Plant growth-promoting rhizobacteria improved growth and physio-biochemical properties of geranium (*Pelargonium graveolens* L.) under salinity stress

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Abstract

The use of plant growth-promoting bacteria (PGPRs) to modulate salinity is of utmost importance for enhancing plant growth and adaptation in saline environments. This study aimed to investigate the effects of *Azotobacter chroococcum* and *Pseudomonas putida* on the growth and biochemical characteristics of rose-scented geranium under salinity stress. The experiment was conducted using a factorial design with four levels of PGPR treatment (control, *Azotobacter*, *Pseudomonas*, and *Azotobacter* + *Pseudomonas*) and three levels of salt at 0, 60, and 120 mM NaCl. The results indicate that salinity stress resulted in a decrease in plant yield, with the high est reduction observed at 120 mM salinity. This led to reductions in plant weight (31%), root weight (37%), total chlorophyll (33%), relative water content (RWC, 19%), essential oil yield (25%), as well as increases in malondialdehyde (MDA, 45%), catalase activity (179%), and superoxide dismutase activity (100%). However, the inoculation of geranium plants with PGPRs, particularly the simultaneous application of *Azotobacter* and *Pseudomonas*, resulted in stress mitigation. This was evident through an increase in biomass, photosynthetic rate, RWC, as well as a reduction in the activity of antioxidant enzymes and MDA in the leaves. Among the different treatments, the combined application of *Azotobacter* and *Pseudomonas*, along with a salinity stress level of 60 mM, resulted in the highest production of secondary metabolites, including total phenols, flavonoids, and essential oil content. In conclusion, the combined treatment of *Azotobacter* and *Pseudomonas* is recommended as an effective approach to mitigate salinity stress and increase plant yield in rose-scented geranium.

Keywords: *Azotobacter, Pseudomonas*, Growth regulation, Salt stress, Secondary metabolites

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Introduction

Salinity stress negatively affects plant physiological processes such as photosynthesis and antioxidant systems. High salt concentrations disrupt photosynthetic efficiency, reducing carbon

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dioxide availability and energy production. Salinity stress also induces oxidative stress by producing reactive oxygen species (ROS) at a rate that exceeds the plant's antioxidant defense mechanisms (Sapre et al., 2022). This imbalance leads to damage to cellular structures and impairment of plant development. However, plants have adaptive mechanisms to restore photosynthetic activity and enhance antioxidant defenses. Understanding these effects is important for developing strategies to improve plant tolerance to salinity stress (Nawaz et al., 2020a). In recent years, researchers and farmers alike have shown an increasing interest in exploring alternative approaches to alleviate the negative impacts of salinity stress on plants. In recent times, there has been a growing enthusiasm among researchers and farmers to explore effective and environmentally friendly methods to mitigate the adverse effects of salinity stress on plants (Neshat et al., 2022; Sapre et al., 2022).

One promising strategy is the utilization of plant growth-promoting rhizobacteria (PGPR). These beneficial bacteria colonize the rhizosphere, the region surrounding the root system, and form a mutually beneficial relationship with plants. PGPR have been found to enhance plant growth and development through various mechanisms, including facilitating nutrient uptake, boosting stress tolerance, and promoting hormonal balance (Diagne et al., 2020). Azobacter and Pseudomonas are two types of bacteria that have shown potential in mitigating the negative impacts of salinity stress on plants. Azobacter is a nitrogen-fixing bacteria that can form a symbiotic relationship with certain plants, providing them with a source of fixed nitrogen and promoting growth (Ullah et al., 2022). Pseudomonas bacteria produce plant growth-promoting substances and enzymes that improve nutrient availability, water uptake, and antioxidant activity in plants. Both bacteria offer a natural and sustainable solution to enhance crop productivity in saline environments (Abdel Latef et al., 2021).

Geranium (*Pelargonium graveolens* L.) is a slow-growing perennial plant from the Geraniaceae family. Most plants in this family are herbaceous and rarely acquire a woody nature. Geraniums come in various types and are flowering plants that hold economic importance (Mazeed et al., 2022). The hydraulic extract derived from the aromatic geranium leaf is abundant in flavonoid compounds and essential fatty acids (Jaradat et al., 2022). It also contains multiple vitamins, including vitamin A, E, and coumarin. This extract plays a significant role in the breeding efforts of geraniums, aiming to produce different varieties and hybrids with distinct shapes, colors, and more beneficial compounds (Mazeed et al., 2022). The extraction of essential oils (EOs) is primarily performed using the leaves and aerial parts of the plant (Bergman et al., 2020).

The positive effects of PGPRs on mitigating salinity stress have been reported in various plants such as canola (Abdel Latef et al., 2021; Ghassemi-Golezani and Abdoli, 2023), *Casuarina obesa* (Diagne et al., 2020), and cherry tomato (El-Beltagi et al., 2022). However, limited information is available regarding the effect of co-applied *Azotobacter* and *Pseudomonas* species on modulating salinity in geranium. This research aimed to address this knowledge gap by exploring the potential of PGPR in enhancing geranium's tolerance to salinity stress by investigating physiological and biochemical attributes in geranium plants. The findings will provide valuable insights into the mechanisms by which PGPR promotes plant growth and physiological adaptations, contributing to the development of sustainable solutions for managing salinity stress in geranium and potentially other ornamental and medicinal species.

Materials and Methods

Plant Materials and Experimental Treatments

Cuttings of the geranium plant were initially grown in a culture medium containing sand. After one month, when rooting and leafing (4 leaves) were observed, they were transplanted into plastic pots filled with agricultural soil with an EC of 1.1 dS m^{-1} and a pH of 7.1. The experimental design was conducted in a factorial arrangement based on a randomized complete block design (RCBD) with three levels of salinity and four levels of PGPRs in three replications. Four levels of PGPRs were applied to the plant roots by soaking: control (no

bacteria), *Azotobacter*, *Pseudomonas*, and a combination of *Azotobacter* and *Pseudomonas*. *Azotobacter chroococcum* and *Pseudomonas putida* were used in a soluble form, isolated, and purified by the Soil Biological Research Department of the Soil and Water Research Institute, Karaj, Iran. The inoculum population used was approximately 10^8 colony-forming units (CFU). One month after transplantation, salt stress was induced by applying sodium chloride (NaCl) at three levels: 0 (control), 60, and 120 mM. Each pot received 200 mL of the NaCl solution. To prevent salt accumulation, the pots were rinsed with water (without salt) after every four irrigations with salt water. The stress period lasted for 40 days, and the plants were harvested in the middle of the flowering stage. The total plant growth period was 100 days, during which no fertilizers or pesticides were applied.

Plant Weight

The fresh weight of shoots and roots after harvesting was measured with a digital scale accurate to 0.01 g. The aerial parts were cut from the collar with scissors, and all the aboveground parts (stem, flower, and leaf) were measured. The roots were gently separated from the soil and weighed with a digital scale.

Total Chlorophyll (Chl)

The Chl content assay was conducted using the method developed by Arnon (1949). A 0.1 g leaf sample was ground in a mortar with 3 mL of 80% acetone and diluted to a final volume of 15 mL. The extract was clarified by centrifugation at 5000 × g for 10 min. The absorbance was measured using a spectrophotometer (Shimadzu UV-160) at wavelengths of 645 nm and 663 nm. Total Chl (mg g^{-1}) contents were calculated using the equation:

$$[(20.2 \times A645) + (8.02 \times A663)] \times V / 1000 \times W$$

where A represents the absorbance at the specified wavelengths, V is the final volume of 80% acetone in mL, and W is the weight of the fresh leaf sample in g.

Relative Leaf Water Content (RWC) Measurement

RWC was measured by weighing fully developed leaves, hydrating them in distilled water for 4-5 hours, drying them with filter paper, and reweighing. The leaves were then dried at 70 °C for 48 hours to obtain the dry weight. RWC was calculated using the equation:

$$RWC = (FW - DW) / (TW - DW) \times 100$$

where FW is the fresh weight, DW is the dry weight, and TW is the turgid weight (Ritchie et al., 1990).

Malondialdehyde (MDA) Concentration

MDA concentration was determined using the method of Heath and Packer (1968). A 0.5 g fresh leaf sample was ground with trichloroacetic acid (TCA), centrifuged, and the supernatant mixed with TBA. After boiling and cooling, absorbance was measured at 532 nm and 600 nm. The concentration was calculated in μmol g^{-1} FW using calibration standards.

Enzyme Assay

Enzyme assays for catalase (CAT) and superoxide dismutase (SOD) activities were performed using fresh samples homogenized in potassium phosphate buffer with EDTA-Na_2 and ascorbate. CAT activity was measured by the decline in H_2O_2 concentration, and SOD activity by the reduction of NBT in the presence of riboflavin, with absorbance readings taken at room temperature (Nasirzadeh et al., 2021).

Determination of Total Phenolic Content (TPC)

TPC was measured using Folin–Ciocalteu reagent and spectrophotometry at 725 nm, with results expressed as mg Gallic acid (GA) g^{-1} dry weight (Xu and Chang, 2007).

Determination of Total Flavonoid Content (TFC)

TFC was measured using the aluminum chloride colorimetric method, with absorbance at 415 nm

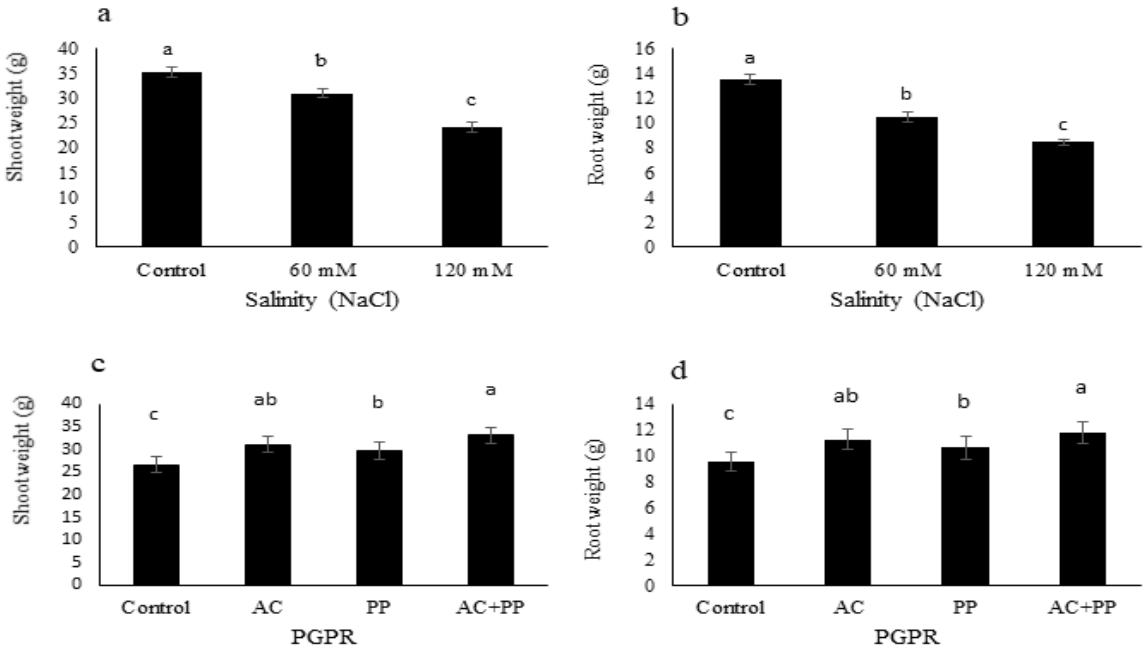

Fig. I. Shoot weight and root weight of geranium plants under salinity (a and b) and *Azotobacter chroococcum* (AC) and Pseudomonas putida (PP) inoculation (c and d). Different letters above the column show statistical significance (P≤0.05).

and results quantified using a quercetin calibration curve (Zhishen et al., 1999)n.

Essential Oil (EO) Content and Yield

EO content was determined by hydro-distillation of 100 g dried plant material using a Clevenger-type apparatus, with yield calculated from EO content and plant dry weight (Sefidkon et al., 2006).

Data Analysis

Data were analyzed using SAS (version 9.3, SAS Institute, Cary, NC) with mean comparisons made using the LSD test at a 5% probability level ($P \leq 0.05$).

Results

Plant Weight

The shoot weight and root weight of the plants decreased significantly with increasing levels of salinity stress. The lowest shoot weight and root weight were observed in the 120 mM salinity stress treatment. The shoot weight decreased by 12% and 31% in the 60 and 120 mM salinity stress treatments, respectively, compared to the control treatment (Fig. I a). The root weight decreased by 22% and 37% in the 60 and 120 mM salinity stress treatments, respectively, compared to the control treatment (Fig. I b). These results indicate that salinity stress negatively impacts plant growth, with higher levels of salinity stress resulting in greater reductions in shoot and root weight.

The results showed that the growth stimulants had a significant positive effect on both shoot and root weight. The fresh weight of aerial parts and roots increased significantly in the growth-stimulating treatments compared to the control treatment. There was no significant difference between the *Azotobacter* and *Pseudomonas* treatments in terms of fresh weight. The combination of *Azotobacter* and *Pseudomonas* treatments resulted in the highest fresh weight of both shoot and roots. The fresh weight of shoot and root in the combined *Azotobacter* and *Pseudomonas* increased by 17% and 24%, respectively, compared to the control treatment for shoot weight (Fig. I c), and by 18% and 23%, respectively, for roots (Fig. I d). These results suggest that the application of growth stimulants, particularly the combination of *Azotobacter* and

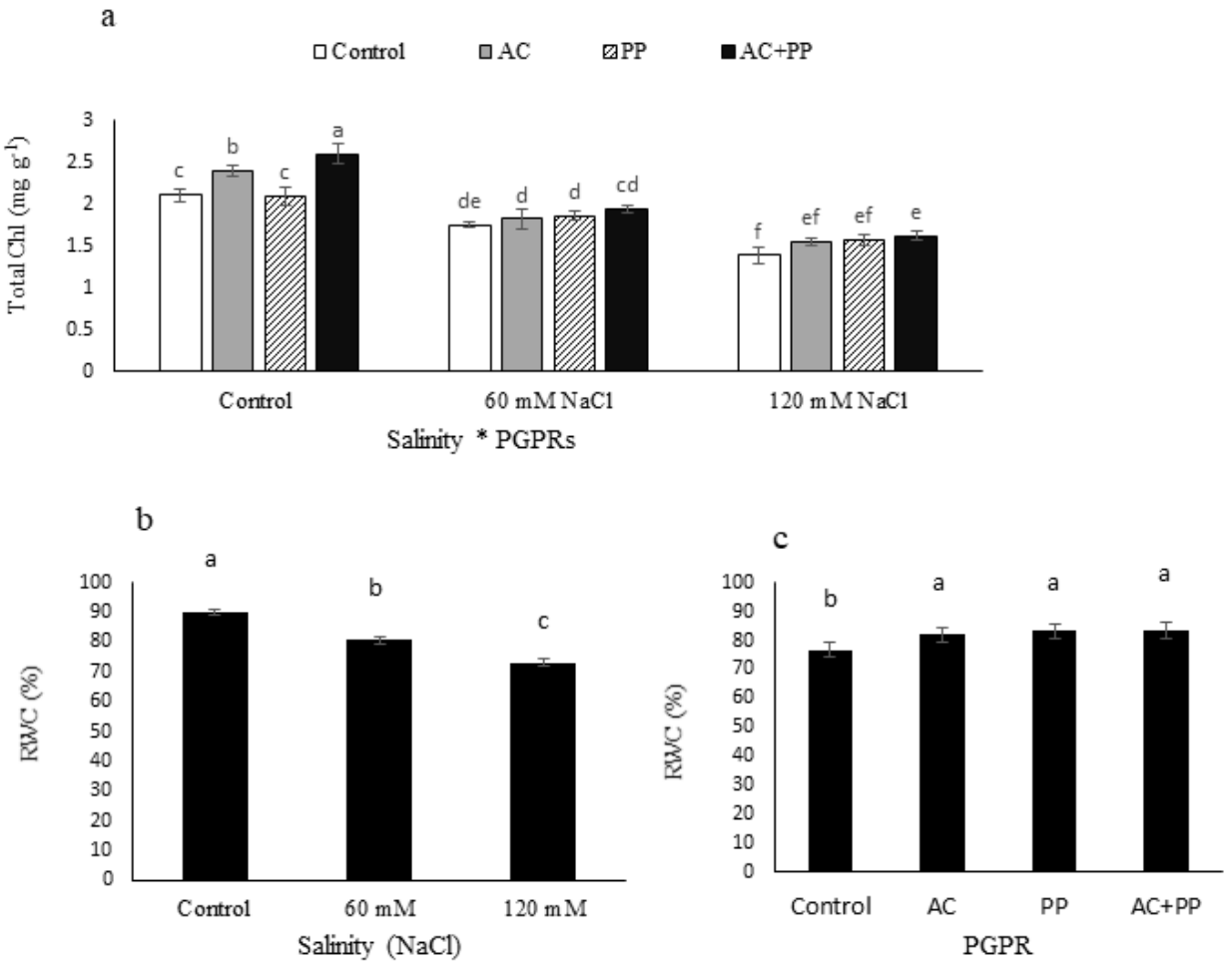

Fig. II. Total chlorophyll (Chl, a) and relative water content (RWC, b and c) of geranium plants under salinity and *Azotobacter chroococcum* (AC) and *Pseudomonas putida* (PP) inoculation. Different letters above the column show statistical significance (P≤0.05).

Pseudomonas, can enhance plant growth and increase shoot and root weight.

Chl and RWC

The results indicated that the mutual effect of salinity stress and growth stimulants had a significant impact on the total Chl content of the plants. The salinity stress treatments led to a decrease in total Chl compared to the control treatment. However, when the growth stimulants were applied in combination with salinity stress, the total Chl content improved compared to the control treatment without growth stimulants. The highest total Chl content was observed in the control treatment and the combination of *Azotobacter* and *Pseudomonas* in the absence of salt stress. In the 120 mM salt stress treatment, the combination of *Azotobacter* and *Pseudomonas* resulted in a 17% increase in total Chl compared to the control treatment without growth stimulants. These findings suggest that the application of growth stimulants can mitigate the negative effects of salinity stress on Chl content and help improve the plant's photosynthetic capacity (Fig. II a).

Salt stress led to a significant decrease in the relative water content (RWC) of the leaves. As the level of salinity stress increased, the RWC showed a significant decreasing trend, reaching its lowest value in the treatment of 120 mM salinity stress with a 19% decline relative to the control (Fig. II b). Additionally, the application of PGPRs caused a

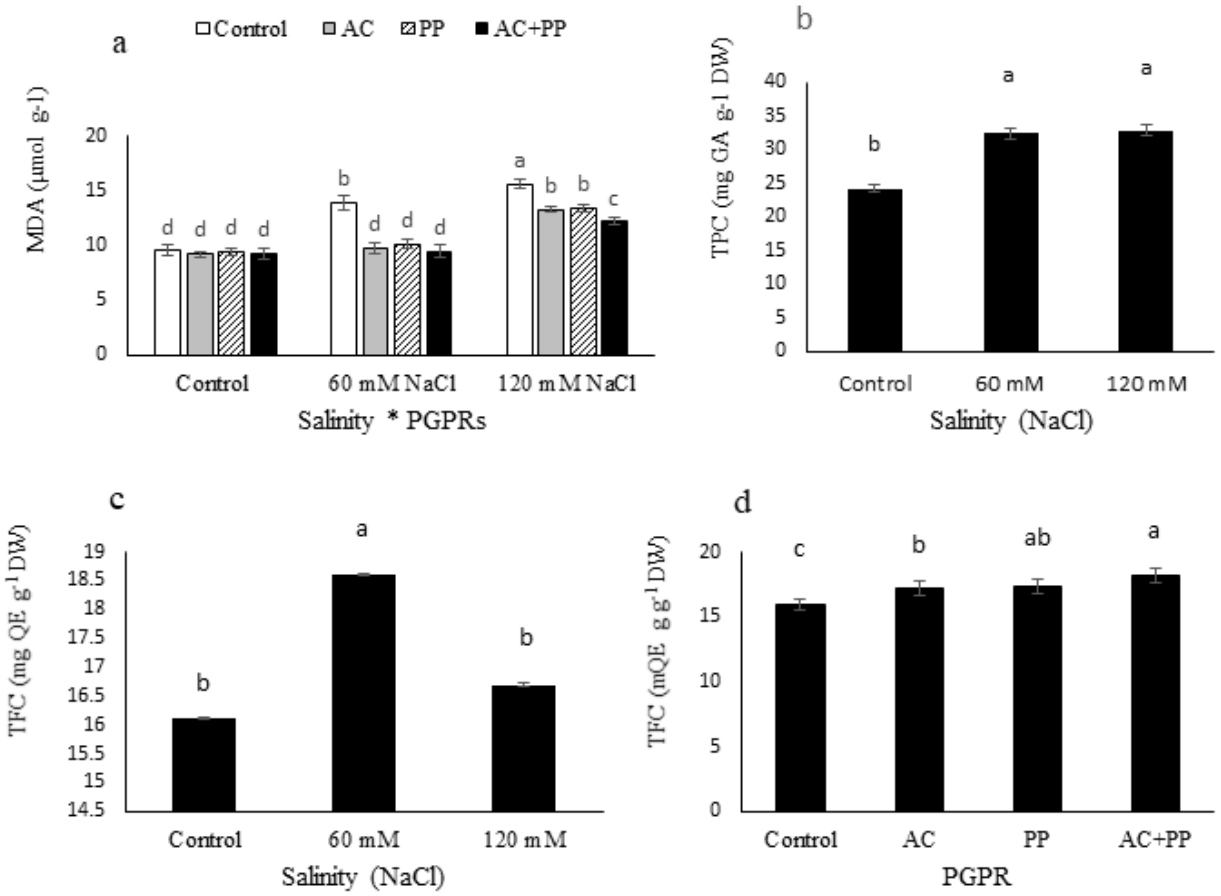

Fig. III. Malondialdehyde (MDA, a), total phenolic content (TPC, b), and total flavonoid content (c and d) of geranium plants under salinity and *Azotobacter chroococcum* (AC) and *Pseudomonas putida* (PP) inoculation. Different letters above the column show statistical significance (P≤0.05).

significant increase in RWC compared to the control treatment. However, there was no significant difference between different PGPR treatments in terms of RWC (Fig. II c). These findings suggest that salt stress negatively affects the water content of the leaves, but the application of PGPRs can help mitigate this effect and improve the water status of the plant.

MDA, TPC, and TFC

The interaction between salinity stress and PGPRs had a significant effect on MDA levels. Salinity stress treatments increased the amount of MDA, indicating lipid peroxidation and oxidative stress. However, when PGPRs were applied in combination with salinity stress, the levels of MDA decreased compared to the control treatment. The highest amount of malondialdehyde was observed in the 120 mM salinity stress treatment and the control treatment without PGPRs. In the 120 mM salt stress treatment, the combination treatment of *Azotobacter* and *Pseudomonas* resulted in a significant decrease of 22% in MDA compared to the control treatment without PGPRs (Fig. III a). These findings suggest that the application of growth stimulants can mitigate oxidative stress caused by salinity stress and reduce lipid peroxidation in plants.

The results suggest that salinity stress had a significant impact on the total phenolic content (TPC) and total flavonoid content (TFC) in plants. The 60 mM salinity treatment led to a significant increase in both TPC and TFC compared to the control. However, the 120 mM salinity treatment did not show a significant difference in the amount of TPC and TFC compared to the control treatment. The lowest amount of TPC was observed in the control treatment, while the highest amount was seen in the 60 mM salinity stress treatment. Furthermore, the 60 mM salinity

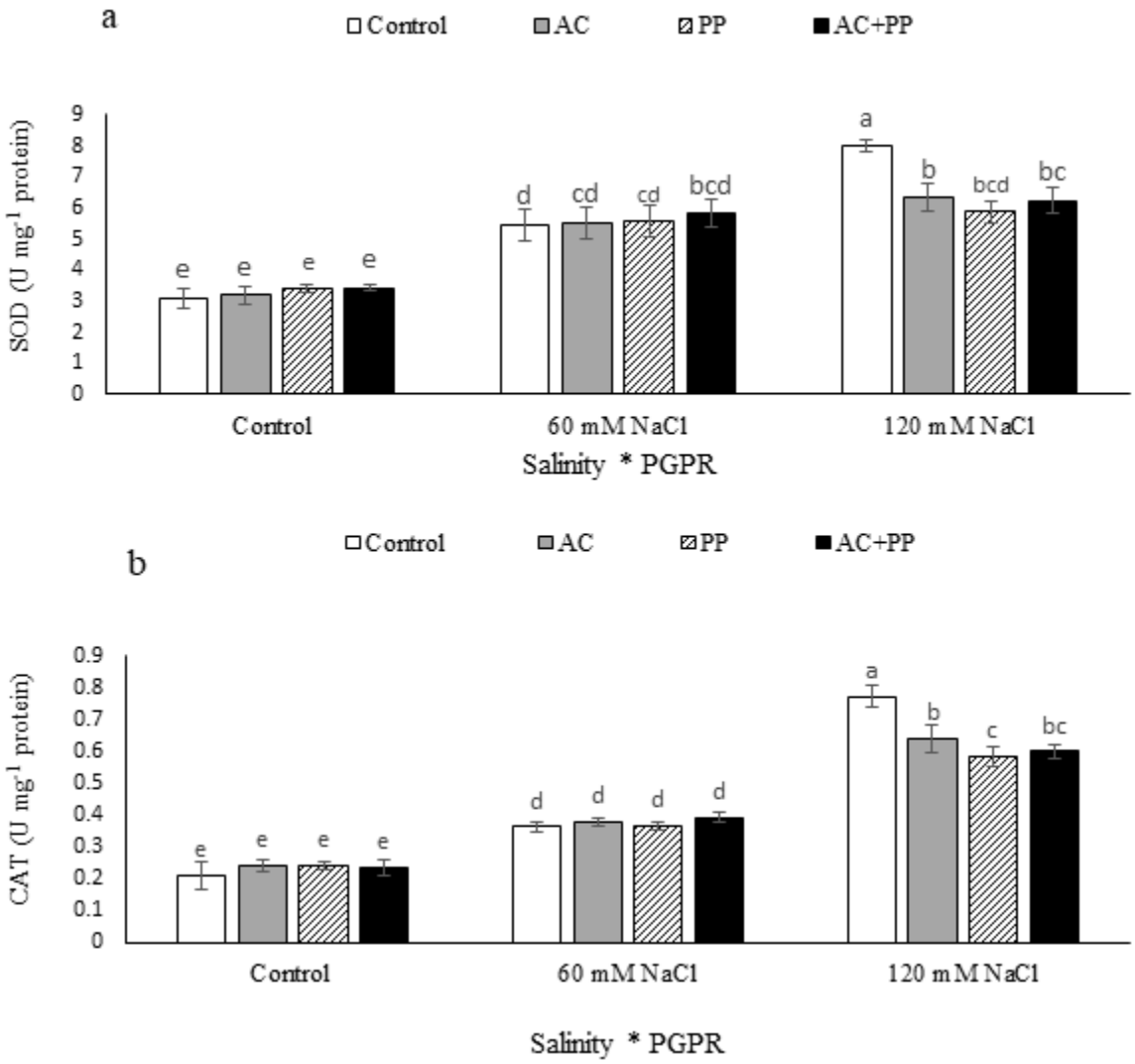

Fig. IV. Superoxide dismutase (SOD, a) and catalase (CAT, b) activity of geranium plants under salinity and *Azotobacter chroococcum* (AC) and *Pseudomonas putida* (PP) inoculation. Different letters above the column show statistical significance (P≤0.05).

stress treatment caused a 34% increase in TPC (Fig. III b) and a 15% increase in TFC compared to the control treatment (Fig. III c). Additionally, the application of PGPRs increased the TFC content compared to the control treatment. The highest amount of TFC was observed in the treatments of *Pseudomonas* and the combination of *Azotobacter* and *Pseudomonas*. The lowest amount of total flavonoids was observed in the control treatment. Specifically, the TFC content in the Pseudomonas treatment increased by 10% compared to the control treatment (Fig. III d). These findings suggest that salinity stress can enhance the production of phenols and flavonoids in plants, and the application of PGPRs further increases the phenolic compounds.

SOD and CAT Activity

Salinity at 120 mM and the control treatment without PGPRs showed the highest amount of SOD activity. Specifically, the 120 mM salinity stress treatment led to the highest activity of SOD with a 158% increase compared to the control. However, in the 120 mM salinity stress treatment, the application of *Azotobacter* resulted in a significant decrease of 21% in SOD enzyme activity compared to the treatment without PGPRs. This suggests that the use of *Azotobacter* may have a mitigating effect on SOD enzyme activity under salinity stress conditions (Fig. IV a). On the other hand, the average comparison results showed that the PGPRs decreased the activity of the CAT compared

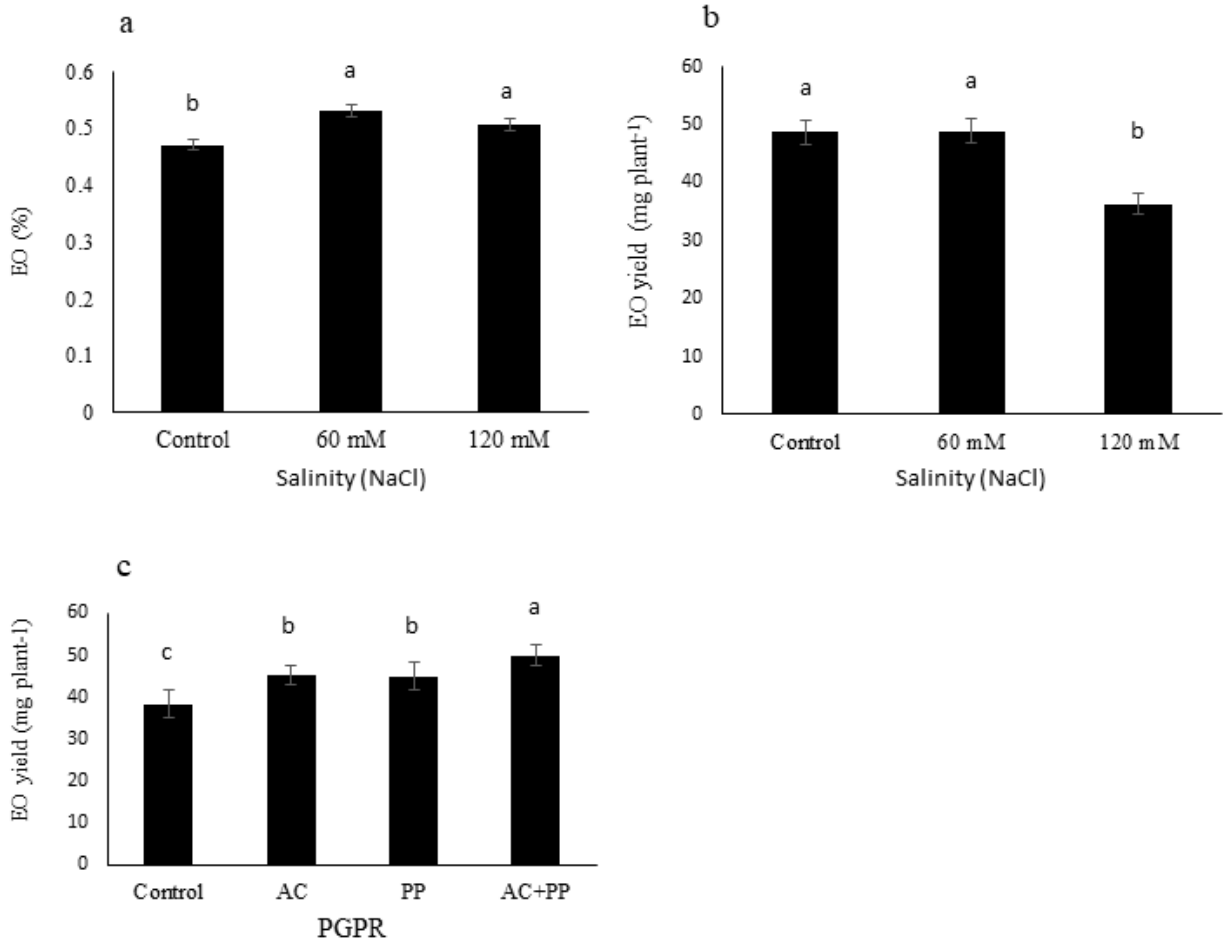

Fig. V. Essential oil (EO) content (a) and EO yield (b and c) of geranium plants under salinity and *Azotobacter chroococcum* (AC) and *Pseudomonas putida* (PP) inoculation. Different letters above the column show statistical significance (P≤0.05).

to the control. The highest activity of CAT was observed at salinity at 120 mM without PGPRs with a 268% enhancement relative to the control (Fig. IV b). Overall, salinity at 120 mM led to the highest activity of antioxidant enzymes. However, the application of *Azotobacter* under this salinity stress condition reduced the activity of antioxidant enzymes compared to the control.

EO Content and EO Yield

Salinity stress increased the essential oil (EO) content compared to the control treatment, with no significant difference between the salinity at 60 and 120 mM. The highest EO content was observed in both the 60 and 120 mM salinity stress treatments (Fig. V a). Conversely, the control treatment had the lowest EO content. Additionally, salinity at 120 mM stress significantly decreased the EO yield compared to the control treatment, while the 60 mM treatment did not show a significant difference. The lowest yield of EO was observed in the 120 mM salinity, with a 26% decrease compared to the control treatment. Furthermore, the PGPRs significantly increased the EO yield compared to the control treatment. There was no significant difference in the yield of essential oil between the *Azotobacter* and Pseudomonas treatments. The combination treatment of *Azotobacter* and Pseudomonas showed the highest EO yield. Specifically, the EO yield increased by 30% in the combination treatment of *Azotobacter* and *Pseudomonas* compared to the control treatment (Fig. V b,c).

Discussion

Salinity had an extensive effect on the weight loss of plant shoots and roots. Faced with salinity stress, plants react to the stressful situation and try to adapt to harsh environmental conditions.

One of the effects of salinity stress on the weight loss of aerial parts of plants is a significant reduction in the volume of water in the aerial parts. This causes the weight of the aerial parts of the plants to decrease (Nawaz et al., 2020a). Under salinity stress conditions, the rhizosphere system of the plant is also affected. In an effort to obtain more water and minerals, plants produce additional roots in selected tissues, such as exposed roots. This action causes the roots to grow more, resulting in an increase in root weight. Increasing the concentration of salt in the soil increases the external osmotic pressure and decreases the plant's capacity to absorb water. This causes the amount of water the plant can receive to decrease, leading to reduced plant vigor. Salt stress can disrupt the process of water secretion from leaves and other parts of the plant. This can lead to the accumulation of salts in plant tissues, further reducing plant vigor. Additionally, salinity can lead to disturbances in the activity of enzymes and metabolic processes of the plant (Shalaby and Ramadan, 2024). These disturbances cause a decrease in water secretion and an increase in evaporation from the surface of the leaves, which consequently affects the weight of the plant. In general, salinity stress causes a decrease in plant weight. However, its actual effect depends on factors such as plant type, plant age, salt concentration, duration of salinity stress, and environmental conditions.

Azotobacter and *Pseudomonas* are two genera of bacteria that are commonly known as beneficial plant bacteria. These bacteria can have a positive effect on the growth and weight of aerial parts of plants under salt stress conditions. These beneficial bacteria can help plants reduce the concentration of solutes in the soil through a cooperative relationship between the plant and the bacteria. This leads to an increase in the absorption of water and salts from the soil and, as a result, an increase in the growth and weight of the aerial parts of the plant. Beneficial bacteria can regulate the production of plant hormones and help regulate plant growth and development. This hormonal regulation can help increase the growth and weight of aerial parts of plants under salt stress conditions. *Azotobacter* and *Pseudomonas* can produce antioxidants that provide the plant with more resistance against oxidative stress, one of the effects of salinity stress (Minuț et al., 2022). Khodadadi et al. (2020) pointed out the positive role of *Azotobacter* in increasing the fresh and dry weight of barley plants under salinity stress conditions, which aligns with the results of Azar's research. Also, Abdel Latef et al. (2021)stated that plant inoculation with *Azotobacter* helped adjust to salinity stress, resulting in a significant increase in plant weight.

The decrease in the amount of photosynthetic pigments under salinity stress can mainly be due to the destruction of the chloroplast structure and photosynthetic apparatus, photooxidation of chlorophylls, their reaction with oxygen radicals, destruction of the precursors of chlorophyll synthesis, and inhibition of chlorophyll biosynthesis. New changes and activation of Chl-decomposing enzymes, including chlorophyllase, and hormonal disorders also play a role. Leaf Chl content is considered an important factor in determining leaf photosynthetic capacity. Reducing chlorophyll content as a non-porous factor can lead to a decrease in leaf photosynthetic capacity (Cui et al., 2022). In addition, salinity stress interferes with the absorption of essential elements such as iron and magnesium, which are essential for Chl synthesis (Rashmi et al., 2023). Salinity stress causes premature aging of leaves, chloroplast breakage, and reduction of chlorophyll. The reduction of chlorophyll leads to decreased photosynthesis, and plants that maintain more chlorophyll during stress have higher photosynthesis efficiency and are more resistant to stress (Feizi et al., 2021). Some growth regulators, such as abscisic acid and ethylene, whose levels increase under stress conditions, stimulate the activity of this enzyme. Also, the decrease in greenness may be due to changes in nitrogen metabolism in relation to the production of proline amino acid compounds, which are produced under stress conditions for osmotic regulation (Rossi et al., 2020). *Azotobacter* and *Pseudomonas*, as useful plant bacteria, can have a positive effect on leaf chlorophyll levels under salt stress conditions. Beneficial bacteria can help increase chlorophyll production by regulating the activity of enzymes

related to Chl activity (Abdel Latef et al., 2021). These enzymes play a role in the processes of Chl secretion and biochemical reactions related to the production of Chl and other plant pigments. Salinity stress usually increases oxidative stress in plants, which can lead to a decrease in leaf chlorophyll levels. Beneficial bacteria can help protect the plant's antioxidant system against oxidative stress by producing antioxidants and enzymes (Yaghoubian et al., 2021). The important role of *Azotobacter* in increasing nitrogen and other elements needed by the plant under stress conditions to boost Chl production can be highlighted. *Azotobacter* and *Pseudomonas* can help produce plant hormones that may assist in better absorption and use of nutrients, thus leading to an increase in leaf Chl levels (Abdel Latef et al., 2021). Aslani et al. (2024)reported an increase in the Chl content of *Salvia* under salt stress conditions with the use of *Pseudomonas*.

The relative water content of plants reflects their water status and is determined by the relationship between water supply to the leaf tissue and transpiration rate (Javardi et al., 2023). Water is essential for all metabolic processes in plant cells, and the ability of plants to recover from stress and yield depends on their relative water content. Decreased water levels result in reduced turgor pressure, leading to cell damage, wilting, and hindered plant growth. Conversely, maintaining a relatively high water content helps plants counteract reactive oxygen species and osmotic stress caused by drought, potentially leading to higher yields (Nawaz et al., 2020b). In response to salinity stress, plants reduce stomatal conductivity through a decrease in relative water content. Osmotic regulation improves drought tolerance by enabling cell enlargement, promoting plant growth, and partially opening stomata to maintain CO_2 fixation during severe water deficit. *Wheat* plants accumulate various organic and inorganic solutes in their cytosol to reduce osmotic pressure and maintain cell turgor. Salinity negatively affects photosynthesis by altering the internal structure of chloroplasts, mitochondria, and the levels of chlorophyll and minerals. Pagán et al. (2022) have reported a significant decrease in relative water content during salinity stress treatment. *Pseudomonas* and *Azotobacter* are effective in increasing the relative water content of leaves due to their ability to improve root system function, enhance water absorption by the leaves, and enhance leaf structure and function, particularly under stress conditions. They also improve stomatal function and reduce water evaporation, leading to higher relative water content (Nehela et al., 2021). Additionally, these bacteria can increase soil water levels through activities such as the hydrolysis and transformation of organic matter, further contributing to an increase in the relative water content of leaves. Bacteria present in the rhizosphere of plant roots can create a more favorable moisture environment by secreting enzymes and establishing positive interactions with the plant, resulting in a higher relative water content in leaves (Yaghoubian et al., 2021). Similarly, Nehela et al. (2021)demonstrated that PGPRs, along with biochar, mitigate salinity stress by increasing the RWC.

Salinity stress can increase the production of reactive oxygen species (ROS) and non-oxygenated free radicals (NROS), leading to lipid oxidation and the formation of MDA. This process damages plant cell membranes and allows for further penetration of oxygen and heavy metals, resulting in increased ROS production and lipid oxidation ((Hasanuzzaman et al., 2021). Additionally, salt stress can decrease the activity of antioxidants responsible for inhibiting and neutralizing ROS, further exacerbating oxidative damage. Enzymes such as superoxide dismutase, peroxidase, and catalase, which play a role in neutralizing ROS, may also have decreased activity under salt stress conditions, leading to increased MDA production (Hasanuzzaman et al., 2020). *Azotobacter* and *Pseudomonas* can positively impact the reduction of MDA, which serves as an indicator of plant performance and response to oxidative stress. These bacteria can secrete antioxidant enzymes like superoxide dismutase, peroxidase, and ascorbate peroxidase, contributing to a balance between free radical production and destruction in plants. As a result, oxidative damage can be reduced through the elimination of free radicals and a decrease in MDA production (Abdel Latef et al., 2021). Furthermore, bacteria can improve the physiological and morphological characteristics of plants, leading to a reduction in oxidative stress and subsequently a

decrease in MDA production (Yaghoubian et al., 2021).

Moderate salinity and PGPRs led to increased TPC and TFC. PGPRs can have positive effects on increasing the TPC and TFC in plant leaves. The first point is that plant growth-stimulating bacteria can increase the production of certain phenols by increasing the absorption of minerals by plant roots. Nitrogen, phosphorus, and calcium are the materials required for the synthesis of phenols, and these bacteria can facilitate the improvement of the absorption of these elements and thus increase the production of TPC and TFC in the plant. Secondly, *Azotobacter* and *Pseudomonas* can directly stimulate the biochemical pathways related to the synthesis of phenols and flavonoids. These bacteria can affect the production of phenylalanine ammonia lyase (PAL) enzymes, which play an essential role in the synthesis of phenols (Darakeh et al., 2021). Also, these bacteria can improve the performance of the antioxidant system by creating positive effects on the antioxidant activity of the plant and thus increase the amount of phenols in the plant. Antioxidant enzymes can have great activity in reducing oxidative stress and preventing plant damage. In general, PGPRs can have positive effects on increasing the phenolic content in plant leaves by stimulating the absorption of minerals, stimulating the synthesis pathway of phenols, and improving the function of the plant's antioxidant system (Khan et al., 2023). In a similar study, an increase in TPC and TFC of black cumin was reported by Darakeh et al. (2021).

Salt stress showed to increase the activity of the antioxidant enzymes. Catalase is responsible for breaking down hydrogen peroxide (H2O2) into water and oxygen. Salt stress can lead to an increased production of H_2O_2 in cells, and in response, the activity of catalase increases to mitigate the risks associated with elevated H2O2 levels (Hasanuzzaman et al., 2020). Similarly, salt stress can also boost the activity of the superoxide dismutase enzyme, which aids in the decomposition of the superoxide free radical ($O_2^{\bullet-}$) into water and oxygen. Studies indicate that salt stress can induce the production of superoxide in cells, prompting an increase in the activity of superoxide dismutase to neutralize this free radical and prevent oxidative stress (Hasanuzzaman et al., 2020). Several biochemical pathways contribute to the activation of catalase and superoxide dismutase under salinity stress. These pathways include the direct influence of salts on enzyme activity, the regulation of minor elements and their impact on genes associated with enzyme activity, and the physiological effects of salinity on signaling systems related to sulfur oxide and plant adaptation to limited water supply. These pathways play critical roles in controlling catalase and superoxide dismutase activity in response to salt stress, helping to maintain oxidant balance and manage oxidative stress by reducing reactive oxygen species (Alharbi et al., 2022). Plant growth-stimulating bacteria can have a significant impact on the activity of catalase and superoxide dismutase enzymes through biochemical pathways. These bacteria can enhance catalase activity by preventing the accumulation of substances like hydrogen peroxide and promoting the production of plant growth hormones and the absorption of mineral elements. Furthermore, plant growth-stimulating bacteria can increase superoxide dismutase activity in plants by regulating the absorption of mineral elements, producing cytokinins, and, in some cases, directly transferring the enzyme to the plants. These mechanisms help to boost the activity of superoxide dismutase, thereby preventing oxidative stress (Neshat et al., 2022).

Geranium EO plays an important role in cosmetics and pharmaceuticals. Also, the role of EO in stress conditions is to protect the plant. The increase in EO content of different plants is influenced by external stimuli caused by the change in the size and number of EO secreting glands per unit area, which causes a change in the amount of essential oil (del Rosario Cappellari et al., 2019). Omer et al. (2022)reported an increase in the percentage of essential oil under saline conditions in lemongrass plants under conditions of moderate salinity stress with the use of growth-promoting bacteria in flax, which confirms the results of the present research. Plant growth-stimulating bacteria may have a positive effect on increasing essential oil production in plants. Plant growth-stimulating bacteria can change the regulation of plant growth

hormones by changing the structure and physiology of the plant. These changes can stimulate the secretory glands of plants and increase the production of EO. *Azotobacter* and *Pseudomonas* can increase the activity of secretory glands by regulating the absorption and distribution of mineral elements in the plant (Khodadadi et al., 2020). Some bacteria are able to supply the required elements to plants, and these mineral elements may affect the activity of essential oil secretory glands (Khodadadi et al., 2020). In a similar study, Mirzaei et al. (2020)reported an increase in the percentage of lemongrass EO with the use of *Azotobacter* and drought stress conditions. As in the present study, it has been reported that the EO content of *coriander* leaves increased under moderate cadmium stress; on the other hand, severe cadmium stress caused a significant decrease in essential oil yield (Ahmad et al., 2018), which could be due to significant weight loss. In a similar study on *cilantro*, a decrease in EO yield was reported under extreme salinity stress (more than 100 mM), which is in line with the results of the present study (Hazrati et al., 2022). In addition, Hashemi et al. (2022) showed that *Azotobacter* caused a significant increase in EO yield.

Conclusion

The research findings suggest that the application of plant growth-promoting rhizobacteria (PGPR) can be an effective strategy for enhancing the growth, physiological, and biochemical properties of geranium plants under salinity stress. The results demonstrate that the PGPR treatment significantly improved plant growth parameters, such as increased shoot length, root length, and biomass production. Additionally, the PGPR-treated plants exhibited enhanced physiological properties, including increased chlorophyll content, antioxidant enzyme activities, and osmolyte accumulation, which contributed to improved stress tolerance in the plants. These findings indicate that PGPR can be a valuable tool for mitigating the negative effects of salinity stress on geranium plants and enhancing their overall performance. Further studies are warranted to explore the underlying mechanisms of this beneficial interaction and determine optimal application methods and dosage for different plant species.

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