Doi: https://doi.org/10.17584/rcch.2019v13i2.9292

How does the physiological activity and growth of tomato plants react to the use of a soil-mineral compound?

¿Cómo reacciona la actividad fisiológica y el crecimiento de las plantas de tomate al uso de un compuesto suelo-mineral?

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Tomato plant experiment.

Photo: I.S. Pereira

ABSTRACT

The tomato crop has a high productive potential that can be depleted by biotic and abiotic stresses. Increased plant resilience to stress conditions has been reported with foliar applications of soil-mineral compounds; however, it is necessary to better understand how plants react to the use of this compound. Thus, this study evaluated the effect of foliar applications of a soil-mineral compound on the physiological and growth attributes of tomato plants. This experiment was carried out in Lagoa Formosa/MG during 2016. Different rates of the soil-mineral compound were used during the crop cycle, forming four distinct managements. The management consisted of different doses of the mineral compound in four stages after transplanting the tomato seedlings. The experiment design used randomized blocks. The following physiological evaluations were performed: total soluble protein, hydrogen peroxide, nitrate reductase enzyme activity, urease, superoxide dismutase (SOD), peroxidase, phenylalanine ammonia lyase, and lipid peroxidation (LP). The growth assessments were plant biomass and yield. Foliar applications of the soil-mineral compound increased the activity of the SOD enzyme by 4.17 and 6.25%. The use of the soil-mineral compound also increased the LP activity and reduced the antioxidant enzyme activity. The foliar application of the soil-mineral compost at doses of 0.5, 0.750, 1.0 and 1.0 kg ha^{-1} at 15, 25, 40 and 60 days after transplanting, respectively, increased the yield of the table tomatoes by 20%.

Additional key words: resistance inducers; oxidative metabolism; productivity; fertilizers.

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RESUMEN

El cultivo de tomate presenta un alto potencial productivo pero puede afectarse debido al estrés biótico y abiótico. Se ha informado un aumento de la resistencia de la planta a las condiciones de estrés con la aplicación foliar de compuestos minerales del suelo, sin embargo, aún es necesario comprender mejor cómo reacciona la planta al uso de este compuesto. Por lo tanto, este estudio evaluó el efecto de la aplicación foliar del compuesto mineral del suelo sobre los atributos fisiológicos y de crecimiento de las plantas de tomate. Este experimento se llevó a cabo en Lagoa Formosa / MG durante 2016. Se usaron diferentes tasas del compuesto mineral del suelo durante el ciclo del cultivo, lo que constituye cuatro manejos distintos. El manejo consistió en diferentes dosis del compuesto mineral en cuatro etapas después del trasplante de las plántulas de tomate. El diseño experimental utilizado fue de bloques al azar. Se realizaron las siguientes evaluaciones fisiológicas: proteína soluble total, peróxido de hidrógeno, actividad de la enzima nitrato reductasa, ureasa, superóxido dismutasa (SOD), peroxidasa, fenilalanina amoniaco liasa y peroxidación lipídica (LP). Las evaluaciones de crecimiento fueron biomasa vegetal y rendimiento. La aplicación foliar del compuesto mineral del suelo aumentó la actividad de la enzima SOD en 4,17 y 6,25%. El uso del compuesto mineral del suelo también aumentó la actividad de LP y redujo la actividad de las enzimas antioxidantes. La aplicación foliar de compost mineral del suelo a dosis de 0,5; 0,750; 1,0 y 1.0 kg ha^{-1} a los 15, 25, 40 y 60 días después del trasplante, respectivamente, aumentó el rendimiento de tomates de mesa al 20%.

Palabras clave adicionales: inductores de resistencia; metabolismo oxidativo; productividad; fertilizantes.

Received for publication: 19-04-2019 Accepted for publication: 31-07-2019

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) is one of the most consumed vegetables globally, mainly because of its high nutritional value and antioxidant potential (Du *et al*., 2017). Overall tomato production increased from 27.6 million tons in 1960 to 177 million tons in 2016 (FAOSTAT, 2016). In Brazil, table tomato cultivation stands out because of its socioeconomic importance, as it generates jobs, improving the income of rural workers and growers (Silva *et al.,* 2013). Many factors can lead to a reduction in crop productivity, affecting the quality of the final product, which include problems caused by pests and diseases (Zaidi *et al*., 2018).

Disease management has become a challenge in agriculture as it requires pesticides, often with high toxicity (Carrascosa *et al*., 2015). Chemical pesticides, particularly soil fumigants, have been severely restricted in recent decades because of the environmental consequences (Van Bruggen and Finckh, 2016), the residual effects on soil and the long period necessary for degradation. In addition, the use of pesticides in the environment impacts human health, and resistance is acquired by pathogens for the chemical groups. The use of pesticides should be minimized and rationalized to promote more sustainable agriculture (Lamichhane *et al*., 2015).

Phytosanitary management needs to be improved to minimize or mitigate its impact on the environment (Dayan *et al*., 2009). Plants have mechanisms to respond to different types of stress, whether abiotic (Shah *et al*., 2014) or biotic (Shah and Zeier, 2013). These natural defense mechanisms remain inactive or latent until activated after exposure and/or contact with inducing agents (Mandal, 2010).

The defense mechanisms of a plant are genetically controlled, depending on the expression after contact with the host. So, plants can activate the defense mechanism in several ways, including through oxidative activities (Teixeira *et al*., 2017; Xue and Yi, 2017), enzymes involved in the phenylpropanoid route (Sangeetha and Sarada, 2015), and enzymes involved in lipid peroxidation, among other forms. The use of resistance-inducing products for different crops is increasing, for example on vine (Xue and Yi, 2017), wheat (Moya-Elizondo and Jacobsen, 2016), pepper (Siddiqui and Meon, 2009), and peach (Jiao *et al*., 2018), mainly.

When plants recognize the resistance inducers applied, the intracellular signal transduction pathways are activated (Shah *et al*., 2014). Perception occurs when the molecules of the inducing agent bind to receptor molecules that are probably located in the plasma membrane of the plant cell; this reaction triggers the activation of various defense mechanisms, culminating in protection against pathogens (Graham and Myers, 2011). These inductions alter the physiology of the plant and can interfere directly with productivity.

Application of resistance inductors that have Silicon (Si) is a potentially sustainable option to improve biotic and abiotic stresses in several plants (Zhu and Gong, 2014; Liang *et al*., 2015; Cooke and Leishman, 2016; Etesami, 2018).

Despite the importance of the use of these products, there are few studies on resistance inductors based on silicon oxide and aluminum in the tomato to table culture. One source of these nutrients available to growers is through soil-mineral compost. Thus, this study evaluated the effect of foliar applications of a soil-mineral compound on the physiological and growth attributes of tomato plants.

MATERIAL AND METHODS

This experiment was conducted in the 2016 growing season in the commercial area of Lagoa Formosa, Minas Gerais, Brazil (18°30'01.6" S and 46°30'48.2" W) in the first crop of the year. The cultivar "Dominador" was used, which has an indeterminate growth habit, high vigor, average cycle duration of 120 d and fruits with an aptitude for salads. The seedlings were produced in trays and transplanted to the field when they had two leaves, 28 d after sowing.

Cultural management including fungicides, insecticides and herbicides was carried out in all treatments. Each plot was composed of five rows, with a 6 m length, 2 m row spacing, and 0.6 m between plants. The useful area of each plot consisted of the three central lines, discarding 0.5 m at each border. The plants were irrigated with a central pivot and received fertirrigation according to the nutritional needs of the crop.

The experiment design used a randomized block with four treatments and five replications. The treatments consisted of four management types of the soil-mineral compound during the crop cycle: management 1 was the control; management 2 consisted of foliar applications at the dose of 0.75 kg ha^{-1} per application at 15, 25, 40 and 60 days after transplanting (DAT); management 3 consisted of foliar applications of 0.5 kg ha^{-1} at 15 DAT, 0.750 kg ha^{-1} at 25 DAT, 1.0 kg ha^{-1} at 40 DAT, and again at 60 DAT; and management 4 consisted of foliar applications of 1.0 kg ha^{-1} per application at 15, 25, and 40 DAT.

The soil-mineral compound was a fine, balanced powder, prepared by milling, micronization and standardization of special clays that are commercially distributed in Brazil. The soil-mineral compound was a fine powder composed of Al_2O_3 (20.6%), SiO_2 (17.4%), S (9.8%), CaO (1.3%), TiO_2 (0.34%), MgO (0.18%), Fe_2O_3 (0.16%), and P_2O_5 (0.10). The doses were as indicated in the commercial product (Rocksil®).

The foliar applications were done with costal spraying with a CO_2 injection. The bar contained a fan-type nozzle, with a constant pressure of 2.0 bar.

Biochemical evaluations

Leaf samples for the nitrate reductase determinations were performed at 8, 26, 40, 47 and 73 DAT, and, for the other analyses, they were taken only at 73 DAT. Completely expanded leaves were collected from the middle third of the plants.

The activity of the enzymes nitrate reductase, urease, lipid peroxidation, peroxidase enzymes, superoxide dismutase, phenylalanine ammonia-lyase, hydrogen peroxide, and total proteins were determined.

The NR analysis was performed according to the method proposed by Mulder *et al*. (1959). The urease was evaluated throughout extraction, and the analysis of the plant material was done according to the methodology adapted from Hogan *et al*. (1983).

Samples of 200 mg of fresh biomass of leaves were macerated with 4.0 mL of 0.1 mol L^{-1} potassium phosphate buffer pH 6.8. Then, the samples were transferred to Eppendorf flasks and centrifuged at 10,000 rpm for 30 min at 4°C (Kar and Mishra, 1976). Then, the samples were stored at -20°C until determination of the total protein content of the leaf (Bradford, 1976), superoxide dismutase activity (SOD) (Beuchamp and Fridovich, 1971), peroxidase activity (POD) (Teisseire and Guy, 2000), and phenylalanine ammonia-lyase activity (PAL) (Umesha, 2006). With the fresh leaf biomass the hydrogen peroxide content (H_2O_2) (Alexieva *et al*., 2001) and lipid peroxidation (LP) (Heath and Packer, 1968) were also evaluated.

Biomass attributes

One plant per replicate was harvested at 47 and 83 DAT for determination of the root, stem and leaf biomass. The samples were dried in a forced-air oven at 65°C for 48 h before the determination of the dry biomass. Productivity harvests were also performed at 73, 81, 92, 102, 110, 119, 124, 130 DAT.

Statistical analysis

The data were evaluated for normality and homogeneity using Levene and Shapiro-Wilk tests, both at the 5% significance level. A variance analysis was performed, and, when significant, the Tukey test was applied at the 5% level of significance. For some analyses, regression analysis was also performed. The analyses were performed using statistical software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

For the nitrate reductase variable, the variance analysis presented significance only for the management; there was no effect from the harvesting times or interaction. The other attributes of nitrogen metabolism and the activity of antioxidant enzymes had no effect from the management. There was a difference in the productivity attributes.

The nitrate reductase enzyme did not present a significant difference during the evaluation periods although some management showed a tendency for increasing (Fig. 1). The nitrate reductase enzyme acts on nitrogen assimilation in plants and reduces nitrate to nitrite through NADPH (nicotinamide adenine dinucleotide phosphate hydrogen) energy. Thus, the increase in this enzyme activity contributes to the increase of the assimilation of nitrogen (N) and the growth and development of the plant (Taiz *et al*., 2016).

Resistance induction represents an extra energy expenditure for plant defense and reduces nitrate reductase metabolism and consequently plant growth.

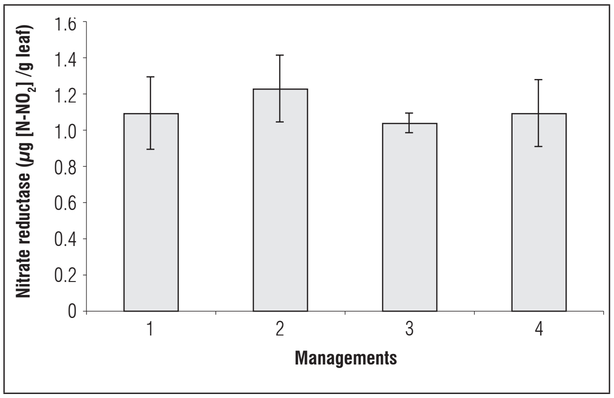

Figure 1. Activity of the enzyme nitrate reductase when submitted to different managements for foliar applications of the soil-mineral compound. Nitrate reductase (μg N-NO_2/g leaf). The vertical bars indicate ± standard error.

Thus, we verified that the foliar applications of the soil-mineral compound may not have activated resistance mechanisms related to the nitrogen metabolism enzymes.

According to Lawlor (2002), the role of nitrogen in the production of dry phytomass and, consequently, remobilization to reserve organs is directly related to photosynthesis. Photon energy is converted into chemical energy and stored in ATP (Adenosine triphosphate) and secondary metabolites, primarily NADH, which is used in the synthesis of carbon and nitrogen assimilates, particularly amino acids (Lawlor, 2002).

It was found that the activity of nitrate reductase reduces during harvests, more accentuated after flowering. We hypothesize that the fruiting drain competes for the N present in the plant, which explains this reduction in enzyme activity.

The protein content and the activity of the urease enzyme did not differ between the management types; however, it was noted that treatments 2, 3 and 4 increased the total protein content (Fig. 2A).

The quantification of the protein content in the leaf during the reproductive growth evidences the redistribution of amino acids from leaves to reproductive growth. A higher protein content in the leaves may be an indicator of greater assimilation of atmospheric CO_2 because the main enzyme of photosynthesis is composed of N, the ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco) (Taiz *et al*., 2016).

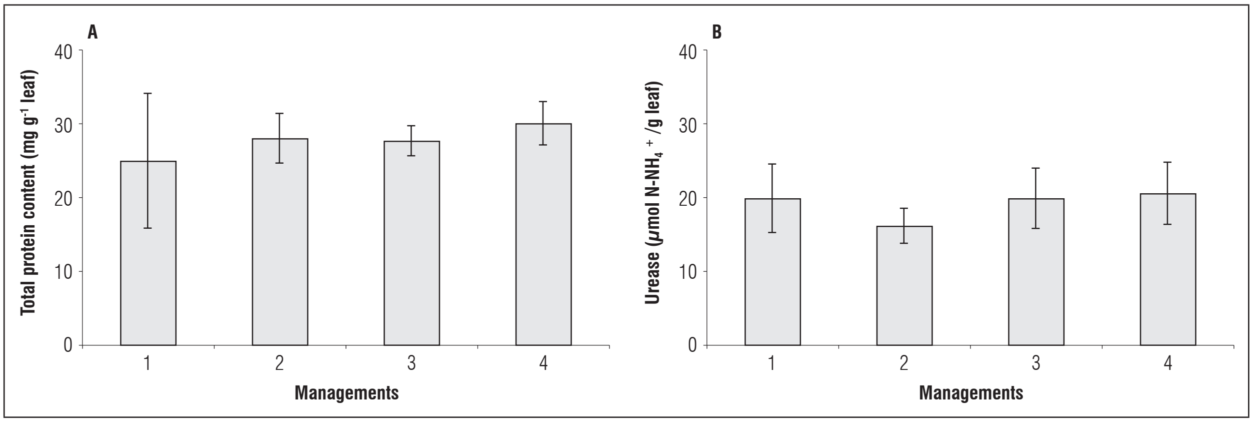

Figure 2. Protein content (A) and urease enzyme activity (B) in tomato leaves at 73 DAT when submitted to different management types for foliar applications of the soil-mineral compound. The vertical bars indicate ± standard error.

Oxidative metabolism

The managements 2, 3 and 4 presented higher levels of hydrogen peroxide than management 1 (control), without significant differences (Fig. 3A). Management 4 presented, on average, values of hydrogen peroxide production that were approximately 56% higher than treatment 1 (control), which demonstrated that the resistance inducer activated the oxidative metabolism in some signal transduction pathways in the plant tissue.

The increase of this free radical indicates that the defense signaling of the plants probably activated genes related to pathogenesis. Thus, the increase in the synthesis of hydrogen peroxide does not always result in negative effects in plants, such as destruction of membranes, because this molecule can function as a signal agent in plants, which includes responses to pathogen elicitors, stomatal closure, acquired systemic resistance, and programmed cell death (Chen and Gallie, 2005).

For the enzyme POD, there was a positive trend in managements 2, 3 and 4, without statistical significance (Fig. 3B). The increase in the activity of the POD enzyme as well as the increase in H_2O_2 content was a protective measure to degrade the reactive oxygen species (ROS) in the plants (Barbosa *et al*., 2014). PODs are related to the synthesis of lignin and suberin, which increase the hardness of tissues and the production of quinones and active oxygen, which have antibiotic activities (Stout *et al*., 1994).

The activity of the SOD enzyme was reduced in managements 3, 4 and 2, in relation to treatment 1 (control) (Fig. 3C). This enzyme is responsible for the degradation of superoxide ERO in hydrogen peroxide, so it is later degraded by CAT and POD enzymes (Mittler, 2002). An increased SOD and POD activity is directly related to plant tolerance to environmental stresses, such as saline stress (Koca *et al*., 2007). However, when the activity of the enzyme is saturated before reaching the maximum concentration of reactive oxygen species, lipid peroxidation may occur.

The activity of the PAL had a different behavior (Fig. 3D). This enzyme is responsible for catalyzing the conversion of phenylalanine to trans cinnamic acid, the first step for the biosynthesis of phenylpropanoids. This compound is the basis for the synthesis pathway of secondary metabolites that exhibit antioxidant activity, such as flavonoids and tannins (Dias *et al*., 2015). A different behavior can be explained by a different route of action for defending plants against stress. Alternative products, such as chitosan and plant extracts, may increase the activity of phenylalanine-ammonia-lyase (PAL) (Lorencetti *et al*., 2015).

The levels of lipid peroxidation were similar among all treatments (Fig. 3E). Lipid peroxidation is used as a basis to measure the damage caused by the action of reactive oxygen species on the unsaturated lipids of cell membranes. This leads to membrane destruction, failure of cellular mechanisms and, in extreme cases, cell death (Lima and Abdalla, 2001). Therefore,

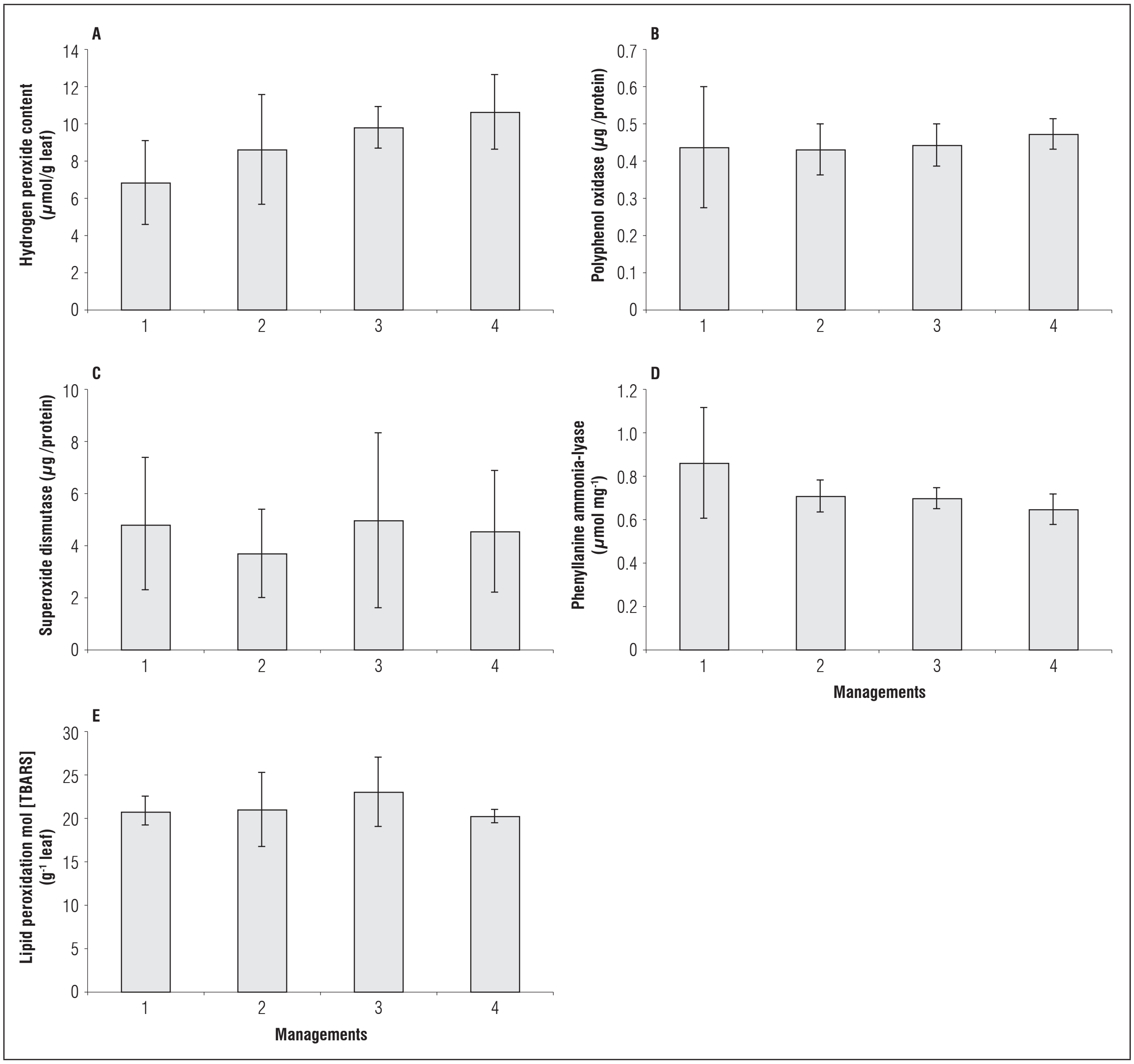

Figure 3. Hydrogen peroxide content (A), polyphenol oxidase activity (B), superoxide dismutase μ^{-1} (C), phenylalanine ammonia-lyase (D), and lipid peroxidation (E) of tomato leaves at 73 DAT, when submitted to different management types for foliar applications of the soil-mineral compound. The vertical bars indicate ± standard error.

because there was no difference between the treatments and the control, there was no cellular damage from the foliar application of the soil-mineral compound.

Silicon increases the synthesis of phenolic compounds in plants and increases the activity of the following enzymes: polyphenol oxidase (PPO), peroxidases (POD) and phenylalanine ammonia-lyase (PAL) (Gomes *et al*., 2005). As a consequence, it tends to reduce the rate of pathogen growth and the incidence of pest insects. The application of Si increased the accumulation of phenolic compounds in walls of epidermal cells of *Triticum aestivum*, and consequently, increased the resistance of plants infected by *Blumeria graminis* f. sp. *tritici* (Bélanger *et al*., 2003).

A PPO plays an important role in plants since it provides resistance to attack from pathogens and diseases. It has also been reported that PPO may exert a direct relationship with photosynthesis since it assists in the maintenance of system homeostasis

(Boeckx *et al*., 2015). This enzyme performs the oxidation of diphenol in quinone, a beneficial compound for the photosystem. In addition, during this process, PPO removes excess O_2 from the system, avoiding the possible formation of superoxide radicals (Boeckx *et al*., 2015).

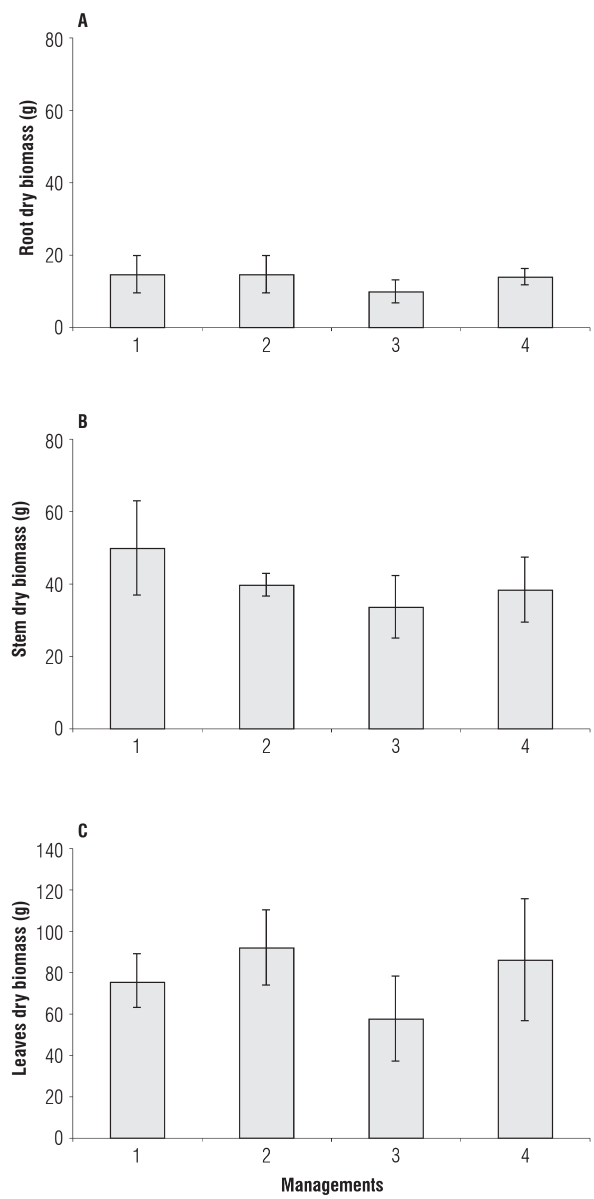

Figure 4. Root (A), stem (B), and leaf (C) biomass of tomato plants when submitted to different management types for foliar applications of the soil-mineral compound. The vertical bars indicate ± standard error.

Although the foliar applied soil-mineral compound had Si, no change in the activity of SOD and PAL enzymes was observed. The interaction of Si with these enzymes is probably more complex and depends on other factors, such as dose, culture, form of application, and interaction with other nutrients in the application, among other factors, which needs more studies to be better understood.

Biomass attributes

About 95% of the dry mass accumulated by plants during their cycle is derived from the photosynthetic activity, and the rest comes from the soil (Benincasa, 2004). The root dry and stem dry biomass did not differ between the management types (Fig. 4AB). There was a trend for a higher dry leaf biomass in management 4, without significant differences (Fig. 4C).

Si, after being absorbed, is translocated and deposited just below the cuticle, forming a double layer of silicon-cuticle. This contributes to protection from abiotic stresses, such as: elemental toxicity, salinity, and frost, among others; as well as protection from biotic stresses, such as pests and diseases (Ranganathan *et al*., 2006). The accumulation of Si makes leaves more upright and rigid and increases the interception of light and, consequently, photosynthetic efficiency (Gonçalves, 2009).

Productivity

All treatments with foliar applications of the soil-mineral compost increased yield per plant and yield. The highest production per plant and productivity were obtained in management 3, with 3.11 kg/plant and 31,152 kg ha^{-1}, respectively (Fig. 5, A and B). The use of the soil mineral compost increased tomato yield by 20% in management 3, a good option for increasing crop productivity.

Si, applied in the form of aluminum silicate on vines (*Vitis vinifera*), contributes to the control of mildew (*Plasmopara viticola*) (Gomes *et al*., 2011). The use of a soil-mineral compost in guava (*Psidium guajava*) contributes to the control of anthracnose (*Colletotrichum gloeosporioides*) and reduced the diameter of colletotricum lesions (*Colletotrichum gloesporioides*) in fruits (Gomes *et al*., 2016). The aluminum oxide (Al_2O_3), silicon dioxide (SiO_2) and sulfur (S) present in the soil-mineral compost inhibited in vitro tests on the

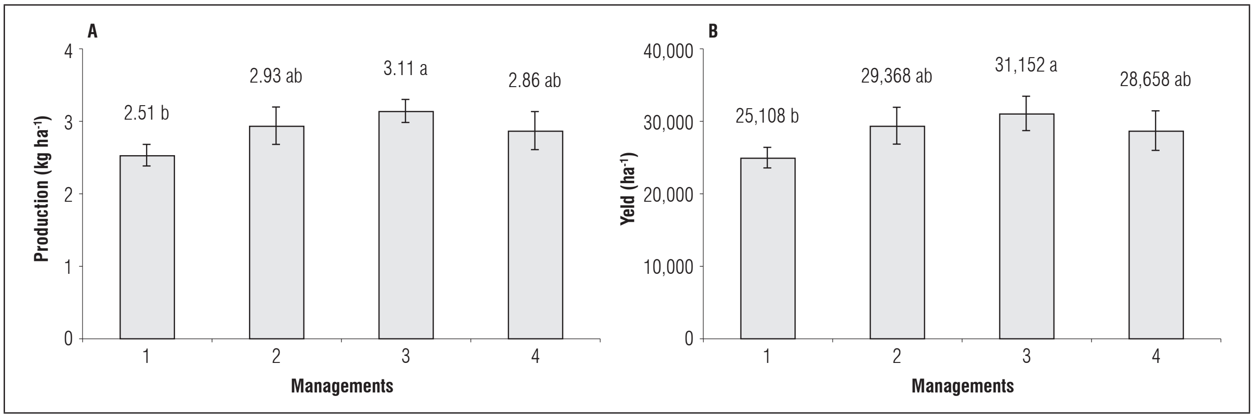

Figure 5. **Production per plant (A) and yield (B) of tomato plants when submitted to different management types of foliar applications of the soil-mineral compost. Means with different letters indicate a significant statistical differences according to Tukey test ($P \leq 0.05$) ($n=5$) ± standard error.**

mycelial growth of colletotrichum (*Colletotrichum gloesporioides*) (Gomes *et al*., 2016).

We hypothesize that foliar applications of aluminum oxides can trigger the production of reactive oxygen species in plants, which stimulates the production of antioxidant enzymes.

Si was identified as a resistance inducer in some monocotyledons, such as rice (*Oriza sativa*), corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.), which actively absorb and accumulate large amounts of silicon (Liang *et al*., 2015). This was also observed in some dicotyledonous crops, such as cotton (*Gossypium hirsutum*), soybean (*Glycine max* (L.) Merr.), and tomato (*Solanum lycopersicum* L.), and also in some cucurbitaceous species (Liang *et al*., 2015).

Si can favor the upright position of leaves, indirectly favors photosynthesis, and makes the opening and closing of stomata more efficient (Pereira *et al*., 2003). The productivity of sugarcane increased with the foliar applications of Si (Elawad *et al*., 1982). Foliar applications of potassium silicate (K_2SiO_3) increase the chlorophyll content and growth of strawberry plants (*Fragaria* × *ananassa*) (Wang and Galletta, 1998).

The effectiveness of the application of Si in the mitigation of stress depends on the species (Mitani and Ma, 2005) and the level of stress suffered by the plant (Hodson *et al*., 2005). In this experiment, the plants were not subjected to severe stress conditions, which may have contributed to the stress metabolism not responding significantly to the soil-mineral compound treatments. Biochemical and molecular responses using Si occur when the plant is subject to stress conditions (Liang *et al.*, 2015).

Although the treatments with foliar applications did not modify the activity of the evaluated enzymes, the increase in productivity showed that the management types with the compost soil mineral contributed in another way to an increased tomato productivity. We hypothesized that there may have been an increase in proteins, lignins, and efficiency of the photosynthetic activity, but more studies are required for a better understanding.

CONCLUSION

The foliar applications of the soil-mineral compost at doses of 0.5, 0.75, 1.0 and 1.0 kg ha^{-1} at 15, 25, 40 and 60 DAT, respectively, increased the yield of the table tomatoes.

No effect from the foliar applications of the soil-mineral compound was observed on the enzymes related to stress metabolism as a total protein, hydrogen peroxide, nitrate reductase enzyme activity, urease, superoxide dismutase, peroxidase, phenylalanine ammonia lyase and lipid peroxidation under the conditions of this study.

Conflict of interests: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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