Assessment of the effects of selenium application on leaves or substrate on the growth of sunflower plants

Avaliação dos efeitos da aplicação de selênio nas folhas ou no substrato sobre o crescimento de plantas de girassol

DOI: 10.34188/bjaerv5n4-041

Recebimento dos originais: 06/05/2022
Aceitação para publicação: 30/06/2022

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ABSTRACT
Though Selenium (Se) is a nonessential micronutrient to plants, its presence under appropriate concentrations helps in the plant antioxidant system, thus benefiting crop growth and productivity. Considering the need for acclimatization of crops due to climate changes and the need for diversification of the energy matrix, this study aimed to assess the different concentrations and forms of application of selenium (sodium selenite and sodium selenate) suitable for the cultivation of sunflower plants through growth analysis (height, diameter and number of leaves) and relative chlorophyll contents. The experimental design was completely randomized, and consisted of 18 treatments with 5 replications each, with applications of selenium in the substrate or by leaf spraying. All 18 treatments were independently assessed. Analysis of variance was performed and means were independently compared using the Tukey test (P ≤ 0.05) in the AgroEstat program. Sunflower plants supplemented with 0.8 mg L⁻¹ of sodium selenite in the substrate presented values higher than those of the other treatments for plant height variable. For relative contents of chlorophyll, concentrations of 0.8 mg L⁻¹ and 1.7 mg L⁻¹ of selenate in the substrate and 3.3 mg L⁻¹ of selenite in the substrate stood out. For the other variables, treatments containing selenium were equivalent to the control. It was concluded that the treatments whose application was directly made on the substrate showed higher values than those that received foliar application.

Keywords: Biofuel, Sunflower, Selenium.

RESUMO
O selênio (Se) é um micronutriente não essencial às plantas, que quando presente em concentrações adequadas auxilia no sistema antioxidante vegetal, beneficiando assim o crescimento e a produtividade das culturas. Sabendo da necessidade de aclimatação dos cultivos às mudanças climáticas e da necessidade de diversificação da matriz energética, este estudo objetivou avaliar as diferentes concentrações e formas de aplicação de selênio (selenito e selenato de sódio) adequadas ao cultivo de plantas de girassol através de análises de crescimento (altura, diâmetro e número de folhas) e teores relativos de clorofila. O delineamento experimental foi inteiramente casualizado, composto por 18 tratamentos com 5 repetições cada com aplicações de selênio no substrato ou por pulverização foliar. Todos os 18 tratamentos foram avaliados de forma independente. Realizou-se análise de variância e as médias foram comparadas independentemente pelo teste de Tukey (P ≤ 0,05) no programa AgroEstat. As plantas de girassol suplementadas com 0,8 mg L⁻¹ de selenito de sódio no substrato obtiveram valores superiores aos demais tratamentos para a variável altura da planta. Para os teores relativos de clorofila as concentrações de 0,8 mg L⁻¹ e 1,7 mg L⁻¹ de selenato no substrato e 3,3 mg L⁻¹ de selenito no substrato se sobressaíram. Nas demais variáveis, os tratamentos contendo selênio se equipararam ao controle. Concluindo que os tratamentos cuja aplicação foi diretamente no substrato mostraram maiores valores do que os que receberam aplicação foliar.

Palavras-chave: Biocombustível, Girassol, Selênio.
1 INTRODUCTION

The search for diversification of the Brazilian energy matrix has contributed to advances in technologies for the production of biofuels, less dependence on foreign oil, more jobs and more sustainable production chains (QUEIROZ et al., 2021). Within this matrix, biodiesel has been produced using vegetable or animal fat. Among the vegetables used, soybean, castor bean, canola, palm oil and sunflower stand out (FERREIRA et al., 2018).

Sunflower (*Helianthus annus* L.) is an annual crop oilseed. It is used in cultivation between crops in a large part of the Brazilian territory, as it tolerates moderate climatic variations. Among its main uses are animal feed and biodiesel production (MOURA et al., 2017).

The northeast of Brazil is a region historically affected by irregular rains and degraded soils, and these phenomena have a significant influence on agriculture. Climate changes give the region a prospect of increased temperatures and irregular rainfall. This scenario reinforces the need of strategies for crops that improve their acclimatization, reinforcing the capacity of the plantations' defense system (CARLOS et al., 2019).

Selenium (Se) is a nonessential micronutrient to plants, however, when present in appropriate concentrations, helps in the plant antioxidant system, thus benefiting crop growth and productivity. The forms of selenium that can be bioaccumulated by vegetables are selenate and selenite. In tropical and subtropical regions, selenate tends to be more available to plants in the soil, because selenite has a strong interaction with the clay portion of the soils becoming more aggregated to the soil (OLIVEIRA et al., 2018).

The concentration of selenium in the soil can have beneficial or toxic characteristics for plants. High concentrations of this element cause the plant to substitute sulfur for Se in amino acids cysteine and methionine, thus causing stress to the crop (FERREIRA et al., 2020). Shahraki et al. (2022), when carrying out tests with selenium in plants of *Calendula officinalis* L., found improvements in the development of flowers and leaves of the species.

It is believed that selenium can act as a mitigator of abiotic stresses through its antioxidant properties. Considering the need for acclimatization of crops due to climate changes, and the need for diversification of the energy matrix, this study aimed to assess the different concentrations and forms of application of selenium (selenite and selenate) suitable for the cultivation of sunflower plants through growth analysis (height, diameter and number of leaves) and relative chlorophyll contents.
2 METHODOLOGY

The experiment was carried out in July 2021 under greenhouse conditions at Instituto Federal de Educação, Ciência e Tecnologia do Ceará (IFCE) located in the municipality of Maracanaú-CE, Brazil.

Selenium was applied as sodium selenate (Na₂SeO₄, molecular weight 188.94 g mol⁻¹) and sodium selenite (Na₂SeO₃, molecular weight 172.94 g mol⁻¹) in two application modes: 1) directly in the substrate on the day of sowing; 2) spraying on leaves 12 days after sowing (DAS). The selection of concentrations used was based on the studies by Boldrin et al. (2012) and Subramanyam et al. (2019). Sunflower seeds (Helianthus annus L.) of cultivar BRS 323, provided by EMBRAPA (Brazilian Agricultural Research Corporation), were used.

The seeds were sown in 5 L pots containing sand and earthworm humus in a 4:1 ratio (sand volume: humus volume) with or without selenium, as shown in Figure 1. Watering was carried out daily at 80% of field capacity per means of replacing evapotranspiration water.

The experimental design (Figure 1) was completely randomized, consisting of 18 treatments with 5 repetitions each with applications of selenium in the substrate or by leaf spraying. Applied to the substrate: 1) sand + humus; 2) 0.8 mg L⁻¹ of Na₂SeO₄ + sand + humus; 3) 1.7 mg L⁻¹ of Na₂SeO₄ + sand + humus; 4) 3.3 mg L⁻¹ of Na₂SeO₄ + sand + humus; 5) 6.7 mg L⁻¹ of Na₂SeO₄ + sand + humus; 6) 0.8 mg L⁻¹ of Na₂SeO₃ + sand + humus; 7) 1.7 mg L⁻¹ of Na₂SeO₃ + sand + humus; 8) 3.3 mg L⁻¹ of Na₂SeO₃ + sand + humus; 9) 6.7 mg L⁻¹ of Na₂SeO₃ + sand + humus; 10) sand + humus + 0.025% Tween 20 detergent; 11) 20 mg L⁻¹ Na₂SeO₄+ sand+ humus + 0.025% Tween 20 detergent; 12) 40 mg L⁻¹ Na₂SeO₄+ sand+ humus + 0.025% Tween 20 detergent; 13) 80 mg L⁻¹ Na₂SeO₄+ sand+ humus + 0.025% Tween 20 detergent; 14) 120 mg L⁻¹ Na₂SeO₄+ sand+ humus + 0.025% Tween 20 detergent; 15) 20 mg L⁻¹ Na₂SeO₃+ sand+ humus + 0.025% Tween 20 detergent; 16) 40 mg L⁻¹ Na₂SeO₃+ sand+ humus + 0.025% Tween 20 detergent; 17) 80 mg L⁻¹ Na₂SeO₃+ sand + humus + 0.025% Tween 20 detergent; 18) 120 mg L⁻¹ Na₂SeO₃+ sand + humus + 0.025% Tween 20 detergent.
Figure 1. Selenium application modalities and concentrations employed.

For treatments with foliar application of selenium, detergent Tween 20 at 0.025% was added to the aqueous solutions to reduce surface tension and facilitate penetration. Leaf spraying took place using a manual spray pump, at 6 am to avoid losses due to evapotranspiration. 15 mL of solution were applied per plant. Plants sprayed with distilled water containing 0.025% Tween 20 detergent were used as a control group.

Analyses of biometric variables (height, diameter and number of leaves) and relative chlorophyll contents were performed. Growth assessments were made at 19 days after sowing (DAS). The diameter was measured using a digital caliper. The height of the aerial part was measured using a graduated ruler from ground level to the apical bud. The number of leaves was obtained from counting directly on each plant. The relative chlorophyll contents were determined in the first fully expanded leaf, counting from the apex with the aid of a portable meter (model Minolta SPAD – 502).

All 18 treatments were independently assessed. Analysis of variance was performed and average were independently compared using Tukey’s test (P ≤ 0.05) in the AgroEstat program. The graphics were produced in Sigma Plot 11.0 program.

3 RESULTS AND DISCUSSION

Plants added with selenite in the substrate at 0.8 mg L⁻¹ presented values higher than those of the other treatments for height variable (Figure 2). Those treatments that received concentrations of 0.8 mg L⁻¹ of selenite in the substrate obtained an average value of 16 cm plant⁻¹, which represents an increase of 20% compared to the average of the treatments spray control, 20 mg L⁻¹, spray
selenate, 40 mg L\(^{-1}\), and spray selenite, 40 mg L\(^{-1}\). It is believed the difference between the concentrations comes from the concentration and form of application of selenium, since it implies efficiency or toxicity in plants. High doses of selenium are harmful, but in appropriate amounts they can act on the antioxidant system by decreasing reactive oxygen species (FERREIRA et al., 2020). Subramanyam et al. (2019) performed the analysis of antioxidant enzymes in rice plants subjected to salt stress added with doses of selenium. The authors concluded that the application of selenium mitigated the effects of salinity.

For stem diameter (Figure 3), the treatments with application to the substrate: control, 0.8 mg L\(^{-1}\) of selenate, 0.8 mg L\(^{-1}\) of selenite and with leaf spraying application, control and 40 mg L\(^{-1}\) of selenate, did not differ statistically from each other, with an average value of 3.37 mm plant\(^{-1}\). In the stem diameter parameter, values close were found by Brito et al. (2020), when cultivating sunflower supplemented with upland algae, with the average of the best treatment of 3.4 mm plant\(^{-1}\). Moreira et al. (2019), when cultivating sunflower with industrial residue of mineral supplement at 20 DAS, also found an average around 3 mm plant\(^{-1}\) in the most efficient treatments.
Figure 3. Stem diameter of *Helianthus annuus* L. at 19 DAS with application of selenite or sodium selenate at different concentrations through the substrate or by leaf spraying. The bars represent the mean values of five replicates. Values followed by distinct letters represent statistically significant differences between treatments, according to the Tukey test ($P \leq 0.05$).

The values referring to the number of leaves (Figure 4), for the most part, did not differ statistically from each other. However, the 0.8 mg L$^{-1}$ treatment of selenite in the substrate was superior to the treatment 6.7 mg L$^{-1}$ of selenite in the substrate, with 19% increase, presenting an average value of 5 leaves plant$^{-1}$. It was observed, through visual assessment of the leaves, that selenium did not cause phytotoxic visual effects to the plants. This positive effect coincides with what is indicated by Palencia et al. (2016), who tested selenium to cultivate strawberry, and while evaluating the variable number of leaves, the plants containing selenium stood out by 10% compared to those of the control treatment.

Figure 4. Leaf number of *Helianthus annuus* L. at 19 DAS with application of selenite or sodium selenate at different concentrations through the substrate or by leaf spraying. The bars represent the mean values of five replicates. Values followed by distinct letters represent statistically significant differences between treatments, according to the Tukey test ($P \leq 0.05$).
When analyzing the relative chlorophyll contents of (Figure 5), the concentrations added to the substrates - 0.8 mg L\(^{-1}\) and 1.7 mg L\(^{-1}\) of selenate and 3.3 mg L\(^{-1}\) of selenite - did not show statistical differences and were superior to those of the other groups, with average SPAD index value of 39.7. When such treatments are compared to those of the smallest group, with 1.7 mg L\(^{-1}\) of selenite added to the substrate, there is an increase of 37%. Astaneh et al. (2018), when it evaluated the relative chlorophyll contents also found higher values for the treatment incremented with selenate in the garlic crop. Brito et al. (2020) supplemented sunflower cultivars with uplifted algae and obtained, in their best treatment, the value of 35 (SPAD index) when evaluating the relative chlorophyll contents. Chlorophyll is an important pigment that acts on the chloroplast of plants, capturing light to carry out photosynthesis. The higher the index obtained through the relative chlorophyll contents, the greater the capacity of the sheet to absorb light (MAIA JÚNIOR et al., 2017).

Figure 5. Relative chlorophyll contents of *Helianthus annuus* L. at 19 DAS with application of selenite or sodium selenate at different concentrations through the substrate or by leaf spraying. The bars represent the mean values of five replicates. Values followed by distinct letters represent statistically significant differences between treatments, according to the Tukey test (P ≤ 0.05).

Appropriate doses and forms of application of selenium in plants can influence nitrogen absorption and promote the synthesis of proteins that provide greater plant growth (AL-KAZZAZ, 2018). Chlorophyll contents correlate with nitrogen concentrations, as from 50% to 70% of the nitrogen present in the leaves is contained in the chloroplast (ARGENTA et al., 2001).

It is believed that due to the controlled conditions of the experiment and the inexistence of biotic or abiotic stress, the positive effect of selenium was evident only in some treatments and in a few variables analyzed when compared to plants under control conditions (absence of selenium). Selenium can act in the defense mechanism of plants, specifically when plants are subjected to stress.
(BARBOSA et al. 2014; FERREIRA et al. 2020). Antioxidative enzymes are associated with an increase in reactive oxygen species due to biotic and abiotic stresses (NUNES et al., 2017).

4 CONCLUSION

Sunflower plants supplemented with 0.8 mg L\(^{-1}\) of sodium selenite in the substrate presented values higher than those of the other treatments for the plant height variable. For the relative contents of chlorophyll, the concentrations of 0.8mg L\(^{-1}\) and 1.7 mg L\(^{-1}\) of selenate in the substrate and 3.3 mg L\(^{-1}\) of selenite in the substrate stood out. For the other variables, the treatments containing selenium were equivalent to the control.

Thus, in general, treatments whose application was directly on the substrate showed higher values than those that received foliar application. Thus, further studies are needed to define the ideal concentration of selenium for sunflower plants. Furthermore, assessments under stress conditions and the effects on antioxidant enzymes are suggested.
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