussain *et al.*, 2021; Mundada *et al.*, 2021; Venancio *et al.*, 2022; Wade *et al.*, 2022).

Silicon (Si) is the second most abundant element in the lithosphere (27.7%), only after O_2 (47.4%) (Sommer *et al.*, 2006). Despite its abundance in soil, cations, organic compounds, pH, temperature, and water content all influence Si availability to plants as silicic acid (H_4SiO_4) or mono silicic acid [$Si(OH)_4$] (Kurdali *et al.*, 2019). The diversity in Si concentration in leaves and shoots is caused by different plants' Si absorption and passing mechanisms (Bhardwaj & Kapoor, 2021). Higher plant Si adsorption is characterized as active uptake (Si uptake > water uptake), passive uptake (Si uptake = water uptake), and rejective uptake (Si uptake water uptake) based on water uptake capacity (Kaur & Greger, 2019). Furthermore, higher plants are classified as accumulators [>4% Si; rice (*Oryza sativa*)], intermediate [2-4% Si; soybean (*Glycine max*)], and non-accumulators (2% Si; tomato) based on Si accumulation in tissues (Marmiroli *et al.*, 2022).

Despite being classified as a rejective uptaker and a non-accumulator of Si, the tomato has shown improvement biostimulation in biotic and abiotic stress such as high pH (Bautista *et al.*, 2020), salinity (Hoffmann *et al.*, 2020), water deficit (Chakma *et al.*, 2021) and pathogen attack (Wu *et al.*, 2022). In addition, Si has positive effects on the postharvest shelf life of tomato fruits and their quality characteristics (Pinedo-Guerrero *et al.*, 2020). However, there is little information on the effect of Si on plants that were not subjected to any kind of plant stressor. For this reason, the aim of the present study is to determine de effect of edaphic silicon nutrition of tomato (*Solanum lycopersicum*) on the biostimulation of growth, yield and quality parameters under greenhouse conditions.

MATERIAL AND METHODS

Study area

The research was carried out in a greenhouse, located in the Quinto sector, parroquia Matriz belonging to the Chambo canton, in Chimborazo province, Ecuador. (01°10'29"S, 78°34'95"W, 2,570 m altitude). The experimental site is located in a warm and temperate climate zone, with an average annual temperature of 12°C, average annual rainfall of 1,462 mm; and 86.0% relative humidity.

Growth conditions

The experiment was established in a greenhouse with an area of 160 m^2. The beds were prepared with a height of 0.15 m and a width of 0.8 m, spaced 1.5 m from center to center. To said surface, 64 kg of organic fertilizer was added. The vegetable species used was tomato of the Miramar variety, which was transplanted manually with a planting distance of 0.25 m between plants. The evaluated treatments consisted of three doses of silicon (0.06, 0.12 and 0.18 g/plant) and a control (0.0 g/plant). The silicon used was SIO-100 containing 80 to 83% SiO_2. This was applied to the soil every two weeks.

Growth parameters

Plants were fractionated into the different organs (root, stem, leaf and fruit) to determine their biomass (110 days after transplanting). Plant tissues were left to air dry, then in a forced air oven at 105°C, for 48 hours to estimate the dry biomass. The number of fruits per plant was determined when 50.0% of the fruits set on each plant for each treatment and the polar and equatorial diameter was obtained using a caliper (cm). The yield (kg/plant) was determined with the total production harvested in each treatment. Plant height (cm) was measured 75 days after transplanting and root length (cm) was evaluated 110 days after transplanting.

Antioxidant composition

For antioxidant activity, phenolics, flavonoids, carotenoids, vitamin C and total soluble solids (TSS), at least 2 kg of injury free tomato fruit for each treatment was harvested by hand, weighed and delivered quickly to the laboratory. All tomato fruits were collected at commercial maturity. The tomatoes were washed with tap water, cut into pieces and ground with a commercial blender (7011, Waring® Laboratory Science, Stamford, CT, USA) in order to obtain a homogeneous puree. Part of each sample was immediately used for TSS content. The other part was frozen at -20℃ and used to determine the other above mentioned parameters.

The phenolic content was analyzed spectrophotometrically using the modified Folin-Ciocalteu method (Singleton *et al.*, 1999; Eberhardt *et al.*, 2000). Each sample (2 g) was extracted with 10 mL methanol for 24 h. 125 µL of the diluted extract was mixed with 500 µL distilled water in a test tube followed by the addition of 125 µL of Folin-Ciocalteu reagent and allowed to stand for 3 min. Then, 1,250 µL of 7% sodium carbonate solution was added and the final volume was made up to 3 mL with distilled water. Each sample was allowed to stand for 90 min at room temperature and measured at 760 nm against the blank on a spectrophotometer (Beckman DU 650). The linear reading of standard curve was from 0 to 300 µg of gallic acid/mL. Results were expressed as mg gallic acid equivalent/g (mg GAE/g).

The flavonoid content was determined as described by Zhishen *et al.* (1999) on triplicate aliquots of the homogeneous juice (0.3 g). Fifty microliter aliquots of the methanolic extract were used for flavonoid determination. Samples were diluted with distilled water to a final volume of 0.5 mL, and 30 µL of 5% $NaNO_2$ was added. After 5 min, 60 µL of 10% $AlCl_3$ was added and finally 200 µL of 1 M NaOH was added after 6 min. The absorbance was read at 510 nm using a Beckman DU 650 spectrophotometer. The linear reading of the standard curve was from 0 to 250 µg catechin/mL and flavonoid content was expressed as mg of catechin equivalents/g (mg Catechin/g).

Total carotenoids determination was conducted as described by Lee (2001). The method uses a mixture of hexane/ethanol/acetone (2/1/1 by vol.) containing 0.05% butylated hydroxytoluene (BHT). During the extraction process, some precautions were taken, like working in a reduced luminosity room and wrapping glass materials in aluminium foil to avoid lycopene loss by photo-oxidation. The absorbance of the hexane extract was read at 450 and 503 nm respectively using a Beckman DU 650 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). Total carotenoids were expressed as mg β-carotene equivalents/g (mg β-CaE/g).

Ascorbic acid (AsA) and dehydroascorbic acid (DHA) contents were determined as reported by Kampfenkel *et al.* (1995) on triplicate samples of the homogenate juice (0.1 g). AsA and DHA were extracted by using 6% metaphosphoric acid and detected at 525 nm in a Beckman DU 650 spectrophotometer. The linear reading of the standard curve was from 0 to 700 µmol AsA. The assay used for the determination of AsA and DHA is based on the reduction of Fe^{3+} to Fe^{2+} by AsA and spectrophotometric detection of Fe^{2+} complexed with 2,2´-dypyridl. DHA is reduced to AsA by preincubation of the sample with dithiothreitol (DTT). Subsequently the excess DTT is then calculated by the 2,2´-dypyridl method. The concentration of DHA is then calculated from the difference of total AsA and AsA (without pretreatment with DTT). Vitamin C content is the sum of both (AsA + DHA) contents.

SST was expressed by the °Brix of the fresh juice. The measurement was taken by placing a drop of filtered juice on the prism of a digital refractometer with automatic temperature compensation (Atago PR-100 NSG Precision Cells, Inc, Framing dale, NY). The antioxidant activity was determined by the ferric reducing antioxidant power (FRAP) assay method (Benzie & Strain, 1996). The antioxidants were extracted from 0.3 g of homogenate (three replicates) with absolute methanol or hexane at 4°C under constant shaking (300 rpm) overnight. Samples were centrifuged at 10,000 g. The supernatants were used for antioixidant activity measurement. To measure antioxidant activity, 50 µL of tomato extract was added to 1.5 mL of FRAP reagent [1 mM 2,4,6-tripiridyl-2-triazine (TPTZ) and 20 mM ferric chloride in 0.25 M sodium acetate buffer, pH 3.6] and mixed thoroughly. After 4 min at 4°C, absorbance at 593 nm was read against a blank of water. A calibration curve was prepared using freshly prepared ammonium ferrous sulfate. The linear reading of the standard curve was from 0 to 1,200 µM FRAP. Values were obtained from three replicates as mM FRAP/g of tomato (mM FRAP/g fw).

Experimental design and statistics

The treatments were distributed in a completely randomized design with four repetitions. The experimental unit was made up of 10 plants. The data were analyzed with the software R v.4.2.1 (R Core Team, 2022). Analysis of variance (the criteria of normality and homogeneity of variance were met) and comparison of means were performed by Tukey's test ($p<0.05$).

RESULTS AND DISCUSSION

Growth parameters

The addition of silicon in tomato plants increased biomass production. The treatment with 0.18 g/plant was the one that produced the highest fresh and dry biomass in root, leaf and stem (Table 1). According to these results, it is clear that silicon affects plant metabolism, because with doses starting at 0.12 g/plant of silicon, the biomass of the different organs increased, and there was no response with a dose of 0.06 g/plant. This suggests that biomass production requires this element for its increase. These results have been confirmed in other investigations that have documented the positive effect of silicon on crop growth and yield (Gunes *et al.*, 2007; Balakhnina *et al.*, 2012; Pati *et al.*, 2016). On the other hand, the increase in biomass production in plants due to the effect of silicon could be related to the improvement in photosynthesis (Zhang *et al.*, 2018; Ligaba-Osena *et al.*, 2020) or by an effect on the decreased biotic and abiotic stress (Sun *et al.*, 2010; Ligaba-Osena *et al.*, 2020) or a combination of both. However, it is necessary to clarify the role of silicon in crop growth and yield to increase its efficiency (Reyes-Perez *et al.*, 2023).

Table 1. Average values of root, stem, and leaf biomass of tomato plants with different doses of silicon. Chambo, UTEQ, 2022.

Doses of Si (g/plant)	Biomass (g/plant)					
	Fresh			Dry		
	Root	Stem	Leaf	Root	Stem	Leaf
0.00	29.61±3.34 b	760.43±53.99 b	322.88±34.22 c	7.72±0.61 c	123.87±11.39 b	58.18±4.16 c
0.06	28.55±2.52 b	813.55±90.33 b	356.85±46.6 c	8.78±0.82 bc	124.34±11.98 b	93.88±14.3 b
0.12	38.81±1.49 a	1061.63±17.47 a	519.78±13.62 b	9.68±0.41 ab	143.05±8.62 b	100.03±8.83 b
0.18	41.60±3.74 a	1165.95±10.85 a	660.37±50.9 a	10.64±0.76 a	177.11±6.22 a	130.42±10.5 a
CV (%)	8.38	5.64	8.41	7.25	6.92	10.59
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

The values shown correspond to the average and standard deviation. Different letters ^{abc} in the same column indicate statistical differences.

The yield, the number of fruits both in the bunch and in the plant, as well as the equatorial and polar diameter, were higher with the addition of 0.18 g/plant of silicon. On the other hand, the treatment with 0.06 g/plant of silicon and the control treatment produced fewer fruits with no statistical difference between them (Table 2). Like biomass production, fruit yield increased with the addition of silicon, however, unlike biomass production, fruit yield was higher with the highest dose of silicon in this study. These results suggest that, in the management of tomato cultivation, it is necessary to include in the fertilization at least 0.18 g/plant of silicon, to obtain the highest yield in biomass and fruit production.

Table 2. Average values of number of fruits per bunch and per plant in tomato with different doses of silicon. Chambo, UTEQ, 2022.

Doses of Si (g/plant)	Number of fruits/bunch	Number of fruits/plant	Diameter (cm)		Yield (kg/plant)
			Equatorial	Polar	
0.00	4.60±0.42 c	23.00± 2.08c	6.66±0.16 d	5.20± 0.10c	2.74±0.20 c
0.06	5.20±0.56 c	25.98±2.78 c	7.01±0.09 c	5.56±0.07 b	3.01±0.20 c
0.12	6.68±0.36 b	33.38±1.81 b	7.49±0.07 b	5.77±0.09 b	3.76±0.19 b
0.18	8.66±0.31 a	43.29±1.57 a	8.00±0.13 a	6.00±0.15 a	4.41±0.14 a
CV (%)	6.72	6.72	1.61	1.91	5.29
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

The values shown correspond to the average and standard deviation. Different letters ^{abcd} in the same column indicate statistical difference.

The increase in biomass and number of fruits with the inclusion of silicon revealed a general trend of improvement in the crop. In this regard, Gowda *et al.* (2015) applied silicon in combination with the recommended dose of NPK fertilizer, and reported that tomato plants had a greater number of branches and an increase in fruit yield, the same as the observed in the present study. The improvement in fruit yield could be explained by the effect that silicon has on the roots of the plants. In many researches, it has been documented that silicon increases root mass and length (Chakma *et al.*, 2021), as could be corroborated in the present study (Figure 1). The improvement of the root system increases the absorption capacity of nutrients and water, consequently, the plant has greater availability of nutrients, so it can increase the production of biomass and fruits. This effect has been observed in crops such as potato (*Solanum tuberosum*) (Pilon *et al.*, 2014), wheat (*Triticum aestivum*) (Ahmed *et al.*, 2016), corn (*Zea mays*) (Sirisuntornlak *et al.*, 2019), melon (*Cucumis melo*) (Alam *et al.*, 2021), and grape tomato (*Solanum lycopersicum* var. cerasiforme) (Chakma *et al.*, 2021).

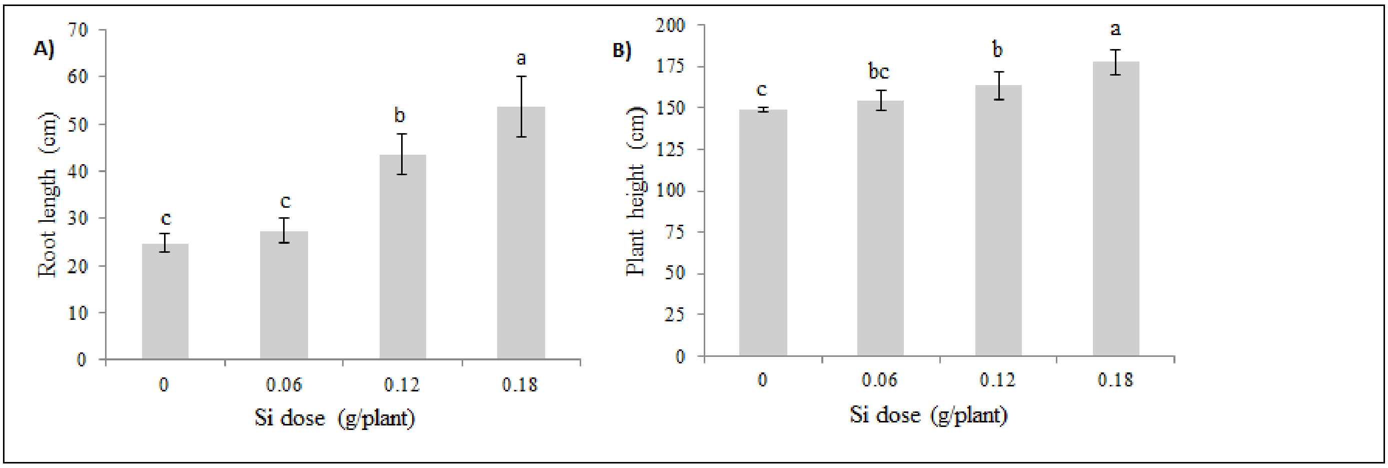

Figure 1. A) Root length and B) height of tomato plants with different doses of silicon. Different letters between columns indicate significant difference P < 0.05. Chambo, UTEQ, 2022.

Table 3. Average values of number of antioxidant capacity and concentration of different antioxidants, as well as total soluble solids of the tomato under different doses of silicon. Chambo, UTEQ, 2022.

Doses of Si (g/plant)	Antioxidants (mM FRAP/g)	Phenols (mg GAE/g)	Flavonoids (mg Catechin/g)	Carotenoids (mg β-CaE/g)	Vit C (mg/100 g)	SST (%)
0.00	95.96±0.02 d	5.82± 0.01d	1.27±0.01 d	102.58±0.01 d	62.85±0.01 d	3.30±0.01c
0.06	96.76±0.02 c	5.92±0.01 c	1.59±0.01 c	106.32±0.01 c	73.53±0.01 c	3.51±0.01b
0.12	97.30±0.02 b	6.51±0.01 b	1.74±0.01 b	142.04±0.01 b	82.77±0.02 b	3.61±0.01a
0.18	102.75± 0.02a	6.76±0.02 a	1.86±0.01 a	167.29± 0.02a	89.52±0.02 a	3.62±0.01 a
CV(%)	0.02	0.19	0.69	0.01	0.02	0.28
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

The values shown correspond to the average and standard deviation. Different letters ^{abcd} in the same column indicate statistical difference.

Antioxidant composition

Regarding the antioxidant capacity, the content of polyphenols, flavonoids, carotenoids and vitamin C increased as the dose of silicon increased (Table 3). Carotenoids increased 63% by including 0.18 g/plant of silicon in respect to control treatment. The flavonoids and vitamin C, in this same treatment (0.18 g of silicon) increased by around 40% compared to the control group. The polyphenols showed an increase of 16% when comparing the group with 0.18 g/plant of silicon with the control group. Finally, including 0.18 g of silicon increased the antioxidant capacity of tomato by 16% compared to not including silicon in the tomato fertilization program.

The results found in this study show the importance of including silicon in the tomato crop, since the increase in antioxidant activity confers greater protection to the plant against different types of stress, which has been widely documented (Zhang *et al.*, 2019; Pinedo-Guerrero *et al.*, 2020; Sun *et al.*, 2022; Peña-Calzada *et al.*, 2023). In addition, it offers the possibility of obtaining a product of higher nutritional quality and with nutraceutical characteristics for the consumer, since antioxidants are associated with the prevention of carcinogenic and vascular diseases (Ali *et al.*, 2020). In particular, the high increase in carotenoids could be associated with the fact that silicon increases the concentration of chlorophyll in plants (Song *et al.*, 2014; Lu *et al.*, 2017). In this sense, if we take into account that there is a direct relationship between the concentration of chlorophyll and carotenoids (Guavita-Vargas *et al.*, 2018), it is possible to assume that silicon increases the concentration of carotenoids, as observed in the present study.

In addition, the effect of carotenoids on the health of plants and consumers, are also related to the characteristic color of the fruit, therefore, a higher content of carotenes in fruit helps to give a better appearance of the fruit in terms of freshness and, consequently, its acceptance by the consumer increases (Liu *et al.*, 2009).

On the other hand, the total antioxidant activity in tomato is commonly classified as hydrophilic and lipophilic. Hydrophilic compounds are mainly phenolic compounds and vitamin C, while lipophilic compounds are carotenoids, and lipophilic phenols (Pinedo-Guerrero *et al.*, 2020). Based on our results, it is clear that the application of silicon influences the antioxidant capacity of tomato plants, since all the antioxidants evaluated increased in the treatments with silicon, as well as the total antioxidant capacity. In different studies, the effect of silicon under stress conditions, either biotic or abiotic, has been evaluated. These studies have concluded that the inclusion of silicon helps to mitigate the effects of stress by increasing the synthesis of antioxidants. For example, the application of exogenous Si helped to alleviate oxidative stress in several plant species, such as cucumber (*Cucumis sativus*) (Pavlovic *et al.*, 2013), tomato (Shi *et al.*, 2014), strawberry (*Fragaria* × *ananassa*) (Muneer *et al.*, 2017), and rice (Khan & Gupta, 2018). The authors of these investigations associated the improvement of crops under stress, to an effect induced by silicon on the activity of antioxidant enzymes, therefore they conclude that silicon has the potential to stimulate the synthesis of antioxidants. Statement that is reinforced by the results found in this research.

Something important to highlight is that most of the studies that have been carried out with silicon have induced some stress in the plant, so the hypothesis has been put forward that the level of the stress factor, measured through the concentration of reactive oxygen species in plant tissues, is what determines the antioxidant synthesis rate (Eraslan *et al.*, 2008). However, the results found in the present study could refute this hypothesis, since no stress factor was included in the tomato crop, even so, the concentration of antioxidants increased as the dose of silicon increased. Therefore, it is necessary to carry out additional studies that help to clarify this situation and to have a better understanding of the effect of silicon on the synthesis and expression of antioxidants. Likewise, it is necessary to identify and describe the adjacent mechanisms of the effect of silicon on the synthesis of antioxidants. At the moment, it has been suggested that silicon could be involved in the positive regulation of metabolism genes, signal transduction, defense and stress response in plants (Kurabachew *et al.*, 2013). However, more research is needed in this regard to clarify these mechanisms of action.

Under the experimental conditions of the present study, including 0.18 g of silicon/plant increases the growth and yield of the tomato crop. In addition, it increases the concentration of antioxidants and the total antioxidant activity. Therefore, it is suggested to include silicon in tomato fertilization programs as a sustainable alternative to improve crop growth and productivity.

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