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Original Research

Influence of Exopolysaccharide-Producing Bacteria and SiO_2 Nanoparticles on Proline Content and Antioxidant Enzyme Activities of Tomato Seedlings (*Solanum lycopersicum* L.) under Salinity Stress

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Abstract

A greenhouse experiment was conducted to evaluate the effects regarding inoculation of exopolysaccharide (EPS)-producing bacterium, the extracted EPS and silicon nanoparticles on *Solanum lycopersicum* L. seeds under salinity stress, in a completely randomized factorial design with three replicates. The inoculated seeds with silicon nanoparticles (8 gr L^{-1}), bacterial EPS (0.01 M), and 1 mL of bacterial suspension (1×10^8 CFU mL^{-1}) were sown in pots and irrigated with water at different salinity levels (0.3, 2, 4, 6, and 8 dS m^{-1}). Results showed that treatment application could enhance salinity tolerance of tomato seeds and improve plant growth so that combined treatments of EPS and silicon nanoparticles (S.E.N), bacteria and silicon nanoparticles (S.B.N), and EPS with silicon nanoparticles and bacteria (S.E.B.N) were the best treatments for plant growth and improvement regarding salinity levels. The mentioned treatments significantly ($p<0.01$) increased root and shoot fresh or dry weight in comparison to the control sample. In addition, treatments significantly ($p<0.01$) decreased proline content and antioxidant enzyme activities. Thus, it can be concluded that applied treatments are suitable for agricultural and environmental applications and bring about less damage caused by salinity stress.

Keywords: catalase, *Citrobacter freundii*, peroxidase, silicon nanoparticles, superoxide dismutase

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Introduction

In recent decades environmental challenges have become a main concern of policymakers. Considering environmental damage is essential in the global move toward sustainable development**.** An important abiotic stress in the world is salinity, and because of soil salinization as well as lack of adequate irrigation water, it is considered a major problem in Iran. Salinity is an important reason for reducing plant growth in different regions [1-2]. Salty saturated soil extract that effects plant growth contains electrical conductivity (ECe) of more than 4 dS m^{-1} at 25°C [3]. Plant growth is limited under salinity stress, which is due to different distinct reasons, including toxicity of ions, reducing water absorption, disturbing the balance of ions, a reduction in photosynthesis, or some of these causes in combination [4]. Various parameters define salinity tolerance in a plant, and plants require specific mechanisms for osmotic pressure. Fahramand et al. [5] indicated that osmotic capability is reduced due to increasing of various substances, including glycine betaine, proline, polyamines, sucrose, ions, and soluble substances. Chawla et al. [6] showed that protection of oxidative stress occurs by the mechanism regarding enzymatic antioxidant, which contains catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD). Tomato is one of the most important horticultural crops in the world and is semi-sensitive to salinity conditions at the germination stage. The threshold for tolerance to tomato salinity in the germination stage is reported to be 1.7 dS m^{-1} [7]. Tomatoes need much water because of their rapid growth and development. Regarding nutrition, absorption, and nutrient distribution in different parts of the plant, concentrations of acid and various vitamins and the amount of vitamin C in tomato can also be influenced by such factors as salinity [8]. Considerable attempts are being done to improve salt tolerance in tomato due to the vast salinization of Iranian soils. Studies on the use of biotechnology and nanotechnology methods and their role in improving salinity tolerance have recently increased.

One of the approaches in solving the salinity stress problem is the use of halophilic or halotolerant EPS-producing bacteria. It is believed that bacterial inhabitants of saline soils apply related strategies for resisting high salt concentrations in order to develop multiple adaptations to keep their population active while coping with such extreme environmental conditions. It is also believed that EPS production and biofilm formation are those of the adaptation mechanisms that are able to protect the cells from high salinity stress by retaining a nutrient-rich water layer around the cells [9]. Based on previous studies, EPS can facilitate nutrient and water maintenance, cell adhesion, and protection of cells from stress. Moreover, several researchers have shown the role of bacterial EPS to bind the sodium ions and reduce its toxicity in the soil [10].

Nanotechnology has affected various aspects of human life in recent years. This technology has widespread effects on economics, production, and the environment. Nanotechnology is able to improve the methods of assessing, managing, and reducing environmental hazards and providing opportunities for the production of new and safe products. Nanotechnology plays an important role in the protection of the environment in soil and climate change, and it is a strategy for protecting the environment and natural resources and achieving sustainable development. The use of nanoparticles is superior to other chemical and biological agents to deal with environmental stresses, because these nanoparticles are more responsive and more flexible in reactions due to their small size and high cross-sectional area. Considering the abundant capabilities of nanotechnology in removing and controlling environmental pollution and preventing its release, it can be considered as an effective green technology and an effective tool for achieving sustainable development [11]. In relation to coping with environmental stresses, the use of silicon nanoparticles can affect the salinity stress in plants. Silicon nanoparticles are effective on water translocation, hence increasing the efficiency of water usage [1]. Nanoparticles can have effects of agriculture, water resources, environment, and other similar practical matters [12]. In this regard, nanofertilizers may prevent extensive loss of money occurred because of the low efficiency of using chemical fertilizers [13].

The present article is a step to show the importance of using nanoparticles and bacteria and bacterial products for studying the environment and coping with salinity stress. Considering the harmful effects and damage caused by the growth of population and industry on the environment and considering the increasing salinity of the soil, there must be conducted research on the effects of bacteria and bacterial exopolysaccharide associated with silicon nanoparticles. These studies should be focused on increasing salt tolerance of seeds of tomatoes and finding a practical and eco-friendly solution to cope with salinity stress.

Material and Methods

Treatment Preparations

Salinity resistant bacterium *Citrobacter freundii* (strain ATHM38 with the accession number of KX553903 in the NCBI database) with growth potential in 0-25% salt, and 0.168 gr L^{-1} exopolysaccharide (EPS) production in a period of 24 hr., used in the study, was isolated previously from saline soils of the Roudasht region near Isfahan, Iran (32° 18′ N, 52° 23′ E, 1,497 m above mean sea level). In order to extract EPS, 10 mL of overnight bacterial culture with the turbidity equivalent to 0.5 McFarland was transferred to 240 mL BHI (brain heart infusion) of broth (5%) salt, and 2% sucrose transmitted at the speed of 180 rpm for 48 hrs. After that, the culture medium containing the bacteria was centrifuged for 30 minutes at a speed of 13,000 rpm. Then the supernatant

Table 1. Some chemical and physical properties of the experimental soil.

Texture	*EC (dS/m)	pH	N (%)	P (mg/Kg)	K (%)	*OC ($cmol^{+}/kg$)	*CEC (%)	$CaCO_3$ (g/cm^3)	*ρb (%)	Porosity
Sandy loam	1.42	8.22	0.08	28	153	1.7	19	42	1.5	56

*EC = electrical conductivity, *OC = organic matter, *CEC = cation exchange capacity, *ρb = bulk density

material was filtrated and removed. Next, it was mixed with an equal volume of EPS. The prepared mixture was kept overnight at 4°C for depositing the EPS, and then it was centrifuged for 30 minutes at a speed of 13,000 rpm again, and the sediment was collected. The obtained sediment was washed twice with ethanol and dried by air [10]. Then the EPS solutions with concentrations of 0.01 to 0.1 M were prepared, and the seed germinations were tested in these solutions with three replicates. The optimal EPS concentration was determined according to the highest germination percentage. To prepare the bacterial inoculants by a population of 1×10^8 cells mL^{-1}, the bacteria were incubated in the BHI culture medium broth (5%) of salt for 24 hrs. at 32ºC on a rotary shaker at 180 rpm, and they were then centrifuged at 13,000 rpm for 30 min. for sedimentation, and finally the supernatant material was separated and the produced sediment was washed and re-deposited in the physiologic serum to obtain a density of 1×10^8 cells mL^{-1} [14]. Silicon nanoparticles with the size of 50 nm (purchased from the Department of Chemistry, Amir Kabir University, Tehran/Iran) were prepared at a concentration of 8 gr L^{-1} according to the procedure described by Siddiqui and Al-Whaibi [15].

This study was carried out in the greenhouse of Islamic Azad University of Isfahan. It was done through a completely randomized design with three replications in February 2016. A total of 105 plastic pots (20 cm diameter) were prepared for the plant cultivation. The amount of soil required for all pots was weighed using a scale that weighed 4 kg of soil for each. Some chemical and physical properties of experimental soil are shown in Table 1. Next, 105 *Solanum lycopersicum* seeds (cultivar PS) were selected and sterilized in 96% alcohol solution for 30 seconds and then in a 5% sodium hypochlorite solution for 1.5 to 2 minutes, and finally they were washed with distilled water [16].

The treatments were prepared as follows:

1) Control sample: seed (S)
2) Inoculated seeds with silicon nanoparticles (8 gr L^{-1}) (S.N)
3) Inoculated seeds with 1 mL of bacterial suspension (1×10^8 CFU mL^{-1}) (S.B)
4) Inoculated seeds with bacterial EPS 0.01 M (S.E)
5) Inoculated seeds with bacteria (1×10^8 CFU mL^{-1}) and silicon nanoparticles (8 gr L^{-1}) (S.B.N)
6) Inoculated seeds with EPS 0.01 M and silicon nanoparticles (8 gr L^{-1}) (S.E.N)
7) Inoculated seeds with bacteria (1×10^8 CFU mL^{-1}), bacterial EPS (0.01 M), and silicon nanoparticles (8 gr L^{-1}) (S.E.B.N)

In order to inoculate the seeds with silicon nanoparticles and exopolysaccharide, they were placed in sterile petri plates containing silicon nanoparticles (8 gr L^{-1}) and exopolysaccharides (0.01 M) for 2 hrs. before being planted (15 seeds were used for each treatment with 3 replicates at 5 salinity levels). For bacterial inoculation with the seeds, 1 mL of bacterial strain suspension with a population of 1×10^8 CFU mL^{-1} was poured on them after placement of the seeds in the soil, and then they were covered with soil and irrigated with the saline water (0.3, 2, 4, 6, and 8 dS m^{-1}). Irrigation was carried out during the experiment, with the saline water every 4 days, and underneath each pot we placed a plate under to allow the pouring of the drainage of the pot. During the growth period, the pots were kept at a distance of 50 cm from each other in a greenhouse with a daily temperature of 4±25 and a temperature of 4±20 in normal light. Proline content, activities of catalase, peroxidase, superoxide dismutase enzymes, mean root fresh, and dry weight, mean shoot fresh and dry weight (mg per a seedling in 3 replicates) were measured after 15 days of treatments.

Experimental Characteristic Measurements

Preparation of enzyme extracts: Whole tissue (leaves, stems and/or roots) were homogenized (1:5 w/v) separately in an ice cold mortar using 50 mM sodium phosphate buffer, pH 7.0, containing 1M NaCl, 1% polyvinylpyrrolidone, and 1 mM EDTA. After centrifugation (20,000 g, 15 min), the supernatant (crude extract of leaves) was used to determine enzyme activities, which were measured at 25ºC. Protein contents of the extracts were determined by the method of Bradford [17].

Catalase activity (EC 1.11.1.6): The activity of catalase was determined by following consumption of H_2O_2 (extinction coefficient 0.0394 mM^{-1} Cm^{-1}) at 240 nm for 1 min [18]. The assay mixture contained 100 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2, and 50 µl leaf extract in a 3 ml volume. One unit of catalase activity (U) was assumed to be the amount of enzyme that decomposed 1 µmol H2O2/mg soluble protein per minute.

Peroxidase activity: The method of Hopkins and Tudhope [19], with t-butyl hydroperoxide as a substrate, was used. The reaction mixture comprised 50 mM potassium phosphate buffer, pH 7.0, 2 mM EDTA, 0.28 mM NADPH, 0.13 mM GSH, 0.16 U GR, 0.073 mM t-butyl hydroperoxide, and enzyme extract (50 mg protein). One unit of GSH-Px activity was defined as the amount of enzyme that catalyzed the oxidation of NADPH.

Superoxide dismutase activity: The activity of superoxide dismutase was determined by the method of Minami and Yoshikawa [20] with Tris–Ca–codylic sodium salt buffer, pH 8.2, containing 0.1 mM EDTA. The reaction mixture was composed of 1.42% Triton X-100, 0.055 mM nitroblue tetrazolium (NBT), 16 mM pyrogallol, and enzyme extract (50 mg protein). The unit (50% inhibition) was established according to the definition of McCord and Fridovich [21]. Unit was defined as the quantity of enzyme required to inhibit the reduction of NBT by 50% per 1 min.

Proline content: The leaves proline content was measured by the Bates et al. [22] method. Approximately 0.5 g of fresh or frozen plant material was homogenized in 10 ml of 3% aqueous sulfosalicyclic acid and filtered through Whatman's No. 2 filter paper. Two ml of filtrate was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 hr at 100ºC. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated and cooled to room temperature, and the absorbance was measured at 520 nm with a Nikon XS Spectrometer. Appropriate proline standards were included for calculating proline in the sample.

For root and shoot fresh weight (mg) measurements, seedlings were separated carefully from the soil. Root fresh weight and shoot fresh weight were measured using digital scales. Root dry weight and shoot dry weight (mg) measurements were done after roots and shoots were dried in the oven at 70°C for 72 hrs.

Statistical Analysis

This research was done in a completely randomized factorial design with 3 replicates. Analysis of variance (ANOVA) was used to determine the significant differences. Duncan's multiple range test was used to perform the separation of means (1% level probability). SPSS software (v. 20) was used for data analysis. The diagrams were plotted using Excel software. All of the presented data are the mean of 3 replicates.

Results and Discussion

Plant Biomass

According to the results root/shoot fresh/dry weight significantly decreased along with increasing the salinity level while the applied treatments enhanced salinity resistance of plants and significantly increased the mean of root/shoot fresh/dry weights.

The S.E (seed + EPS), S.E.N (seed + EPS + silicon nanoparticles), and S.E.B.N (seed + EPS + bacterial sus. + silicon nanoparticles) treatments at the salinity level of 4 dS m^{-1}, and S.E.B.N treatment at the salinity level of 6 dS m^{-1} increased the mean of roots fresh/dry weight significantly (Figs 1-2). Although S.B.N (seed + bacterial sus. + silicon nanoparticles) treatment at the salinity level of 4 dS m^{-1} could also significantly increase the mean root dry weight compared to the control sample. In the

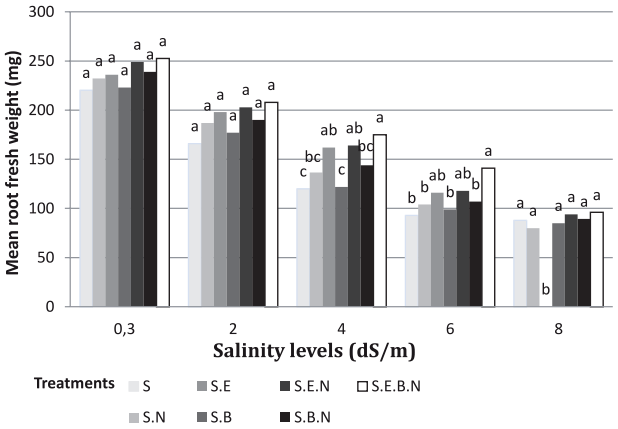

Fig. 1. Effect of treatments on mean root fresh weight (mg) at various salinity levels.
The letters show significance of differences (P≤0.01) – (S): control, (S.N): seed + silicon nanoparticles, (S.B): seed + bacterial sus., (S.E): seed + EPS, (S.B.N): seed + bacterial sus.+ silicon nanoparticles, (S.E.N): seed + EPS + silicon nanoparticles, (S.E.B.N): seed + EPS + bacterial sus.+ silicon nanoparticles.

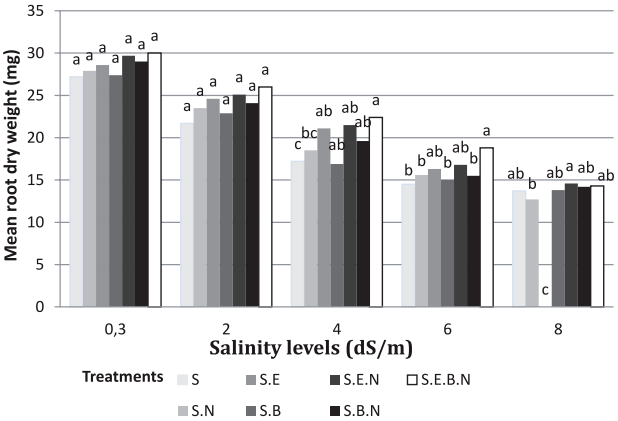

Fig. 2. Effect of treatments on mean root dry weight (mg) at various salinity levels.
The letters show significance of differences (P≤0.01) – (S): control, (S.N): seed + silicon nanoparticles, (S.B): seed + bacterial sus., (S.E): seed + EPS, (S.B.N): seed + bacterial sus.+ silicon nanoparticles, (S.E.N): seed + EPS + silicon nanoparticles, (S.E.B.N): seed + EPS + bacterial sus.+ silicon nanoparticles.

treatment of S.E (seed + EPS) the seeds could not tolerate the salinity stress of 8 dS m^{-1}, so there is no data for this treatment.

The S.B.N (seed + bacterial sus.+ nanoparticles) and S.E.B.N (seed + EPS + bacterial sus.+ nanoparticles) treatments at the salinity level of 0.3 dS m^{-1} significantly increased the mean shoot fresh/dry weights, although at the salinity levels of 2 and 4 dSm^{-1} the shoot fresh weight also increased significantly. The treatments of S.B.N, S.E.N (seed + EPS + silicon nanoparticles), and S.E.B.N at the salinity level of 6 dS m^{-1}, and S.E.B.N treatment at the salinity level of 8 dS m^{-1} significantly increased the mean shoots fresh and dry weights as compared to the control (Figs 3-4).

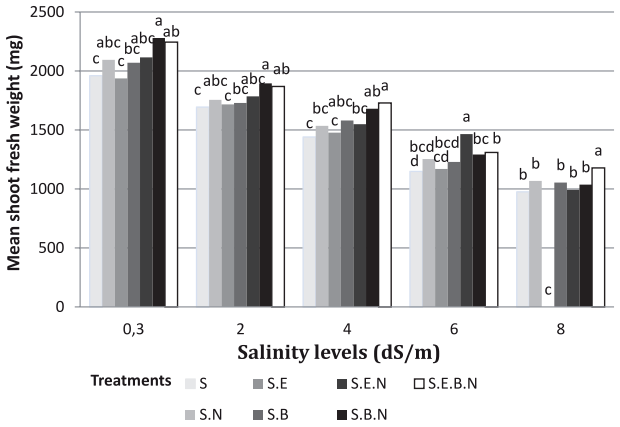

Fig. 3. Effect of treatments on mean shoot fresh weight (mg) at various salinity levels.
The letters show significance of differences (P≤0.01) – (S): control, (S.N): seed + silicon nanoparticles, (S.B): seed + bacterial sus., (S.E): seed + EPS, (S.B.N): seed + bacterial sus.+ silicon nanoparticles, (S.E.N): seed + EPS + silicon nanoparticles, (S.E.B.N): seed + EPS + bacterial sus.+ silicon nanoparticles.

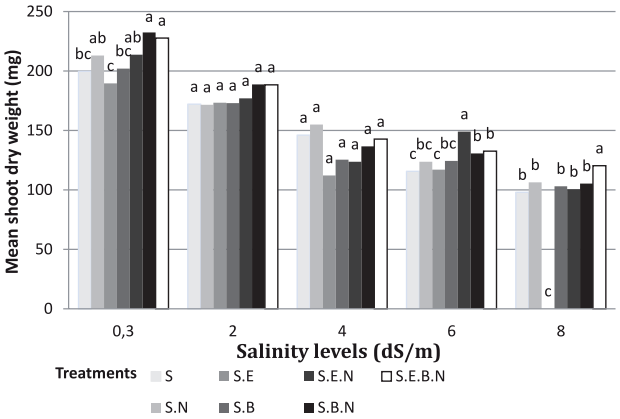

Fig. 4. Effect of treatments on mean shoot dry weight (mg) at various salinity levels.
The letters show significance of differences (P≤0.01) – (S): control, (S.N): seed + silicon nanoparticles, (S.B): seed + bacterial sus., (S.E): seed + EPS, (S.B.N): seed + bacterial sus.+ silicon nanoparticles, (S.E.N): seed + EPS + silicon nanoparticles, (S.E.B.N): seed + EPS + bacterial sus.+ silicon nanoparticles.

Productivity and plant generation are affected by salinity [23]. As the solutes increase, the osmotic pressure of the soil solution increases. So the amount of energy that the plant should use to absorb water from the soil and respiration increase, while water absorption and plant growth and yield decrease [24-25]. The results of this study are in line with the results of the study by Talebi Atouei et al. [26], which stated that bacterial inoculation of seeds increased plant growth under salt stress, so the weight of the shoot and root have increased. This is due to the reduction of ethylene [27] and the production of hormones [28], which stimulate plant growth, as well as the production of polysaccharides by producing bacteria and the improvement of physical and nutritional conditions of soil. Kaci et al. [29] noted that insemination of wheat with polysaccharide caused a significant increase in wheat growth, dry shoot, and root weights. Sandhya et al. [30] also stated that inoculation with EPS producing isolate significantly increased the length of stem, root, and dry biomass of sunflowers in both stressed and non-stressed water conditions. Shilev et al. [31] also observed the increased fresh weight of the plant and improved root development in the sunflower inoculated with *Pseudomonas fluorescens* under stress conditions. This finding was consistent with the observations of Yu et al. [32] regarding the positive and significant effect of *Bacillus subtilis* on fresh pepper weight. Chithrashree et al. [33] also observed that the dry weight of rice was significantly higher in plants inoculated with *Bacillus pumilus* and *Bacillus subtilis* than that in non-bacterial treatments. Bacterial inoculation in saline soil plays an important role in the supply of mobile elements for the plant by increasing the root level [34] and also improves the rate of plant germination and growth by decreasing sodium absorption and regulating osmotic pressure [35]. Concerning the effect of nanoparticles on salinity stress, Haghighi et al. [36] showed that silicon nanoparticles significantly reduced the salinity-induced damage by affecting germination rate, root length, and dry weight of tomato seedlings. Lee et al. [37] also stated that, under salinity stress conditions, silicon plays a crucial role in plant growth and regulates the content of endogenous gibberellin in the plant. Azimi et al. [38] and Lu et al. [39] also showed that the application of silicon nanoparticles significantly increased germination percentage and dry weights of roots and shoots of wheat and soybean, respectively. According to Vaculik et al. [40], silica improves plant growth by increasing cell elongation and expanding primary cell walls. Similar results have been reported by other researchers confirming the positive effects of silicon in reducing salt stress [41-42]. Actually, nanosilicon particles can improve water absorption and distributions in plants due to their hydrophilic characteristics and high absorption surface [43].

According to results in 15-day-old seedlings, the S.E treatment was eliminated at salinity level 8 dS m^{-1}, and this treatment was effective up to 6 dS m^{-1} salinity

level compared to other treatments. The reason is that EPS has many physical and chemical properties, such as suspension, thickness, gel shape, and water retention capacity, and as salinity (for, e.g., 8 dS m^{-1}) increases the suspended and gel features of EPS gels and, consequently, reduces their water retention capacity [44].

Proline content: The results indicated a significant increase in the proline content associated with increasing the salinity level. However, the proline content was significantly decreased by the use of silicon nanoparticles, EPS, and bacterial treatments (Fig. 5). Thus, at different salinity levels, the proline content of S.E.N (seed + EPS + silicon nanoparticles), S.B.N (seed + bacterial sus. + silicon nanoparticles), and S.E.B.N (seed + EPS + bacterial sus. + silicon nanoparticles) treatments was decreased significantly. So, they were the most effective treatments in order to deal with salinity stress. In addition, at the salinity level of 6 dS m^{-1}, S.E (seed + EPS) treatment and at the salinity level of 8 dS m^{-1}, S.N (seed + silicon nanoparticles) treatment decreased proline content significantly as compared to the control. Many species of plants synthesize suitable organic solutions such as proline in the cytosol under salinity stress, which is a considerable factor for salinity tolerance [45-46]. As stated by Ashraf and Foolad [23], the actions related to proline are osmoregulation, detoxification of harmful ions, and reduced salinity damage. On the other hand, as proline content decreases under the influence of treatment application, it can be concluded that under salinity stress conditions, with the increase of extracellular polysaccharides, the accumulation of some enzymes, such as aminopeptidase, amino acid glutamate and betaine, increases their resistance to saline conditions. By increasing the amount of plant growth regulators, nitrogen fixation, and absorption of nutrients and vitamins, the plant's resistance to stress has increased [47]. In addition, EPS have been able to accumulate and maintain moisture under stress conditions due to crosslinking properties [4]. Silicon is also deposited in the cell walls and is combined with organic macromolecules, including cellulose, pectin, glycolic proteins, and lignin, and forms colloidal formations with high absorption levels. Therefore, due to their high adsorption rates, they have an impact on the moisture content of xylem and water transfer and improve water use efficiency [48]. Thus, all the factors mentioned above have caused plants to resist stress conditions. In this respect, Saghafi et al. [16] reported that proline content increased under salt stress in wheat, while it was reduced by the use of EPS-producing bacteria. Liang et al. [49] and Mane et al. [50] reported that salinity tolerance in plants can be increased by silicon, by various actions including immobilization of toxic sodium ion, reducing sodium uptake and increasing the effectiveness of water usage.

Catalase activity: According to Fig. 6, salinity stress significantly increased catalase activity, while the applied treatments significantly decreased it. The S.E (seed + EPS), S.E.N (seed + EPS + silicon nanoparticles), and S.E.B.N (seed + EPS + bacterial sus. + silicon nanoparticles) treatments at the salinity levels of 0.3, 2, 4 and 6 dS m^{-1} significantly decreased the catalase activity as compared to the control sample. Although S.N (seed + silicon nanoparticles) treatment at the salinity levels of 0.3 and 6 dS m^{-1}, S.B.N (seed + bacterial sus. + silicon nanoparticles) treatment at the salinity levels of 0.3, 4, and 6 dS m^{-1}, S.E.N and S.E.B.N treatments at the salinity level of 8 dS m^{-1} could also significantly decrease the catalase activity compared to the control sample. These findings are in line with Upadhyay et al. [51], who reported that bacteria could affect soil particle

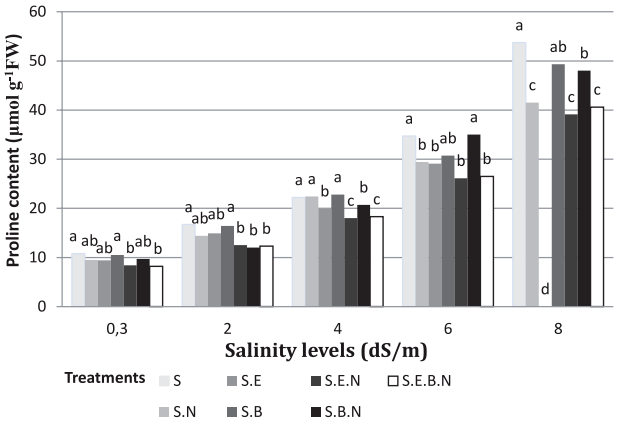

Fig. 5. Effect of treatments on the proline content (µmol g^{-1} FW) at various salinity levels.
The letters show significance of differences (P≤0.01) – (S): control, (S.N): seed + silicon nanoparticles, (S.B): seed + bacterial sus., (S.E): seed + EPS, (S.B.N): seed + bacterial sus.+ silicon nanoparticles, (S.E.N): seed + EPS + silicon nanoparticles, (S.E.B.N): seed + EPS + bacterial sus.+ silicon nanoparticles.

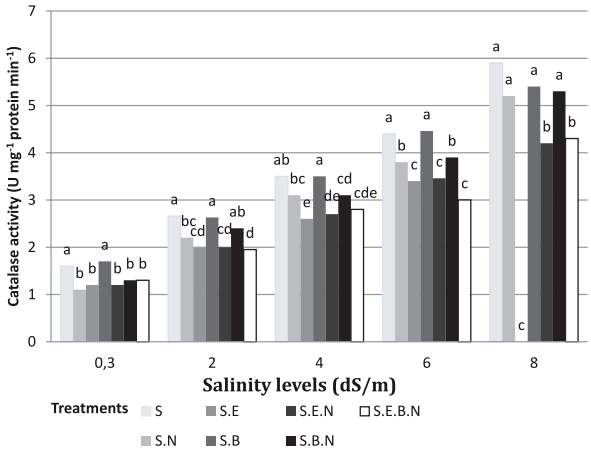

Fig. 6. Effect of treatments on catalase activity (U mg^{-1} protein min^{-1}) at various salinity levels.
The letters show significance of differences (P≤0.01) – (S): control, (S.N): seed + silicon nanoparticles, (S.B): seed + bacterial sus., (S.E): seed + EPS, (S.B.N): seed + bacterial sus.+ silicon nanoparticles, (S.E.N): seed + EPS + silicon nanoparticles, (S.E.B.N): seed + EPS + bacterial sus.+ silicon nanoparticles.

distribution around the roots by producing EPS and improving water availability to the considered plants and lead to decreasing the catalase activity despite saline conditions. The results of Baniaghil et al. [52] indicated a significant increase in the activity of catalase enzymes in corn under salt stress and the positive effect of bacterial inoculation on reduced oxidative damage caused by salt stress, which confirms the results of the present study. Similarly, Omar et al. [53] reported decreased activity of the catalase enzyme in barley seedlings inoculated with *Azospirillum*. Kohler et al. [54] also observed that, under salt stress conditions, there was reduced activity of catalase enzyme in lettuce seedlings inoculated with *Pseudomonas* bacteria compared to control treatment. In the same vein, studying the use of silicon in reducing the oxidative stresses of wheat, Gong et al. [55] stated that silicon could reduce stress-induced damage by increasing catalase activity. These results are inconsistent with the results of this study. It can be deduced that the increase in the activity of antioxidant enzymes in inoculated treatments with silicone was used to induce stress tolerance. The effect of nanoparticles on plants also depends on different factors such as plant species, growth stage of plant, concentration of the nanoparticles, and the experimental situation. In addition, the activity of antioxidant enzymes is also dependent on plant resistance under stress conditions, because the activity of these enzymes in plants indicates the resistance of plants to various stresses. However, salt tolerance has not yet properly defined mechanisms, and antioxidant enzymes are not the only substances to reduce oxidative damage. Decreasing the effects of salinity stress may be due to other defensive factors that can establish better situations in plants [56].

Peroxidase activity: The assessment of peroxidase activity indicated that the activity of peroxidase was significantly increased with increasing the salinity level and a significant decrease in peroxidase activity was observed by the application of treatments (Fig. 7). At different salinity levels, the S.E.N (seed + EPS + silicon nanoparticles) and S.E.B.N (seed + EPS + bacterial sus. + silicon nanoparticles) treatments significantly decreased the peroxidase activity as compared to the control, although S.N (seed + silicon nanoparticles) treatment at the salinity levels of 0.3 and 8 dS m^{-1} and S.E (seed + EPS) treatment at the salinity level of 0.3 dS m^{-1} could also significantly decrease the peroxidase activity compared to the control sample. In this regard, Han and Lee [57] reported that peroxidase activity is reduced and plants are protected against toxicity due to salinity in soybeans by EPS-producing bacteria, which might have produced higher amounts of EPS in greater salinity levels. The produced EPS can reduce the toxic effects. Moreover, by increasing the absorption of water, the bacteria can increase the rate of germination and percentage of it. Since drought and salinity stresses are presumably considerably reduced by silicon, plant growth can fairly be affected by this element [58]. It seems that by forming a layer, the silicon nanoparticles also increase cell wall stability. Similarly, tissue water loss in the treatments inoculated with silicone nanoparticles may be affected by the accumulation of silicon in the lower epidermal cells and reduced transpiration [59].

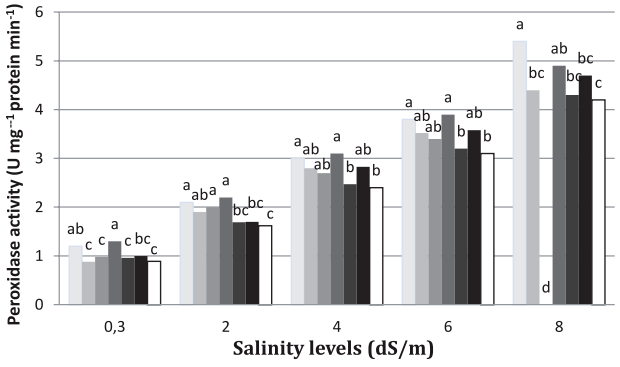

Fig. 7. Effect of treatments on peroxidase activity (U mg^{-1} protein min^{-1}) at various salinity levels.
The letters show significance of differences (P≤0.01) – (S): control, (S.N): seed + silicon nanoparticles, (S.B): seed + bacterial sus., (S.E): seed + EPS, (S.B.N): seed + bacterial sus.+ silicon nanoparticles, (S.E.N): seed + EPS + silicon nanoparticles, (S.E.B.N): seed + EPS + bacterial sus.+ silicon nanoparticles.

Superoxide dismutase activity: The results showed that the activity of superoxide dismutase was significantly increased with increasing the salinity level and treatments application could significantly reduce the activity of this enzyme (Fig. 8). So that at different salinity levels, the S.E.N (seed + EPS + silicon nanoparticles) and S.E.B.N (seed + EPS + bacterial sus. + silicon nanoparticles) treatments decreased the superoxide dismutase activity significantly as compared to the control. In addition, at salinity levels of 2 and 8 dS m^{-1}, S.N (seed + silicon nanoparticles) treatment and at the salinity level of 2 dS m^{-1}, S.E (seed + EPS) treatment decreased the superoxide dismutase activity significantly as compared to the control.

In normal conditions there is a balance between the amount of generation of reactive oxygen species and the activity of the mechanisms eliminating it. In environmental stresses, however, this balance is disturbed and causes oxidative stress in plants [60]. The reports by Karray-Bouraoui et al. [61] showed that the growth and performance of safflower decreased due to salt stress affected by certain changes in physiological and biochemical processes, including ionic balance and antioxidant defense system. Their results also showed the increased activity of antioxidant enzymes along with increasing salinity. Salinity stress causes osmotic stress, and this water deficit causes the formation of reactive oxygen species such as hydrogen peroxide, superoxide, and radical hydroxide. Under such conditions, the plant, by increasing the activity of antioxidant enzymes, attempts to inhibit the active oxygen species found in conditions [62]. As a result, as salinity increases, the activity of enzymes also increases. On the other hand, studying the effect of treatments on different levels of salinity

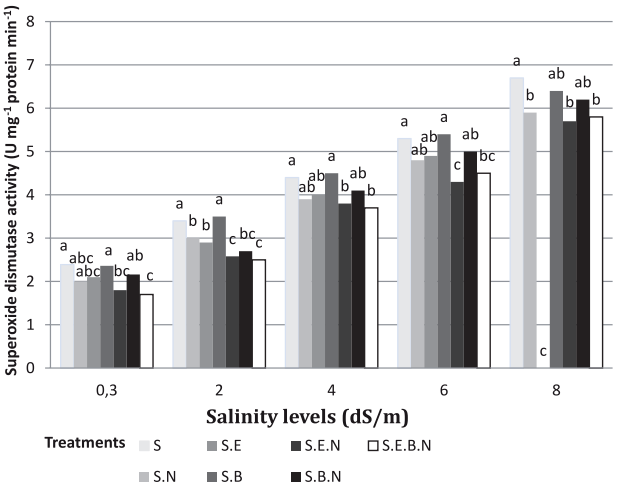

Fig. 8. Effect of treatments on superoxide dismutase activity (U mg^{-1} protein min^{-1}) at various salinity levels.
The letters show significance of differences ($P \leq 0.01$) – (S): control, (S.N): seed + silicon nanoparticles, (S.B): seed + bacterial sus., (S.E): seed + EPS, (S.B.N): seed + bacterial sus.+ silicon nanoparticles, (S.E.N): seed + EPS + silicon nanoparticles, (S.E.B.N): seed + EPS + bacterial sus.+ silicon nanoparticles.

showed that the application of treatments was effective in salinity resistance of seedlings, and consequently the activity of enzymes was decreased. Of course, there is also evidence that bacteria increased plant resistance to different stresses following the increased activity of antioxidant enzymes [63-64], which is inconsistent with the results of the present study. Probably the production of metabolites by growth-promoting bacteria, including growth-promoting hormones, plays a special role in stimulating and expressing antioxidant enzymes.

Concerning the effect of silicon on antioxidant enzymes activities, some of the evidence suggests that silicon reduces the biological and non-biological stresses in plants by enhancing the antioxidant defense system [65-67]. The present results showed that the activity of superoxide dismutase in tomato was enhanced by increasing the salinity level. These results are in line with the results of Hassanein et al. [68], who observed that salt stress increased the activities of antioxidant enzymes in leaves of *Zea mays* plants. In addition, Amira and Abdul Qados [69] also expressed NaCl treatments, causing an increase in activity of superoxide dismutase in *V. Faba* plant leaves, while the application of silicon nanoparticles caused a decrease in the activity of superoxide dismutase as compared to unstressed plants. Increased activity of antioxidant enzymes is a salt-tolerance mechanism in most plants [70].

Generally, since inoculated bacterium used in this study was a salt-resistant bacterium, its activity and growth was not affected by increasing salinity levels. It also could improve soil structure and colonize roots, and therefore increase water absorption efficiency and nutritional elements under stress through increasing root effluent [71]. Bacteria also can compete with harmful species by certain mechanisms such as the production of plant growth stimulants, increased nutrient uptake, and the production of antibiotics and degrading enzymes of the cell wall of plant pathogenic fungi. Inoculating bacteria also have a positive effect on plant growth under salt stress by creating systemic resistance to the plant and increasing the plant's resistance to abiotic stresses [72]. The EPS extracted from the bacteria was also effective in salt tolerance, maybe due to facilitating the adhesion of the bacterium to the root and causing root colonization and biofilm formation, which is necessary to protect the bacteria from adverse environmental conditions [73]. The EPS production under stress conditions leads to better biofilm formation and maintaining the water layers around the cells. The biofilm, which improves soil aggregation and moisture preservation, protects bacterial cells under stress conditions [74]. The EPS can also bond with sodium and decrease its root absorption [75]. However, the specific function and precise role of EPS depends on their structural units and on the environmental characteristics of host microorganisms indicates that further research is needed in this regard [76].

Conclusions

The past century was a century of cultivation of advanced chemicals in agriculture. On the other hand, increasing quality of life in different regions has had a negative impact on food production and the environment. Therefore, agricultural science has moved toward biological factors and the application of biological and nanotechnologies and their integration. According to the results, treatment with salt-tolerant bacterium, EPS, and silicon nanoparticles had certain effects on metabolic processes of the plant, which include decreasing proline content and antioxidant enzyme activities and increasing plant growth under salinity stress. The results also revealed the role of applied treatments in regulating salinity responses, and express that treatments could protect plants against the harmful effects of salinity stress. Therefore, it is possible to use the applied treatments in plant research and planting in saline areas of the country as an affordable and environmentally friendly solution. However, extensive studies should be done in order to achieve the best and optimum yield of plants affected by salinity stress.

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Conflict of Interest

The authors declare no conflict of interest.

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