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Plant Growth and Antioxidative Enzymes in Sunflower Supplemented With Selenium

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Abstract

The use of soil additives such as selenium can positively influence the antioxidative system of plants, making them more tolerant to abiotic stresses. The aim of this work was to evaluate the concentration of sodium selenite or sodium selenate applied to the substrate that causes improve in plant growth and antioxidative enzyme activities in sunflower plants. The treatments were divided in: control (absence of selenium); 0.2 mg L^{-1} of sodium selenate; 0.4 mg L^{-1} of sodium selenate; 0.8 mg L^{-1} of sodium selenate; 0.2 mg L^{-1} of sodium selenite; 0.4 mg L^{-1} of sodium selenite and 0.8 mg L^{-1} of sodium selenite. The analysis of Shoot Dry Mass (SDM) production and activities of the antioxidantive enzymes: Ascorbate Peroxidase (APX), Guaiacol Peroxidase (GPX) and Catalase (CAT) was performed. For SDM and APX the concentration of 0.8 mg L^{-1} of sodium selenite caused higher values. CAT showed greater activity in treatments that received 0.4 and 0.8 mg L^{-1} of sodium selenate and 0.4 and 0.8 mg L^{-1} of sodium selenite than the control treatment. GPX showed superior activity in the treatments 0.8 mg L^{-1} of sodium selenate, 0.2 mg L^{-1} of sodium selenite and 0.8 mg L^{-1} of sodium selenite than the control treatment. It was concluded that selenium promoted improvements in the antioxidant activity and in the production of shoot dry mass of sunflower plants.

Keywords: antioxidant enzymes, sunflower, sodium selenate, sodium selenite

1. Introduction

The cultivation of oilseeds has been encouraged for the production of biofuels, such as biodiesel. The main crops used are soybeans and corn, which are also in great demand for the food industry (Diniz, Sargeant, & Millar, 2018). Therefore, other crops such as palm, peanut, forage radish, castor bean, sesame, canola and sunflower have become an object of study for the development of techniques that improve agricultural management (Cavalcante Filho, Buainain, & Benatti, 2019).

Sunflower (*Helianthus annus* L.) is an annual crop with good acclimatization to different climate and soil conditions. It can be used as ornament, food and for the production of biofuels (Araújo, D. Silva, V. Silva, Magalhães, & Barros, 2018). However, there are regions, such as those with a semi-arid climate, in which water and climate limitations impact the crops negatively. Due to low rainfall regimes, evapotranspiration is higher, which can cause drier soils that accumulate salts, leading plants to a stress condition (Gul, Dinler, & Sarısoy, 2017).

Fertilization and irrigation are widely used techniques to remediate abiotic stresses (Araújo et al., 2018). The use of additives, such as selenium, has shown that there is a possibility of a positive effect on crops subjected to stress, when used in adequate concentrations (Al-Kazzaz, 2018).

Selenium is a trace element that may be naturally present in soils. However, about 70% of the world has selenium deficient soils. In soils, selenium comes from rocky weathering and volcanic magma. Its availability depends on the parameters that guide the chemical and biochemical reactions of the soil. Regions whose climate is temperate and humid are mostly poor in selenium (Hossain et al., 2021). For plants, the presence of selenium can present benefits, such as increased productivity, a positive influence on the quality of products (such as fruits and seeds) and on plant senescence. The bioavailable forms for plants are those soluble in water, which are organic selenium, selenite and selenate (Garduño-Zepeda & Márquez-Quiroz, 2018).

Gül et al. (2018), evaluated sunflower plants under different salt stress conditions in the Black Sea region. The authors found that salt stress conditions reduced the percentage of seedling germination. Habibi (2017) carried out a study in the region of Poland, where the climate is temperate, subjecting sunflower plants added with sodium selenate to salt stress and concluding that selenium helped to reduce damage to the plant. Hachmann et al. (2019), when testing selenium concentrations in cauliflower plants subjected to water stress, observed higher productivity in those treatments that contained selenium.

Considering the scarcity of studies that quantify the ideal selenium concentration applied in soil/substrates for the cultivation of sunflower plants in tropical and semi-arid climates, such as in Northestern Brazil, the aim of this work was to evaluate the concentration of sodium selenite or sodium selenate applied to the substrate that causes improve in plant growth and antioxidative enzyme activities in sunflower plants.

2. Method

The experiment was carried out between August and September 2021, under greenhouse conditions at Instituto Federal de Educação, Ciência e Tecnologia do Ceará (IFCE) Maracanaú campus-CE, Brazil. The geographical location is 24 M 543133.84 m E/9571989.84 m S. The climate is tropical, with dry winters according to the Köppen classification, the average temperature was 26 °C and the average humidity was 65% (Climate-Data, 2022).

The experimental design was completely randomized, with seven independent treatments containing five replications each one. Two sources of selenium were used, sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3), applied directly to the soil on the day of sowing. The concentrations chosen were based on the study of Nasser (2015). The treatments were divided into control (absence of selenium); 0.2 mg L^{-1} of sodium selenate; 0.4 mg L^{-1} of sodium selenate; 0.8 mg L^{-1} of sodium selenate; 0.2 mg L^{-1} of sodium selenite; 0.4 mg L^{-1} of sodium selenite and 0.8 mg L^{-1} of sodium selenite.

Each treatment contained five replications, with three plants in plastic vases of 5 L. The substrate that was used contained four volumes of sand and a volume of commercial earthworm humus, plus the selenium concentration corresponding to the treatment. The sunflower seeds used were cultivar BRS 323, provided by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). The irrigation was daily at 80% of field capacity.

At 19 days after sowing (DAS), a destructive harvest was performed. The material was separated into two parts. The first one for the quantification of Shoot Dry Mass (SDM) and the other one for the enzymatic analyses. In order to obtain the SDM, the plants were placed in paper bags in a forced circulation oven, with a temperature of 60°C, until the material had a constant mass. Subsequently, the dry material was weighed on an analytical balance to obtain the SDM.

In the enzymatic analysis, the material was used in the form of extracts, produced according to the methodology of Nunes et al. (2017). All analyzes used spectrophotometric methods. Catalase (CAT) activity was determined by the methodology of Havir and McHale (1987). The determination of Ascorbate Peroxidase (APX) concentration was performed by the method of Nakano and Asada (1981). Finally, the activity of the enzyme Guaiacol Peroxidase (GPX) was determined by the methodology of Kar and Mishra (1976).

Data were tested by analysis of variance (ANOVA) and means compared by Tukey's test ($P \leq 0.05$). The Sigma Plot 11.0 program was used to perform statistical analysis and graph construction.

3. Results and Discussion

The plants that received selenium in the form of sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3), in most concentrations, had higher values than those obtained in the control treatment. For the variable Shoot Dry Mass (SDM), the treatment with the highest value was the one that received a concentration of 0.8 mg L^{-1} of sodium selenite, with an average value of 0.27 g $plant^{-1}$, as shown in Figure 1. In comparison to the average of the other treatments that received selenium, the percentage of growth was 30% higher. And in relation to the average of the control treatment, the values were 50% higher.

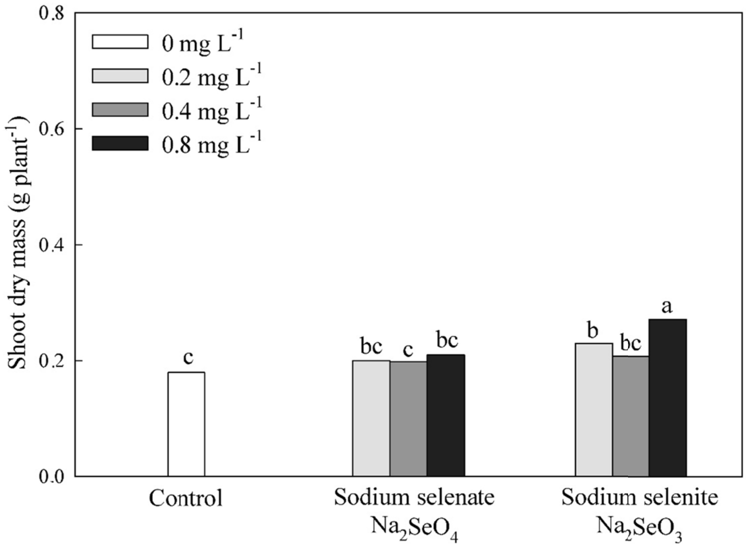

Figure 1. Shoot Dry Mass at 19 days after sowing of sunflower plants plus substrate selenium concentrations. The letters are the representation of the Tukey's test statistics ($P \leq 0.05$), different letters represent that there was a statistical difference between the treatments

The correlation between variables, such as biomass and dry matter are factors directly proportional to productivity. The dry matter values vary accordingly to the radiation absorption capacity, the conversion of this radiation into matter and the disposition of this produced material in the plant's partitions (Guimarães, Echer, & Minami, 2002). At first, the seed provides the necessary resources to initiate the formation of the organs, as the root system and leaves are developed and the interaction with the substrate and photosynthetic processes increase. These factors are correlated with an increase in leaf area, which is represented by SDM values (C. Peixoto, Cruz & M. de F. Peixoto, 2011).

Machado (2022) added selenium to onion cultivation and also obtained higher dry matter values for treatments that received doses of selenium, with a productivity increase of 14.7% in relation to those that did not receive selenium. Similarly, Hachmann (2019) obtained satisfactory values of SDM when adding selenium to the cultivation of vegetables: tomato, pepper, eggplant, cucumber, pumpkin, melon, watermelon and string bean.

Some defense mechanisms are essential to ensure that plants can maintain vitality and productivity. The antioxidative enzymes Ascorbate Peroxidase (APX), Guaiacol Peroxidase (GPX) and Catalase (CAT) are active in cellular defense processes (Soares & Machado, 2007). When analyzing the enzymatic activity for CAT (Figure 2), the treatments that received sodium selenate and the treatments 0.4 and 0.8 mg L^{-1} of sodium selenite outperformed the control treatment. The greatest increase in the activity of this enzyme was noticeable in the treatments 0.4 and 0.8 mg L^{-1} of sodium selenate and 0.4 and 0.8 mg L^{-1} of sodium selenite, which showed no statistical differences between them, obtaining an average value of 20.97 μMol H_2O_2 min^{-1} g^{-1} FM. It represents an increase of 57% compared to the control treatment.

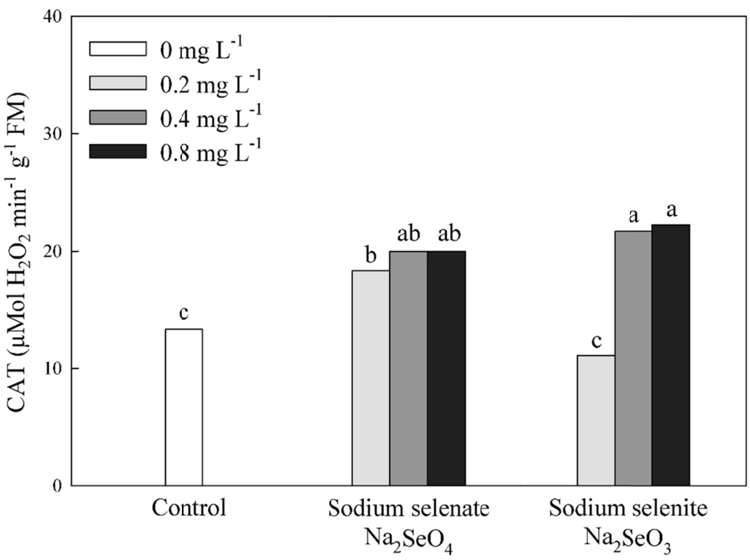

Figure 2. CAT enzyme activity at 19 days after sowing of sunflower plants plus substrate selenium concentrations. The letters are the representation of the Tukey's test statistics ($P \leq 0.05$), different letters represent that there was a statistical difference between the treatments

Mateus et al. (2021) biofortified coffee plants with concentrations of sodium selenate and obtained greater activity in CAT for those treatments that received selenium. CAT is an enzyme that acts in the neutralization of hydrogen peroxide, which is degenerative to crops, transforming it into water and oxygen (Tehrani & Moosavi-Movahedi, 2018). Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-) and hydroxyl (OH^-) trigger cellular mechanisms such as apoptosis and senescence (Gutierrez-Martinez et al., 2020).

CAT when compared to APX (Figure 3) and GPX (Figure 4) had much higher H_2O_2 elimination values. Barbosa, Silva, Willadino, Ulisses, and Camara (2014) state that CAT is the main enzyme in the elimination of H_2O_2, and can be found in peroxisomes, glyoxysomes and mitochondria. Unlike peroxidases, which rely on a regeneration cofactor, CAT can carry out the reaction independently. Gondim, Miranda, Gomes-Filho, and Prisco1 (2013) found increases in the antioxidant system of corn plants subjected to moderate concentrations of H_2O_2, mainly the increase in CAT activity. Thus, suggesting that an activation of the antioxidative system can promote beneficial effects. It is believed that selenium enhanced the antioxidant defense system of sunflower plants due to the increase in enzymatic activity.

APX (Figure 3) is an enzyme that uses ascorbic acid to reduce H_2O_2 to H_2O. High APX activities mean efficient conversion of this ROS (Cheng, Yu, Guo, Chen, & Minrui Guo, 2020). APX was higher in the treatment containing 0.8 mg L^{-1} of sodium selenite, with a mean value of 0.06 µMol H_2O_2 min^{-1} g^{-1} FM. This average was three times higher than that obtained in the control treatment. In relation to the other treatments containing selenium, there was an increase of 53% in the activity of this enzyme. Mateus et al. (2021) also performed APX analyzes and similarly found values three times higher for the selenium-supplemented treatment compared to the control.

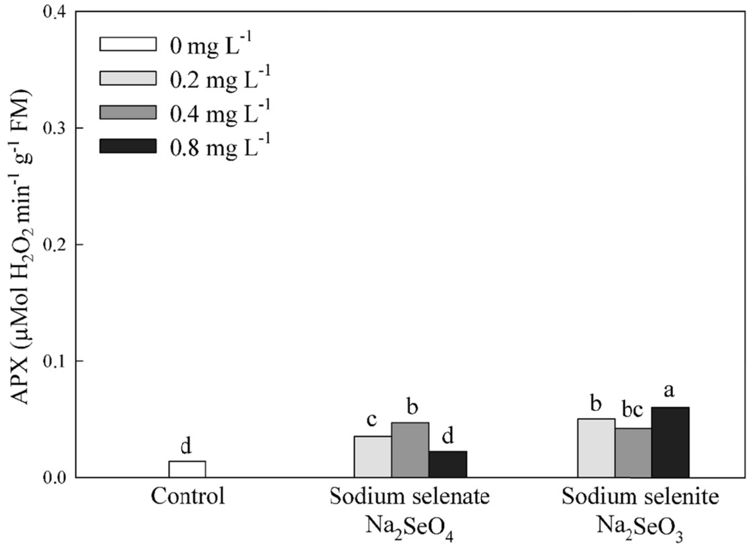

Figure 3. APX enzyme activity at 19 days after sowing of sunflower plants plus substrate selenium concentrations. The letters are the representation of the Tukey's test statistics ($P \leq 0.05$), different letters represent that there was a statistical difference between the treatments

Peroxidases develop many activities in plants along various cellular processes, such as cell wall formation and plant defense response system (Simo, Djocgoue, Minyaka, & Omokolo, 2018). GPX (Figure 4) is an enzyme found in the cytosol, vacuole, cell wall and apoplast, being a peroxidase that acts in the elimination of ROS (Uarrota et al., 2016). GPX had greater activity in the treatments that contained some concentration of selenium. The treatments 0.8 mg L^{-1} of sodium selenate, 0.2 mg L^{-1} of sodium selenite and 0.8 mg L^{-1} of sodium selenite did not differ statistically from each other. An average value of 0.446 µMol H_2O_2 min^{-1} g^{-1} FM was observed, which is twice the average of the control treatment.

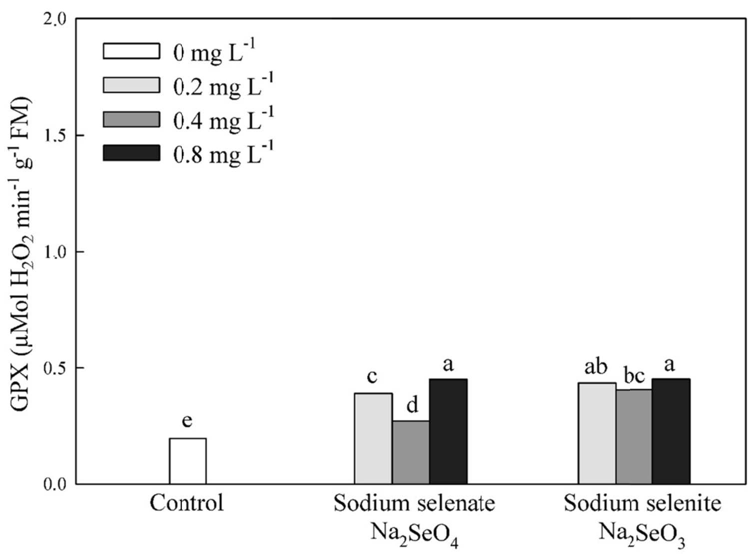

Figure 4. GPX enzyme activity at 19 days after sowing of sunflower plants plus substrate selenium concentrations. The letters are the representation of the Tukey's test statistics ($P \leq 0.05$), different letters represent that there was a statistical difference between the treatments

Different doses of selenium imply its effectiveness. Naseem et al. (2021) found out that high doses of selenium compromise gas exchange in corn crops. Other studies such as the one by Ulhassan et al. (2019), when cultivating Brassica napus, were able to perceive beneficial effects on the antioxidant system in some treatments submitted with dosages of sodium selenite. Selenium, in adequate concentrations, is a regulator of enzymatic reactions involving the elimination of ROS, according to studies carried out by Hernández-Hernández et al. (2019), who showed incremental effects on CAT, APX and GPX activity in tomato crops added to selenium.

4. Conclusion

In the experimental conditions used, selenium promoted improvements in the antioxidative enzyme activities and in the Shoot Dry Mass of sunflower plants. In general, the treatment with a concentration of 0.8 mg L^{-1} of sodium selenite outperformed the control treatment in all variables analyzed. When compared to the other treatments containing selenium, it was superior in the variables MSPA and APX.

Studies involving abiotic stresses, such as salt and water stress are indicated to corroborate the positive effects of selenium on the antioxidant system of sunflower plants. In addition, analyzes that can quantify the selenium concentration absorbed by the plants are necessary to confirm its effectiveness.

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