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Bioestimulante à base de aminoácidos melhora a produtividade de rabanete (*Raphanus sativus* L.) sob estresse salino

Amino acid-based biostimulant improves the productivity of Radish (*Raphanus sativus* L.) under saline stress

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RESUMO - A cultura do rabanete é altamente sensível à salinidade. O uso de um bioestimulante à base de aminoácidos pode, portanto, ser um atenuador do efeito deletério da salinidade na cultura do rabanete. O objetivo foi avaliar a eficácia de um bioestimulante à base de aminoácidos e extrato de algas de água doce em rabanete (*Raphanus sativus* L.) cultivado sob condições de irrigação com água salina. O experimento foi conduzido em casa de vegetação. Os níveis salinos avaliados foram 0,15, 1,8 e 2,8 $dS.m^{-1}$ e foram aplicadas quatro doses de bioestimulante (0, 2, 4 e 6 mL/L). O delineamento estatístico foi inteiramente casualizado, em esquema fatorial 4x3 com 4 repetições. Foram realizadas análises biométricas e de biomassa, teores de sódio e potássio e sólidos solúveis. Observou-se que plantas submetidas a altos níveis de salinidade (2,8 dS m^{-1}) com aplicação de 6mL/L do bioestimulante apresentaram valores elevados em altura (12,2 cm), diâmetro do caule (5mm) e tubérculo (6mm), área foliar (50cm^2), massa fresca e seca da parte aérea (3,5 g) e sistema subterrâneo (5,8 g). A aplicação do bioestimulante 6 mL/L, na salinidade de 2,8 $dS.m^{-1}$, favoreceu o aumento de íons salinos no tubérculo das plantas. A aplicação do bioestimulante proporcionou maior teor de sólidos solúveis. Aplicações do bioestimulante em plantas com estresse salino apresentaram respostas benéficas no desempenho do rabanete. Portanto, a dose de 6 mL/L é recomendada para uma resposta satisfatória do desempenho do rabanete em condições salinas.

Palavras-chave: Estresse abiótico. Culturas agrícolas. Extrato de algas. Semi-árido.

ABSTRACT - The radish culture is highly sensitive to salinity. The use of an amino acid-based biostimulant can, therefore, be an attenuator of the deleterious effect of salinity on the radish crop. The aim was to evaluate the effectiveness of a biostimulant based on amino acids and freshwater algae extract on radish (*Raphanus sativus* L.) cultivated under irrigation conditions with saline water. The experiment was carried out in a greenhouse. The saline levels evaluated were 0.15, 1.8, and 2.8 $dS.m^{-1}$ and four doses of biostimulant were applied (0, 2, 4, and 6 mL/L). The statistical design was completely randomized in a 4x3 factorial scheme with 4 replications. Biometric and biomass analyses, sodium and potassium contents, and soluble solids were performed. Was observed that plants submitted to high salinity levels (2.8 dS m^{-1}) with application of 6mL/L of the biostimulant showed high values in height (12.2 cm), stem (5mm) and tuber (6mm) diameter, leaf area (50cm^2), fresh and dry mass of aerial part (3.5 g) and underground system (5.8 g). The application of the biostimulant 6 mL/L, at the salinity level of 2.8 dS.m^{-1}, favored the increase of saline ions in the tuber of the plants. The application of the biostimulant provided a higher content of soluble solids. Applications of the biostimulant in plants with saline stress showed beneficial responses in the performance of the radish. Therefore, a dose of 6 mL/L is recommended for a satisfactory response in the performance of the radish in saline conditions.

Keywords: Abiotic stress. Agricultural crops. Algae extract. Semiarid.

INTRODUCTION

The demand for plant-based foods is growing worldwide (CARVALHO et al., 2017). However, some regions have unfavorable conditions for cultivation, and it is necessary to intervene with proper management and thus allow efficient productivity of agricultural crops (SILVA et al., 2017). These unfavorable conditions prevail in semi-arid regions, where, in addition to climate impacts, many areas have soils with high salt content, most of which come from irrigation with saline water in arable environments and with inadequate management of inputs (SILVA et al. 2017; SOUZA et al., 2020).

Radish (*Raphanus sativus* L.) belongs to the Brassicaceae family and originates from the Mediterranean (OLIVEIRA et al., 2010). It is a short-cycle crop and therefore requires soil rich in nutrients so that it can develop efficiently (RODRIGUEZ et al., 2017). However, the production of radish in small towns is mostly through irrigation from surface sources, which mostly have water with a high salt content (OLIVEIRA et al., 2022). The use of these waters for radish irrigation has caused serious damage to producers since this crop is classified as moderately sensitive to salinity of 1.2 dS m^{-1} (OLIVEIRA et al., 2010; TAIZ et al., 2017). Irrigation of radish with saline water, together with low-efficiency drainage, negatively interferes with the growth and development of the crop (TAIZ et al., 2017). Thus, an alternative to mitigate the effect of stress on radish plants is through the application of a biostimulant based on amino acids and freshwater algae (SOUZA et al., 2020). And in this way, define improvements in technology and management of the crop that provide an increase in productivity and fruit quality.

Amino acids provide natural stimuli for biological activity, having a very rapid influence on the fixation of carbonic anhydride for photosynthesis while producing a regulatory and balanced endocellular activity. Thus, the balance of amino acids leads to greater efficiency in protein formation (CASTRO, 2014). Once well allocated, they cause gains such as greater efficiency of antioxidant metabolism and greater accumulation of nutrients, as well as direct and indirect effects of reducing abiotic and biotic stresses, especially when associated with seaweed extract (SCHAICH, 2020).

Algae are aquatic, photosynthetic organisms that remove nutrients from the water directly from the surface (AZEVEDO and NAUER 2012). The use of seaweed extracts in cultures has occurred since 1950. Seaweeds can promote vegetative growth, leverage plant productivity, improve the soil microbiota and help plants resist certain abiotic stresses (SINGH, 2014). Seaweed extracts contain all the necessary macro and micronutrients for plants and are rich in auxins and gibberellins (SINGH, 2014; RODRIGUES et al., 2018). In a study by

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Fernandes et al. (2022) with the rice crop, different dosages of freshwater algae extract were evaluated. In this study, the authors observed efficiency when using these extracts, where they noticed an increase in both the growth and the development of the culture in relation to its productivity. However, further studies are needed with other plant species and to evaluate the relationship of this compound with applied stresses and plant reproduction, such as salt stress (RODRIGUES et al., 2018).

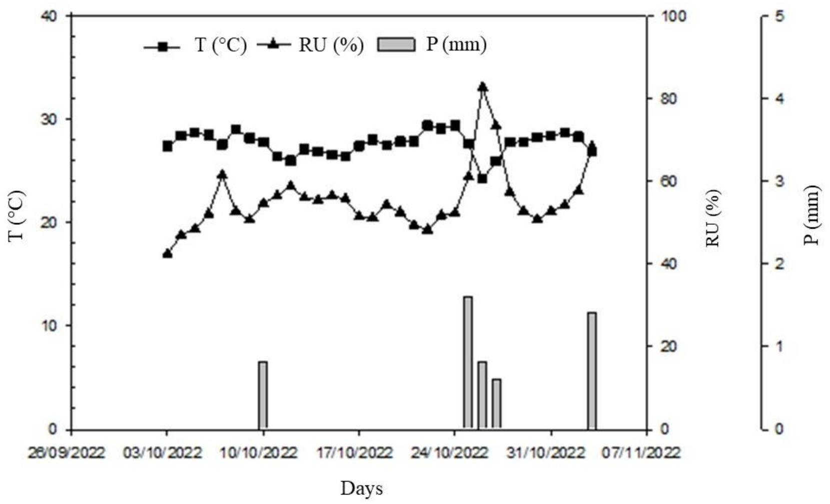
Now, the lack of tests and information about the behaviour of plants under salinity treated alternatively with amino acids and freshwater algae extract does not make it possible to unravel the processes that are influenced by the plants. Therefore, the objective was to evaluate the effectiveness of the biostimulant based on amino acids and freshwater algae extract in radish (*Raphanus sativus* L.) cultivated under irrigation conditions with saline water. Under the hypothesis that the application of the amino acid-based biostimulant provides good agronomic performance in the radish crop under conditions of high saline stress.

MATERIAL AND METHODS

Study area

The experiment was carried out in a greenhouse covered with shade (70%) at the Serra Talhada Academic Unit of the Federal Rural University of Pernambuco, located in the municipality of Serra Talhada, Pernambuco, Brazil (7◦59′ S, 38◦15' W at 431 m altitude). According to the Köppen climate classification, the region has a semi-arid climate, type BSwh (dry and hot with a rainy season in the summer). The average precipitation in the region is 642.1 mm year^{-1}, with an average air temperature of 24.8◦C, relative humidity of 62.5%, and an atmospheric demand greater than 1800 mm year^{-1} (SILVA and ALMEIDA, 2013). The characteristic soil used in the experiment is classified as a typical Haplic Cambisol Ta Eutrophic. The climatic variables observed during the experiment were recorded daily during the experimental period from the automatic station installed in the study area (Figure 1).

Figure 1. Climatic variables. Temperature (T), relative humidity (RH), and precipitation (P)



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Plant material and growing conditions

The genetic material was the cultivar of Radish (*Raphanus sativus* L.) "Gigante siculo" acquired in an agricultural house and sown manually and directly in plastic vases of 1L (11.5 cm in height, 14.5 cm in superior diameter, and 9, 5 cm lower) on October 3, 2022. The Biostimulant based on amino acids and freshwater algae extract, Ferticell-NutriplusN2.5 amino liquid ®, was purchased from Empress Agroplasma Brasil. Irrigation was performed daily at 8:00 a.m. and 5:00 p.m. based on the vessel capacity (VC), following the methodology of Casaroli and Van Lier (2008) using the drainage method over time. For this study, he obtained a value of 0.064 m^3/m^3 of VC. Thus, irrigation was standardized and carried out with the aid of a watering can in all experimental units, using the recommended water for each treatment.

Treatments

The treatments consisted of three levels of irrigation water salinity (0.15, 1.8, and 2.8 $dS.m^{-1}$) and four doses of biostimulant (0, 2, 4, and 6 mL/L). It has already been documented that the radish crop is sensitive to saline levels starting at 2 $dS.m^{-1}$, which is why saline levels close to this value were determined in this study. The water used for irrigation has an electrical conductivity of 0.15 $dS.m^{-1}$ and was increased by diluting sodium chloride (NaCl) according to each treatment. Therefore, to raise saline levels up to 1.8 and 2.8 $dS.m^{-1}$, 105.6 and 169.6 mg were diluted. L^{-1} respectively. The application of the biostimulant started 8 days after the emergence of the seedlings, together with the irrigation water corresponding to each treatment at intervals of 8 days until harvest, totaling three applications of the product.

Analyzes

At the end of the cycle, vegetative structures (leaf, tuber, and root) were carefully removed from the pots, washed, and taken to the laboratory. All plant material was weighed using a digital scale, and the fresh mass weight was obtained, then placed in a paper bag and placed in an oven with forced air circulation at 65 °C for 48 hours to obtain the dry weight, with the results expressed in g $seedling^{-1}$.

To determine the Na^+ and K^+ contents, use the methodology described by Malavolta et al. (1997). 50 mg of dry matter from the tuber and leaves were weighed, added to 10 mL of ultra-pure water, and taken to a water bath at 100°C/1h. The extracts obtained were filtered, and the readings were taken with a flame photometer (Model B462, Micronal).

Soluble solids content was obtained through the use of a benchtop portable digital refractometer (INSTRUTHERM, RTD-95, São Paulo, Brazil), using approximately 1 mL of the sample pulp after pressing for reading (Horwitz and LatimerJunior 2005). The readings obtained were expressed in degree Brix (°Brix).

Experimental design and statistical analysis

The experimental design was completely randomized in a 4x3 factorial scheme with 4 replications, having as factors four biostimulant doses (0, 2, 4, and 6 mL/L) and three irrigation water salinity levels, corresponding to 0, 1.8, and 2.8 $dS.m^{-1}$. The data was tabulated and then submitted to the Shapiro-Wilk normality test. and Levene's homoscedasticity. Data that did not

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show normality were applied to the positive asymmetry technique for data normalization, which can be treated with the following techniques: square root, cube root, and log. Then, analysis of variance was performed and, if there was significance, regression analysis and Tukey's test at 5% probability were performed. Statistical analyzes were performed using the RStudio software (R Core Team, 2018), and the graphs were made using the RStudio and SigmaPlot 14.0 software (Systat Software Inc., 2013).

RESULTS AND DISCUSSION

Growth Response

For plant height, there was a significant interaction between the analyzed factors ($P<0.01$). It is observed that there is a statistically significant difference between salinity levels in plants fed with 6 mL/L of the biostimulant ($P<0.01$), with the highest height values occurring in plants irrigated with saline water at 1.8 and 2.8 $dS.m^{-1}$ (Figure 2A). Therefore, our findings differ from the results found by Cunha et al. (2016), in which they did not observe the influence of the biostimulant on the sunflower and corn crops, respectively. This, therefore, should indicate that the effectiveness of the biostimulant may vary depending on the crop and management conditions.

There was a significant interaction between the analyzed factors for stem diameter ($P<0.01$). It is noted that the application of the biostimulant allowed constant values in the diameter of the stem of the plants, varying between 4 and 6 mm (Figure 2B). For the diameter of the tuber, the factors showed a significant interaction ($P<0.01$) and great variation in the data analyzed. However, it is noted that the dose of 6 mL/L provided an increase in the diameter and length of the tuber in irrigated plants with electrical conductivity of 2.8 dS/m^{-1} (Figure 2C). Bearing in mind that the radish crop is extremely sensitive to salinity (OLIVEIRA et al., 2010), these findings demonstrate the effectiveness of the biostimulant as an attenuator of the deleterious effects of saline stress on the radish crop.

Figure 2. Growth values of radish irrigated with different electrical conductivities and submitted to different doses of amino acid-based biostimulants A) plant height; B) stem

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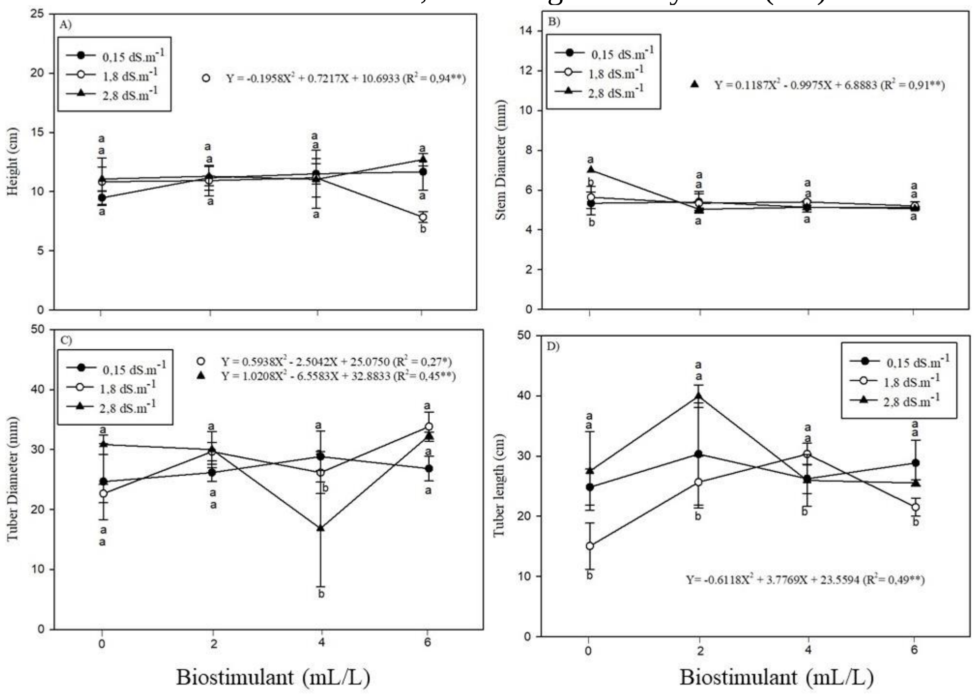
diameter; C) tuber diameter; and D) Tuber length. Different letters indicate statistical differences within each biostimulant level, according to Tukey's test (5%).



The best values observed for the number of leaves occurred at doses of 2 and 6 mL/L of the biostimulant (Figure 3A). considering the salinity conditions in which the plants were submitted, it is considered that the extract had a certain influence on maintaining the foliage of the plants even in saline conditions, thus reinforcing the efficiency of preventing moisture for the plant (TAIZ et al. 2017). On the other hand, leaf area was significantly influenced, mainly at doses of 2 and 4 mL/L of the biostimulant in plants irrigated with 1.8 and 2.8 $dS.m^{-1}$ (Figure 3B). It was observed that the leaf area of stressed plants increases with the increase in biostimulant doses 4mL (45 to 63 cm2) (Figure 3B). Possibly, the biostimulant improved soil moisture conditions and reduced the action of oxidative enzymes in the plant system, increasing the plant's work capacity and consequently the leaf area (TAIZ et al. 2017).

Figure 3. A) number of leaves and B) leaf area of radish plants irrigated with different electrical conductivities, submitted to different doses of amino acid-based biostimulant. Different letters

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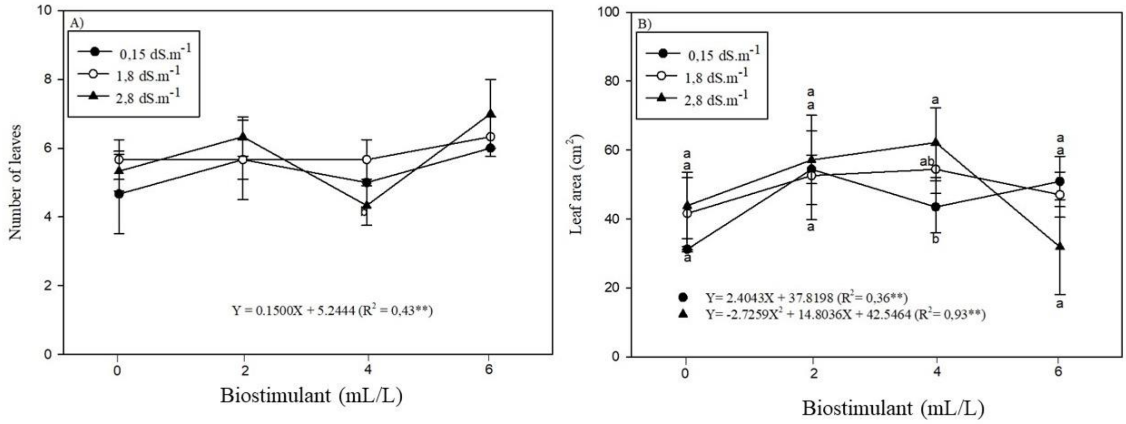
indicate statistical differences within the levels of the biostimulant, according to Tukey's test (5%).



For the fresh mass of the aerial part and underground system, the factors analyzed showed a significant interaction ($P<0.01$). It was observed that the doses of 6 mL/L of the biostimulant allowed a significant increase in plants submitted to irrigation of 1.8 and 2.8 dS.m^{-1}, both in the aerial part (3 to 3.5 g) and in the underground root (5.9 to 6.3 g) (Figure 4A and B). This result shows a positive effect of the biostimulant, which can be attributed to the auxin provided by the seaweed, which is responsible for increasing cell division, elongation, and absorption of superior proteins and nucleic acid, thus allowing greater weight of the aerial part (VENKATESAN et al., 2017; SOUZA NETA et al., 2018).

On the other hand, the dry mass data of the aerial part and the underground vegetal system did not show a significant difference or significant interaction between the factors (Figure 4C and D). According to Albuquerque et al. (2009), plant responses to the biostimulant may vary from nutrient absorption, translocation, and partition, which is influenced by the interaction between the different organic compounds present in the seaweed extract. The result of commercial extracts from seaweed related to plant growth is related to the action of phytohormones, which bring improvements or inhibitions to growth at low and high concentrations (KHAN et al., 2009), thus, the specific action of the seaweed extract will depend on the seaweed used and the handling and processing after harvesting (BHATTACHARYYA et al., 2015).

Figure 4. Growth values of radish irrigated with different electrical conductivities and submitted to different doses of amino acid-based biostimulant A) fresh mass of shoots; B) fresh mass of the underground system; C) dry mass of the aerial part and D) dry mass of the

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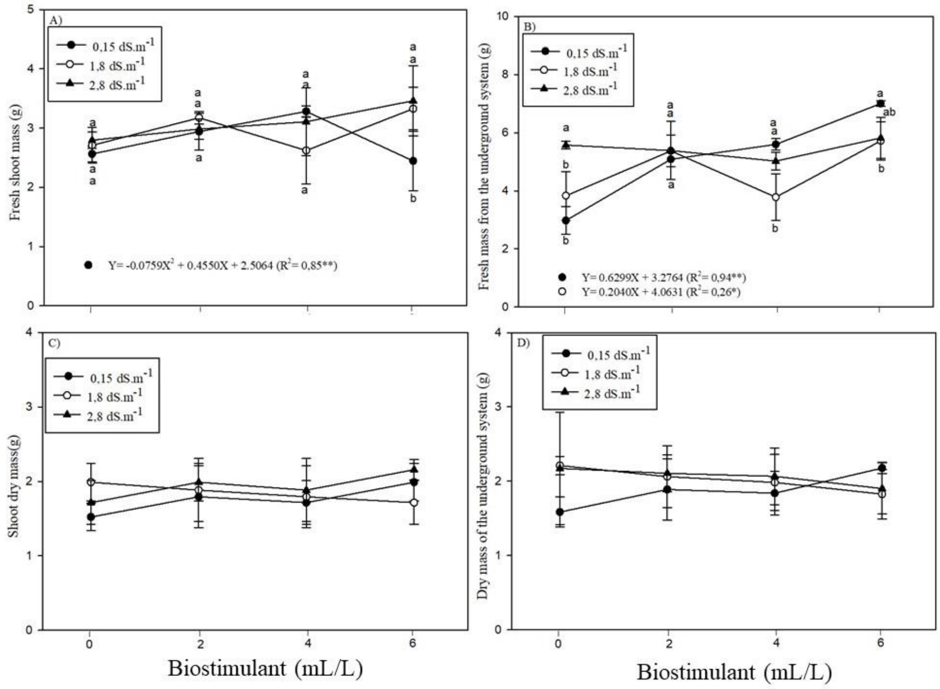
underground system. Different letters indicate statistical differences within each biostimulant level, according to Tukey's test (5%).



Sodium and potassium levels

It is observed that the K^+ content in the tuber was higher at the irrigation water salinity level of 2.8 $dS.m^{-1}$ with a biostimulant dose of 6 mL/L. On the other hand, the dose of 2 mL/L provided greater accumulation of K^+ for the salinity level of the irrigation water 1.8 $dS.m^{-1}$. These results are similar to the behavior observed for the leaves, where the highest levels (2,19, and 2.00 g. Kg^{-1}) of K^+ were observed for irrigation water salinity 1.8 $dS.m^{-1}$, while the lowest levels were observed in irrigation water salinity 2.8 dS. m^{-1}, for all applied doses (Figure 5B). It was possible to observe in our work that the amino acid doses positively favored the development of the tubers since it significantly reduced the accumulation of sodium in the tubers and leaves and increased the accumulation of potassium (OLIVEIRA et al., 2022).

It was observed that the contents of Na^+ in the tubercles were higher with irrigation with a saline level of 2.8 $dS.m^{-1}$, and the highest accumulation occurred with the dose of 6 mL/L of amino acid for this saline level and that the dose of 4mL/L of amino acid provided a smaller accumulation of Na^+. Our results also corroborate with the results found by Youssef, et al. (2022), where he visualized that the application of biostimulants significantly reduced the Na^+ concentration in lettuce, maintaining or even increasing the K^+ concentration. Therefore, the ratio of K^+ to Na^+ increased. Thus, it was verified that the treatment with a dose of 6 mL/L of the biostimulant mitigated this inhibition of the stress induced by NaCl since it favored the increase in potassium levels and reduced the sodium levels. This can be attributed to the presence of growth-promoting substances, total carbohydrates, proline, betaine glycine, and phenolic compounds present in the biostimulant that are capable of inducing alternative genetic traits for adaptation to stress (HERNANDEZ-HERRERA, et al., 2022).

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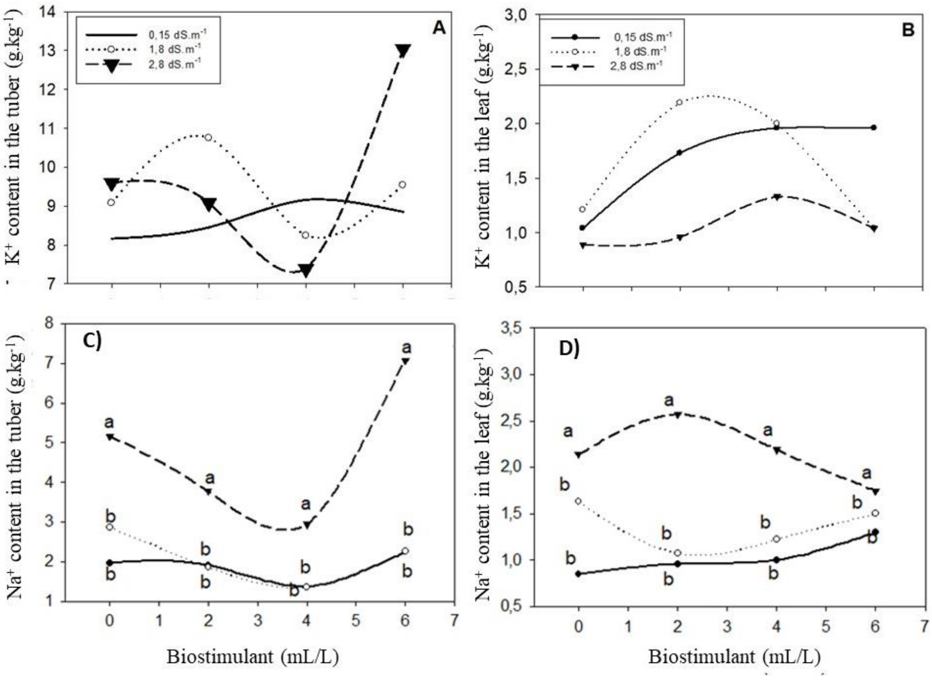
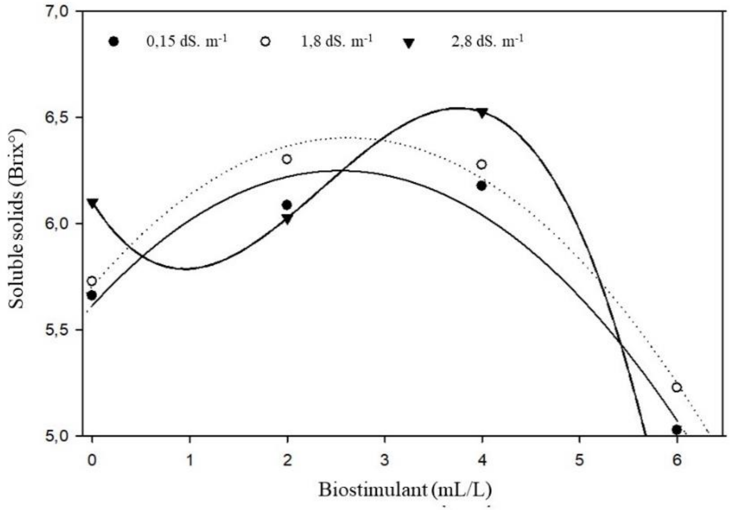
Figure 5. Sodium contents in A) tuber; B) leaves and potassium contents C) tuber; D) leaf of irrigated radish plants with different electrical conductivity submitted to different doses of biostimulant based on the amino acid. Different letters indicate statistical differences within each biostimulant level, according to Tukey's test (5%).



Soluble solids

It was observed that there was an increase in the soluble solids content for all salinity levels (0.15, 1.8, and 2.8 $dS.m^{-1}$) at the level of irrigation with saline water with the application of the dose of 4 mL/L with 6.27 °Brix. The amino acid doses provided a lower content of soluble solids with an application of 6 mL/L, with values of 5.22 °Brix. This result shows that amino acids have learned to increase nutrients for plants, allowing an increase in soluble solids (LIMA et al., 2014). The radish crop is classified as moderately sensitive to a salinity of 1.2 dS m^{-1} (OLIVEIRA et al., 2010), this characteristic may explain the possible cause of the decrease of soluble solids to levels of high salinity.

Figure 6. Content of soluble solids in °Brix in radish tubers subjected to different doses of amino acids and different levels of salinity. According to the F test, the means of this.



CONCLUSION

The use of a biostimulant based on amino acids from seaweed extracts in radish plants subjected to saline stress acts as an excellent alternative to mitigate the deleterious effects of salinity on the crop. It is recommended, therefore, to apply of 6mL/L of the biostimulant to the culture of radish in conditions of production with saline water.

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INTEREST CONFLICTS

The authors declare that the work has no conflict of interest.

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