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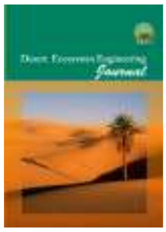

The Effect of the Growth-Promoting Bacteria Bacillus amyloliquefaciens and Bacillus halotolerans and the Application of Biosolids on the Physiological Characteristics of *Salvia hispanica* Under Saline Conditions

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Extended Abstract

Introduction

Salinity stress, resulting from the accumulation of salts in the soil, is one of the most common environmental stresses in arid regions, disrupting plant growth and productivity. Numerous efforts are underway to develop sustainable solutions to enhance plant resistance to environmental stresses. In this regard, the use of innovative approaches such as plant breeding, soil improvement, and biotechnology has become a major research priority at the global level. Plant growth-promoting rhizobacteria (PGPR) can neutralize the destructive effects of stress and enhance plant performance by utilizing adaptive mechanisms. These microorganisms improve plant growth and development indicators through several mechanisms, acting either directly or indirectly. The direct effects of these bacteria include increasing nutrient availability through the solubilization of poorly soluble compounds, the production of siderophores, the activity of ACC deaminase, the synthesis of plant hormones, and nitrogen fixation. Their indirect effects, operating through competitive or inhibitory mechanisms—such as competition with pathogens, production of antibiotics, fungicidal compounds, lytic enzymes, and hydrogen cyanide—reduce the harmful effects of pathogens and promote plant growth. Meanwhile, biosolids have been increasingly considered as a sustainable solution that contributes to plant growth and soil nutrient cycling through biological processes.

Materials and Methods

This research was conducted as a three-factorial factorial experiment within a completely randomized block

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design with three replications over a period of four months (from March 2024 to the end of June 2025) under field conditions in the lands of Ziaratgah village, Shahreza city, Isfahan province, with geographical coordinates of 584514 and 3531579 UTM. The experimental factors included: plant growth-promoting bacteria at four levels (no bacterial inoculation, inoculation with Bacillus halotolerans, inoculation with Bacillus amyloliquefaciens, and simultaneous inoculation with both bacteria); salinity at four levels (irrigation water with a salinity of 0.3 dS/m as control, and salinities of 4, 8, and 12 dS/m); and biosolids at three levels (no biosolids application, and biosolids application at 10 and 30 tons/ha). Soil salinity was monitored during the experimental period using an EC meter, with soil samples collected from a depth of 0 to 30 cm once per month. In each plot, after seed germination and growth to a height of 10 cm, 15 plants were maintained following secondary tillage to achieve uniform density. Blocks were spaced one meter apart, and within each block, 1 × 1 m plots were placed 1.5 m apart. In each plot, four rows of crops were designed with a spacing of 30 cm between rows and 25 cm within rows. Before the experiment, the physical and chemical properties of the soil sample (Table 1) and the biosolids sample (Table 2) were determined. Chia seeds used in this study were provided by Pakan Bazr Company of Isfahan. Biosolids were obtained from urban sewage from the Shahreza urban sewage treatment plant and were air-dried. The dried sludge was subsequently crushed and passed through a No. 20 sieve with openings of 850 μm before use. For plant growth-promoting bacteria, 100 mL of suspension with an approximate cell density of 3×10^8 CFU/mL of each bacterium, as well as a mixture of both, were prepared. Seed inoculation was performed at the beginning of cultivation in the field. For the control group, only the liquid culture medium without bacteria was used instead of the bacterial suspension. Seeds were placed in the suspension of each bacterium for two hours before cultivation. A period of four months elapsed from seed cultivation (beginning in March 2024) to the harvest of plant samples. Subsequently, the mineral elements of the leaves, as well as proline, soluble sugars, total phenols, chlorophyll, and total antioxidants, were measured. Data analysis was performed using SPSS 26 software, and graphs were generated in Excel. The normality of the data distribution was assessed using the Kolmogorov–Smirnov test. Differences between levels were evaluated through analysis of variance (ANOVA). Finally, mean comparisons were performed using Duncan's test at a significance level of 0.05.

Result

The results showed that salinity had a significant effect on reducing the absorption of elements. Specifically, the absorption of phosphorus (20.3%), potassium (32.6%), and calcium (31.7%), as well as chlorophyll content (14.1%), decreased. In contrast, salinity stress led to an increase in antioxidants (160.3%), total phenols (88.2%), and proline (112%). The simultaneous application of biosolids and bacteria increased the amounts of magnesium and calcium in the leaves by 10% and 11%, respectively. Under saline water irrigation conditions, bacterial inoculation increased the absorption of potassium (32.4%), calcium (37.6%), and phosphorus (60.1%), while decreasing sodium absorption by 32.5%. Bacterial inoculation, particularly the combination of both bacteria, increased soluble sugars (39.3%) and proline (137.7%), while decreasing antioxidants (110.2%) and phenols (65.3%).

Discussion and Conclusion

This study demonstrated that salinity represents a major challenge for plants by disrupting ionic homeostasis and inducing oxidative stress. However, inoculation with Bacillus halotolerans and Bacillus amyloliquefaciens—particularly in combination—improves the tolerance of chia (*Salvia hispanica*) to salinity levels of up to 8 dS/m through multiple mechanisms, including the modulation of element uptake, increased osmolyte accumulation, and enhancement of the antioxidant system. In contrast, the use of biosolids alone does not constitute an effective strategy; nevertheless, their potential to interact with microorganisms in providing mineral elements is significant. The findings of the present study emphasize the necessity of integrating microbiological approaches and organic resource management in saline land reclamation programs. Such integration can significantly reduce the damages caused by soil salinization, strengthen plant resistance mechanisms, and, through this, positively affect the physiological performance of chia plants, which will in turn yield favorable economic outcomes.

Keywords: Salty Land Reclamation, Biocontrol, Proline, Chlorophyll.