



Article

# Foliar Application of Cerium Oxide-Salicylic Acid Nanoparticles (CeO<sub>2</sub>:SA Nanoparticles) Influences the Growth and Physiological Responses of *Portulaca oleracea* L. under Salinity

Mohammad Bagher Hassanpouraghdam <sup>1,\*</sup>, Lamia Vojodi Mehrabani <sup>2</sup>, Zahra Bonabian <sup>2</sup>, Mohammad Ali Aazami <sup>1</sup>, Farzad Rasouli <sup>1</sup>, Marcin Feldo <sup>3</sup>, Maciej Strzemeski <sup>4</sup> and Sławomir Dresler <sup>4,5</sup>

- <sup>1</sup> Department of Horticultural Science, Faculty of Agriculture, University of Maragheh, Maragheh 55181-83111, Iran; aazami58@gmail.com (M.A.A.); farrasoli@gmail.com (F.R.)  
<sup>2</sup> Department of Agronomy and Plant Breeding, Azarbaijan Shahid Madani University, Tabriz 53751-71379, Iran; vojodilamia@gmail.com (L.V.M.); bonabizahra7@gmail.com (Z.B.)  
<sup>3</sup> Department of Vascular Surgery, Medical University of Lublin, 11 Staszica St., 20-081 Lublin, Poland; martin@interia.pl  
<sup>4</sup> Department of Analytical Chemistry, Medical University of Lublin, 20-093 Lublin, Poland; maciej.strzemeski@poczta.onet.pl (M.S.); slawomir.dresler@umlub.pl (S.D.)  
<sup>5</sup> Department of Plant Physiology and Biophysics, Institute of Biological Science, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland  
\* Correspondence: hassanpouraghdam@gmail.com; Tel.: +98-9145027100



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**Abstract:** In the present study, the effects of foliar application of salicylic acid (100 μM), cerium oxide (50 mg L<sup>-1</sup>), and cerium oxide:salicylic acid nanoparticles (CeO<sub>2</sub>:SA-nanoparticles, 50 mg L<sup>-1</sup> + 100 μM) on the growth and physiological responses of purslane (*Portulaca oleracea* L.) were examined in non-saline and saline conditions (50 and 100 mM NaCl salinity). Foliar applications mitigated salinity-induced adverse effects, and the highest plant height and N, P, Mg, and Mn content were recorded in the variant with non-saline × foliar use of CeO<sub>2</sub>:SA-nanoparticles. The highest values of fresh and dry weight were noted in the treatment with no-salinity × foliar use of CeO<sub>2</sub>:SA-nanoparticles. The highest number of sub-branches was observed in the foliar treatments with CeO<sub>2</sub>-nanoparticles and CeO<sub>2</sub>:SA-nanoparticles without salinity stress, while the lowest number was noted in the 100 mM NaCl treatment. Moreover, the foliar application of CeO<sub>2</sub>:SA-nanoparticles and cerium-oxide nanoparticles improved the total soluble solid content, K, Fe, Zn, Ca, chlorophyll a, and oil yield in the plants. The salinity of 0 and 50 mM increased the K content, 1000-seed weight, total soluble solid content, and chlorophyll b content. The use of 100 mM NaCl with no-foliar spray increased the malondialdehyde, Na, and H<sub>2</sub>O<sub>2</sub> content and the Na<sup>+</sup>/K<sup>+</sup> ratio. No-salinity and 50 mM NaCl × CeO<sub>2</sub>:SA-nanoparticle interactions improved the anthocyanin content in plants. The phenolic content was influenced by NaCl<sub>100</sub> and the foliar use of CeO<sub>2</sub>:SA-nanoparticles. The study revealed that the foliar treatment with CeO<sub>2</sub>:SA-nanoparticles alleviated the side effects of salinity by improving the physiological responses and growth-related traits of purslane plants.

**Keywords:** *Portulaca oleracea*; elemental content; malondialdehyde; phenolic compound



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## 1. Introduction

Purslane (*Portulaca oleracea*) is an annual succulent plant from the family Portulacaceae. It contains several antioxidant compounds, proteins, vitamins, minerals, polysaccharides, alkaloids, phenolics, flavonoids, and omega-3 fatty acids with many applications in the food and pharmaceutical industries. In traditional medicine, purslane has been used as a febrifuge, disinfectant, anti-bacterial, anti-inflammatory, and wound healing plant [1]. The rapid population growth and the great need for plant material have imposed enormous

pressures on ecological systems. The collection from natural habitats never meets the ever-growing needs of several industries. Therefore, it is necessary to make emergent decisions for the large-scale production of medicinal plants in sustainable agricultural production systems [2].

The Food and Agriculture Organization (FAO) estimates that there are more than 833 million hectares of salt-affected soils around the globe (8.7% of the planet). Most of them can be found in naturally arid or semi-arid environments in Africa, Asia, and Latin America. Moreover, 20 to 50 percent of irrigated soils in all continents are excessively salty, which implies that over 1.5 billion people worldwide face significant challenges in growing food due to soil degradation [3]. In Iran, more than 50% of agricultural soil is in the face of salinity exposure [3]. Salinity impacts plant morphological and physiological characteristics, growth, and productivity by imposing ionic toxicity, ionic imbalances, reduced  $K^+$  absorption, and osmotic and later oxidative stress [4,5]. Salinity induces diverse nutritional disorders in plants via interference in the nutrient uptake and ion competition, translocation, and partitioning toward the growing sites inside plants [6,7]. The decreased potassium uptake triggers water deficiency in the plant resulting in oxidative damage to proteins and fats and disruption of normal cell metabolism [8]. Closure of stomata due to salinity stress reduces NADPH consumption in the Calvin cycle. The generated free radicals reduce the use of NADPH in chemical reactions, thereby disrupting several physiological processes. In salinity stress conditions, the accumulation of soluble sugars increases in favor of adaptation to the salinity stress conditions. The declined water potential and photosynthesis under salinity stress reduce and even stop plant growth, development, and productivity [4,5,7,8]. The outcome of all the interferences mentioned is the oxidative damage to proteins and lipids and the chaos in the normal cell metabolism [5].

Antioxidant enzymes and non-enzymatic antioxidant compounds have prominent roles in ameliorating the side effects of ROS radicals [9]. Excessive accumulation of ROS causes severe damage to cell membrane phospholipids, leading to lipid peroxidation, ion leakage, and reduced plant yield. Plants are equipped with enzymatic and non-enzymatic defense systems to eliminate or reduce ROS production. Foliar application of low-molecular-weight water-soluble substances (osmolytes such as polyamines, proline, and soluble solids) is an efficient strategy to combat stress in plants. The presence of enzymes and antioxidant compounds plays a vital role in reducing the damage caused by oxygen free radicals produced in the cell [9]. Salinity stress causes various nutritional abnormalities in the plant, which may be related to the adverse effects of salinity on the ability to absorb, transport, and distribute nutrients [4,9]. The overall result is oxidative damage to proteins and fats and disruption of normal cell metabolism [5]. In purslane, the concentration of  $NO_3^-$  and soluble protein, Rubisco, ascorbic acid, and phenolic content were increased under 100 mM salinity. The highest Mg, Ca, and K content was recorded in no-salinity conditions. With salinity up to 300 mM, the leaf area index, water content,  $cytb_6f$  concentration, and net photosynthesis potential declined markedly. However, the proline content was improved. This indicates that the plant can withstand salinity of up to 100 mM [10].

In the last decade, the decline in agricultural production due to the enormous environmental changes has been a massive challenge for food security in many countries, especially those with more progressive climate changes. Therefore, it is essential to find a way to accelerate the plant adaptation process to environmental changes. With advances in the multidisciplinary science domains, nanotechnology has emerged as one of the most influential and effective tools in combating environmental stress factors in plants. Nanoparticles (due to their high specific surface area, shape, and size) play an essential role in physiological processes, photosynthesis, and the activity of antioxidant enzymes in plants [7].

Due to their unique electrical, optical, and thermal properties, cerium oxide (lanthanide) nanoparticles have several industrial, biological, therapeutic, and agricultural applications [11]. The widespread use of nanoparticles in various industries has raised concerns about the health of living organisms and humans due to the environmental conditions associated with these materials [11]. The continuous or repeated exposure

(pharmaceutical or ecological) to high doses of cerium oxide nanoparticles disrupts cell wall membranes in *Cyanobacteria* species [12], damages the human lung [13], and harms the liver and spleen tissues in mice [14]. Since cerium oxide can remain in internal organs such as the spleen, liver, and bone marrow for a long time, it is necessary to consider the effect of these substances on human health. In a study on the impact of cerium oxide nanoparticles on reproductive organs and the systematic function of internal organs of rats, the application of an appropriate concentration ( $125 \mu\text{g mL}^{-1}$ ) had no side effects on the health of these organs [11]. Another study found that cerium oxide protected human cells against oxidative stress and inflammation by eliminating the toxic effects of ROS due to the redox reaction of  $\text{Ce}^{3+}/\text{Ce}^{4+}$  [15]. Cerium oxide also stimulates the production of SOD, which acts as a powerful antioxidant and thus helps living organisms to survive under stress [15]. In a study on coriander,  $125 \text{ mg kg}^{-1}$  of cerium oxide in the soil increased root length, improved plant growth, and enhanced catalase activity in the plant. However,  $500 \text{ mg kg}^{-1}$  cerium oxide application resulted in the accumulation of cerium oxide in plant tissues, which may be harmful to human health [16].

However, more in-depth studies should be conducted to confirm the possible human health side-effects of foliar application of small amounts of cerium oxide treatments on edible plants in stress conditions.

In agriculture, cerium oxide can eliminate ROS in stress conditions (light, temperature, salinity, drought, etc.). It maintains ionic homeostasis, chlorophyll biosynthesis, and the potassium-to-sodium ratio in the plant. The effect of cerium oxide nanoparticles depends on the plant species, growth conditions, concentration, and duration of plant exposure to the stress [7,17,18]. The results of studies performed on cotton [18] under salinity stress and foliar application of cerium oxide nanoparticles showed that the foliar treatment had a positive effect on reducing salinity stress, increased chlorophyll content and plant biomass, and decreased malondialdehyde and hydrogen peroxide content in the plant.

Salicylic acid is a phenolic compound that plays an essential role in regulating physiological and biochemical activities in plants. Salicylic acid mediates plant growth and development, flower induction, nutrient uptake and transport, pigment biosynthesis, cell respiration, cell membrane stability, and stomatal movement and enhances the antioxidant defense system [19–21]. The effect of salicylic acid on the plant depends on the plant species, its developmental stage, the concentration of utilized salicylic acid, and environmental conditions [19]. A study on sage showed that salinity reduced yields, but treatment with salicylic acid increased plant tolerance to stress [19]. Similar results have been reported on the reduction in salinity stress through the application of salicylic acid in rosemary [22]. In studies performed on *Trachyspermum ammi* L., it was found that salinity stress (8–12 dS/m) added up the content of sodium, MDA, proline, and the activity of antioxidant enzymes. At the same time, potassium, iron, leaf water content, photosynthetic pigment content, and grain yield were reduced [23]. The application of salicylic acid and iron nanoparticles increased potassium uptake, K/Na ratio, iron content, and activity of antioxidant enzymes (CAT and SOD) in the plant [23].

This study aimed to evaluate the effects of foliar application of salicylic acid, cerium oxide nanoparticles, and  $\text{CeO}_2$ :SA-nanoparticles on the yield (fresh and dry weight of the plant and 1000-seed weight), physiological responses, and elemental content in *Portulaca oleracea* under salinity stress.

## 2. Results

### 2.1. Fresh and Dry Weight (Biomass)

The salinity stress  $\times$  foliar applications had a significant effect on the fresh and dry weight of purslane (Table 1). A significantly reduced amount of fresh and dry weight was shown in response to the salinity stress, whereas the foliar application of salicylic acid,  $\text{CeO}_2$ -nanoparticles, and  $\text{CeO}_2$ :SA-nanoparticles improved the traits. The highest fresh and dry weights were obtained in the plants subjected to the  $\text{CeO}_2$ :SA-nanoparticles foliar application, which increased by 142% and 151% compared to the control, respectively. However, 100 mM of NaCl diminished the dry weight up to 98% compared to the control (Table 2).

**Table 1.** ANOVA for the effect of salinity (0–50 and 100 mM NaCl) and foliar applications (no spray, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles) on *Portulaca oleracea* growth characteristics, oil yield, and content of total soluble solids.

	Plant Fresh Weight (Fresh Biomass)	Root Fresh Weight	Plant Dry Weight (Dry Biomass)	Root Dry Weight	Plant Height	Branch Number	1000-Seed Weight	Total Soluble Solid Content	Oil Yield
Salinity (S)	**	**	**	**	**	**	**	**	**
Foliar (F)	**	**	**	**	**	**	**	**	**
S × F	**	**	**	**	**	**	ns	ns	ns

Significant effects for the main factors and their interaction are indicated with an asterisk: \*\*  $p < 0.01$ ; ns: non-significant.

**Table 2.** Comparison of means for the effects of salinity (0–50 and 100 mM NaCl) and foliar applications (no spray, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles) on the growth characteristics of *Portulaca oleracea* plants.

Salinity	Foliar Spray	Plant Dry Weight (g)	Plant Fresh Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)	Plant Height (cm)	Sub-Branch Number
0	No spray	1.8 ± 0.07f	15.2 ± 1.28f	0.24 ± 0.02c	0.020 ± 0.0e–g	21.8 ± 1.02ef	4.0 ± 0.81d
0	Salicylic acid	2.8 ± 0.09d	27.5 ± 1.50b	0.38 ± 0.07b	0.065 ± 0.0c	25.0 ± 1.41cd	5.6 ± 0.47b
0	CeO <sub>2</sub> -nanoparticles	3.5 ± 0.09b	27.9 ± 2.56b	0.46 ± 0.05b	0.110 ± 0.0b	30.3 ± 1.25b	7.3 ± 0.47a
0	CeO <sub>2</sub> :SA-nanoparticles	4.5 ± 0.13a	36.9 ± 2.26a	0.91 ± 0.11a	0.180 ± 0.0a	39.6 ± 1.70a	7.3 ± 0.47a
50	No spray	1.8 ± 0.25f	13.2 ± 0.52f	0.13 ± 0.02d–f	0.180f ± 0.0g	19.4 ± 1.18g	4.0 ± 0.82d
50	Salicylic acid	2.8 ± 0.12d	23.6 ± 0.60cd	0.16 ± 0.01c–f	0.034 ± 0.0ef	23.0 ± 1.00de	5.0 ± 0.47bc
50	CeO <sub>2</sub> -nanoparticles	2.8 ± 0.12d	22.8 ± 1.51c–e	0.12 ± 0.01ef	0.035 ± 0.0ef	24.3 ± 0.05d	5.3 ± 0.47b
50	CeO <sub>2</sub> :SA-nanoparticles	3.2 ± 0.05c	24.6 ± 3.26bc	0.21 ± 0.06c–e	0.070 ± 0.0c	30.3 ± 0.94b	5.3 ± 0.82b
100	No spray	0.9 ± 0.04h	13.5 ± 1.76f	0.09 ± 0.01f	0.012 ± 0.0g	16.3 ± 0.94h	2.6 ± 0.82e
100	Salicylic acid	1.3 ± 0.08g	19.4 ± 0.58e	0.17 ± 0.02c–f	0.013 ± 0.0g	20.3 ± 0.93fg	4.3 ± 0.47cd
100	CeO <sub>2</sub> -nanoparticles	1.5 ± 0.14g	19.8 ± 0.87de	0.22 ± 0.03cd	0.041d ± 0.0e	23.5 ± 0.48de	4.3 ± 0.47cd
100	CeO <sub>2</sub> :SA-nanoparticles	2.4 ± 0.12e	21.4 ± 2.51c–e	0.25 ± 0.02c	0.050 ± 0.0cd	27.0 ± 0.82c	5.6 ± 0.47b

Means ± SD (n = 3) with different letters within the same column are statistically different according to the LSD test ( $p < 0.05$ ).

## 2.2. Root Fresh and Dry Weight

The findings showed that the foliar application affected the fresh and dry weight of roots under the salinity stress (Table 1). Although the salinity stress reduced root fresh and dry weight, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles recuperated the traits. The highest and the lowest root fresh and dry weight values were observed in the foliar application of CeO<sub>2</sub>:SA-nanoparticles without salinity stress and 100 mM of NaCl without any foliar treatments. The fresh and dry weights of roots in the plants subjected to CeO<sub>2</sub>:SA-nanoparticles exhibited a 3.79- and 9-fold increase, respectively (Table 2).

## 2.3. Plant Height and Number of Branches

Plant height ( $P \leq 1\%$ ) and the number of sub-branches ( $P \leq 1\%$ ) were significantly affected by the foliar application of salicylic acid, cerium oxide, and CeO<sub>2</sub>:SA-nanoparticles under salinity stress (Table 1). The salinity stress considerably reduced the plant height and the number of branches, but the foliar application of salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles mitigated the salinity effects. The highest and the lowest plant height and number of sub-branches were observed in the purslane supplemented with CeO<sub>2</sub>:SA-nanoparticles and under 100 mM of NaCl, respectively. The results showed that CeO<sub>2</sub>:SA-nanoparticles improved these traits in the purslane plants more efficiently than salicylic acid and CeO<sub>2</sub>-NPs under the salinity stress (Table 2).

## 2.4. 1000-Seed Weight

The foliar applications and salinity stress significantly influenced the 1000-seed weight ( $P \leq 1\%$ ) (Table 1). The salinity stress decreased the 1000-seed weight, such that the highest weight was observed in the control plants and the lowest was recorded in the 50 mM salinity variant. The 1000-seed weight was reduced by 25% in the plants exposed to 100 mM NaCl, compared to the control (Table 3). The foliar application of salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles increased the 1000-seed weight of the

plant. The highest 1000-seed weight was noted in the variant of the foliar application of CeO<sub>2</sub>:SA-nanoparticles, which was 27% higher than in the control (Table 4).

**Table 3.** Comparison of means for the effect of NaCl salinity on 1000-seed weight, total soluble solid content, chlorophyll content, and oil yield of *Portulaca oleracea* plants.

Salinity	1000-Seed Weight	Total Soluble Solid Content (°Brix)	Oil Yield (g m <sup>-2</sup> )	Chlorophyll a Content (mg g <sup>-1</sup> FW)	Chlorophyll b Content (mg g <sup>-1</sup> FW)
0	3.0 ± 0.08a	1.6 ± 0.12b	0.64 ± 0.05a	2.40 ± 0.14a	0.95 ± 0.12a
50	2.7 ± 0.31ab	2.1 ± 0.08a	0.52 ± 0.09b	1.66 ± 0.12b	0.89 ± 0.17ab
100	2.4 ± 0.26b	2.2 ± 0.09a	0.45 ± 0.04b	1.33 ± 0.31b	0.65 ± 0.18b

Means ± SD (n = 3) with different letters within the same column are statistically different according to the LSD test ( $p < 0.05$ ).

**Table 4.** Comparison of means for the effect of foliar applications on 1000-seed weight, total soluble solid content, chlorophyll content, and oil yield of *Portulaca oleracea* plants.

Foliar Application	1000-Seed Weight (g)	Total Soluble Solid Content (°Brix)	Oil Yield (g m <sup>-2</sup> )	Chlorophyll a Content (mg g <sup>-1</sup> FW)	Chlorophyll b Content (mg g <sup>-1</sup> FW)
No spray	2.2 ± 0.20c	1.5 ± 0.05c	0.26 ± 0.08b	1.28 ± 0.17b	0.66 ± 0.09c
Salicylic acid	2.7 ± 0.05b	1.9 ± 0.08b	0.56 ± 0.04a	1.68 ± 0.33b	0.78 ± 0.07b
CeO <sub>2</sub> -nanoparticles	2.8 ± 0.09ab	2.2 ± 0.03a	0.63 ± 0.09a	1.96 ± 0.21ab	0.84 ± 0.17b
CeO <sub>2</sub> : SA-nanoparticles	2.3 ± 0.08a	2.3 ± 0.09a	0.68 ± 0.06a	2.30 ± 0.33a	1.04 ± 0.09a

Means ± SD (n = 3) with different letters within the same column are statistically different according to the LSD test ( $p < 0.05$ ).

### 2.5. TSS Content

TSS was significantly affected by the independent effects of the experimental treatments ( $P \leq 1\%$ ) (Table 1). The TSS content was raised by 31% in the purslane plants under 100 mM of NaCl, compared to control (Table 3). The foliar application of CeO<sub>2</sub>:SA-nanoparticles improved the TSS content, compared to the control (Table 4).

### 2.6. Oil Yield

The salinity stress and foliar application of salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles significantly affected the purslane oil yield (Table 1). The salinity of 100 mM caused a 42% reduction in oil yield compared to the control, which exhibited the highest oil yield (Table 3). All of the foliar applications improved the oil yield compared to the control. The highest oil yield was recorded in the purslane plants supplemented with CeO<sub>2</sub>:SA-nanoparticles (161% higher than in the control) (Table 4).

### 2.7. Chlorophyll a and b Content

The chlorophyll a and b contents were significantly affected by the use of NaCl and the foliar applications ( $P \leq 1\%$ ) (Table 5). The salinity stress reduced their content. On the other hand, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles increased their levels. The 100 mM NaCl salinity stress reduced the chlorophyll a and b content by 79% and 57%, respectively, compared to the control. At the same time, CeO<sub>2</sub>:SA-nanoparticles improved these traits by 80% and 49% compared to the control (Tables 3 and 4).

**Table 5.** ANOVA for the effect of salinity (0–50 and 100 mM NaCl) and foliar applications (no spray, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>: SA-nanoparticles) on the content of chlorophylls, total phenolics, flavonoids, and anthocyanins in *Portulaca oleracea* plants.

	Chlorophyll a Content	Chlorophyll b Content	Total Phenolic Content	Flavonoid Content	Anthocyanin Content
Salinity (S)	**	**	**	**	**
Foliar (F)	**	**	**	**	**
S × F	ns	ns	**	**	*

Significant effects for the main factors and their interaction are indicated with an asterisk: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; ns: non-significant.

### 2.8. Total Phenolic, Flavonoid, and Anthocyanin content

The salinity stress with salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles had a significant impact on the content of phenolics ( $P \leq 1\%$ ), anthocyanins ( $P \leq 5\%$ ), and flavonoids ( $P \leq 1\%$ ) (Table 5). The foliar spray with salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles increased the total phenolic, total flavonoid, and anthocyanin content in purslane along with salinity conditions. The highest values were obtained in the foliar treatment with 100 mM NaCl  $\times$  CeO<sub>2</sub>:SA-nanoparticles, and the lowest content of these compounds was recorded in the control (Table 6).

**Table 6.** Comparison of means for the effects of salinity (0–50 and 100 mM NaCl) and foliar applications (no spray, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles) on some biochemical characteristics of *Portulaca oleracea* plants.

Salinity	Foliar Spray	Phenolic Content (mg g <sup>-1</sup> DW)	Flavonoid Content (mg g <sup>-1</sup> DW)	Anthocyanin Content (mg g <sup>-1</sup> FW)	MDA Content (μmol g <sup>-1</sup> FW)	H <sub>2</sub> O <sub>2</sub> Content (μmol g <sup>-1</sup> FW)	CAT Activity (nmol H <sub>2</sub> O <sub>2</sub> mg Protein m <sup>-1</sup> )	Proline Content (μmol g <sup>-1</sup> FW)
0	No spray	38.67 ± 0.47f	4.00 ± 0.81g	1.56 ± 0.36d	20.67 ± 2.05f	1.33 ± 0.16e	0.30 ± 0.08f	1.93 ± 0.23g
	Salicylic acid	43.00 ± 1.63ef	6.33 ± 0.47ef	1.96 ± 0.12d	17.33 ± 1.69ef	1.07 ± 0.12e	0.53 ± 0.04e	2.67 ± 0.24fg
	CeO <sub>2</sub> -nanoparticles	58.67 ± 2.05d	9.50 ± 1.08ac	3.16 ± 0.44c	14.67 ± 0.77f	1.06 ± 0.09e	0.53 ± 0.06e	3.23 ± 0.47f
	CeO <sub>2</sub> :SA-nanoparticles	43.67 ± 1.24ef	9.66 ± 0.99ab	4.23 ± 0.20b	16.33 ± 2.49f	1.07 ± 0.16e	0.73 ± 0.07bc	3.47 ± 0.38f
50	No spray	54.67 ± 3.68d	7.00 ± 0.41df	2.20 ± 0.28d	52.00 ± 2.94b	2.63 ± 0.28bc	0.66 ± 0.04ce	7.60 ± 0.29bc
	Salicylic acid	66.33 ± 1.24c	6.16 ± 0.23f	3.43 ± 0.30bc	49.67 ± 3.29b	2.00 ± 0.14d	0.83 ± 0.09ab	7.57 ± 0.55bd
	CeO <sub>2</sub> -nanoparticles	71.00 ± 3.55c	7.90 ± 0.53ce	3.83 ± 0.12bc	39.33 ± 1.24cd	2.16 ± 0.23d	0.96 ± 0.08a	6.67 ± 0.49de
	CeO <sub>2</sub> :SA-nanoparticles	76.67 ± 4.66b	8.20 ± 0.80bd	5.70 ± 0.49a	35.67 ± 0.94d	2.03 ± 0.18d	0.83 ± 0.03ab	5.77 ± 0.25e
100	No spray	45.67 ± 3.29e	6.50 ± 0.40ef	1.70 ± 0.43b	57.33 ± 2.05a	4.03 ± 0.27a	0.53 ± 0.04e	9.13 ± 0.12a
	Salicylic acid	58.33 ± 2.05d	8.66 ± 0.47bd	2.13 ± 0.12d	49.33 ± 1.24b	2.97 ± 0.09b	0.56 ± 0.11de	8.30 ± 0.24ab
	CeO <sub>2</sub> -nanoparticles	66.33 ± 3.09c	8.56 ± 0.75bd	3.67 ± 0.09bc	43.00 ± 1.69c	2.87 ± 0.12bc	0.70 ± 0.10bd	8.20 ± 0.49b
	CeO <sub>2</sub> :SA-nanoparticles	91.00 ± 1.63a	11.10 ± 0.69a	6.43 ± 0.71a	39.33 ± 1.24cd	2.57 ± 0.10c	0.66 ± 0.70cd	7.00 ± 0.81cd

Means ± SD (n = 3) with different letters within the same column are statistically different according to the LSD test ( $p < 0.05$ ).

### 2.9. Malondialdehyde Content

The results showed that the salinity stress enhanced the MDA content, but the foliar application significantly reduced this parameter ( $P \leq 1\%$ ) (Table 7). The highest MDA content, which increased by 65% compared to the control, was observed in the 100 mM NaCl variant without foliar application. The foliar application of CeO<sub>2</sub>-nanoparticles and CeO<sub>2</sub>:SA-nanoparticles reduced the MDA content more potently than salicylic acid under the salinity stress (Table 6).

**Table 7.** ANOVA for the effect of salinity (0–50 and 100 mM NaCl) and foliar applications (no spray, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles) on the MDA, proline, and H<sub>2</sub>O<sub>2</sub> content and CAT activity in *Portulaca oleracea* plants.

	Malondialdehyde Content	H <sub>2</sub> O <sub>2</sub> Content	Catalase Activity	Proline Content
Salinity (S)	**	**	**	**
Foliar (F)	**	**	**	**
S $\times$ F	**	**	*	**

Significant effects for the main factors and their interaction are indicated with an asterisk: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; ns: non-significant.

### 2.10. Hydrogen Peroxide Content

The H<sub>2</sub>O<sub>2</sub> content in purslane was affected by the salinity stress and the foliar use of salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles ( $P \leq 1\%$ ) (Table 7). The H<sub>2</sub>O<sub>2</sub> content increased in plants under the salinity stress but declined in the foliar application variant (Table 6).

### 2.11. Catalase Activity

The NaCl treatment and foliar application ( $P \leq 5\%$ ) significantly influenced catalase activity in purslane (Table 7). The salinity stress increased catalase activity. Moreover, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles enhanced catalase activity in plants subjected to 50 mM NaCl more efficiently than in the 100 mM treatment. The highest catalase activity was observed in the treatment with 50 mM salinity and CeO<sub>2</sub>-nanoparticles, and the lowest catalase activity was recorded in the control plants. However, CeO<sub>2</sub>-nanoparticles × 50 mM salinity stress produced a 2-fold increase in catalase activity compared to the control. A decrease in catalase activity was observed at 100 mM salinity stress with all foliar applications (Table 6).

### 2.12. Proline Content

The proline content in the purslane plants was considerably affected by the foliar applications under the salinity stress (Table 7). An enhancement of the proline content was induced by the salinity stress, while it was reduced in the plants subjected to the foliar applications. The highest and the lowest proline content was determined in the 100 mM of NaCl treatment and the control, respectively (Table 6).

### 2.13. Nitrogen Content

All foliar applications with salinity treatments (0, 50, and 100 mM) increased the N content in purslane compared to the control, but the highest nitrogen content was obtained in the plants sprayed with CeO<sub>2</sub>:SA-nanoparticles, which showed a 255% increase compared to the control.

### 2.14. Phosphorus Content

Foliar application of salicylic acid and cerium oxide increased the phosphorus content at all levels of salinity stress compared to the control. The highest phosphorus content was observed in the purslane plants exposed to CeO<sub>2</sub>:SA-nanoparticles, which showed a 104% increase compared to the control. The lowest phosphorus content was recorded in the 100 mM NaCl treatment without foliar application, in which it was decreased by 58% compared to the control.

### 2.15. Potassium and Calcium Content

The potassium and calcium content was significantly affected by the salinity stress and foliar applications ( $P \leq 1\%$ ) (Table 8). The salinity stress reduced the potassium content, and its highest and lowest levels were determined in the control and the 100 mM NaCl treatment, respectively (Table 9). The foliar applications improved the potassium content, and the highest and the lowest values were obtained in the plants subjected to the foliar treatments with CeO<sub>2</sub>:SA-nanoparticles and in the control, respectively (Table 10).

**Table 8.** ANOVA for the effect of salinity (0–50 and 100 mM NaCl) and foliar applications (no spray, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>: SA-nanoparticles) on the elemental content in *Portulaca oleracea* plants.

	N Content	P Content	K Content	Na Content	K/Na Ratio	Ca Content	Mg Content	Fe Content	Zn Content	Mn Content
Salinity (S)	**	**	**	**	**	**	**	**	**	**
Foliar (F)	**	**	**	**	**	**	**	**	**	**
S × F	**	*	ns	**	**	ns	**	ns	ns	**

Significant effects for the main factors and their interaction are indicated with an asterisk: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; ns: non-significant.

**Table 9.** Comparison of means for the effects of salinity on K, Ca, Fe, and Zn content in *Portulaca oleracea* plants.

Salinity	K Content (g kg <sup>-1</sup> DW)	Ca Content (g kg <sup>-1</sup> DW)	Fe Content (mg kg <sup>-1</sup> DW)	Zn Content (mg kg <sup>-1</sup> DW)
0	19.50 ± 1.25a	22.80 ± 1.22a	244 ± 8.10a	73 ± 4.11a
50	17.10 ± 1.88ab	17.50 ± 0.80b	204 ± 14.90b	58 ± 3.41b
100	14.80 ± 2.32b	14.90 ± 0.92c	145 ± 19.00c	50 ± 2.14c

Means ± SD (n = 3) with different letters within the same column are statistically different according to LSD test ( $p < 0.05$ ).

**Table 10.** Comparison of means for the effects of foliar applications (no spray, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles) on K, Ca, Fe, and Zn content in *Portulaca oleracea* plants.

Foliar Spray	K Content (g kg <sup>-1</sup> DW)	Ca Content (g kg <sup>-1</sup> DW)	Fe Content (mg kg <sup>-1</sup> DW)	Zn Content (mg kg <sup>-1</sup> DW)
No spray	12.80 ± 2.46c	13.00 ± 1.63c	173 ± 7.41c	50.3 ± 2.40c
Salicylic acid	16.70 ± 1.41b	18.30 ± 1.48b	193 ± 6.49b	60.0 ± 3.40b
CeO <sub>2</sub> -nanoparticles	18.50 ± 0.81ab	20.00 ± 0.90ab	204 ± 12.39ab	61.0 ± 1.25ab
CeO <sub>2</sub> :SA-nanoparticles	20.60 ± 0.94a	21.30 ± 0.85a	221 ± 9.34a	69.0 ± 7.91a

Means ± SD (n = 3) with different letters within the same column are statistically different according to the LSD test ( $p < 0.05$ ).

The salinity stress decreased the calcium content. The treatment with 100 mM NaCl reduced its value by 53% compared to the control (Table 9). In turn, all foliar applications improved the calcium content. However, the highest calcium content was observed in the foliar application of CeO<sub>2</sub>-nanoparticles and CeO<sub>2</sub>:SA-nanoparticles (Table 10).

#### 2.16. Zinc and Iron Content

The zinc and iron contents were significantly affected by the independent effects of the treatments (Table 8). The salinity treatment reduced the Zn and Fe content compared to the control (Table 9). Higher Zn and Fe content (37% and 27%) was obtained in the plants supplemented with the foliar application of CeO<sub>2</sub>:SA-nanoparticles compared to the control (Table 10).

#### 2.17. Na Content and Na/K Ratio

The results showed that the Na content and the Na/K ratio were significantly affected by the interactions of salinity stress and foliar applications (Table 8). The NaCl treatment enhanced the Na content and the Na/K ratio, but all foliar applications reduced these values. The lowest Na content was observed in the plants sprayed with CeO<sub>2</sub>:SA-nanoparticles. All the three foliar treatments reduced the Na uptake in the purslane plants under the salinity stress (Table 11).

**Table 11.** Comparison of means for the effects of salinity (0–50 and 100 mM NaCl) and foliar applications (no spray, salicylic acid, CeO<sub>2</sub>-nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles) on the content of some nutrients in *Portulaca oleracea* plants.

Salinity	Foliar Spray	N (g Kg <sup>-1</sup> DW)	P (g Kg <sup>-1</sup> DW)	Na (mg Kg <sup>-1</sup> DW)	Na/k	Mg (mg Kg <sup>-1</sup> DW)	Mn (mg Kg <sup>-1</sup> DW)
0	No spray	6.67 ± 0.47gh	0.73 ± 0.12ef	2.17 ± 0.30g	0.14 ± 0.01fg	20.67 ± 0.47de	17.67 ± 2.05de
	Salicylic acid	14.73 ± 1.59b	1.07 ± 0.09bc	1.73 ± 0.09gh	0.09 ± 0.00gh	24.33 ± 2.86c	25.67 ± 2.49b
	CeO <sub>2</sub> -nanoparticles	14.97 ± 1.25b	1.23 ± 0.04b	1.57 ± 0.09gh	0.078 ± 0.00gh	31.00 ± 1.63b	28.00 ± 0.81b
	CeO <sub>2</sub> :SA-nanoparticles	23.70 ± 0.95a	1.50 ± 0.08a	1.27 ± 0.28h	0.05 ± 0.00h	36.67 ± 1.69a	34.67 ± 0.94a
50	No spray	8.27 ± 0.44fg	0.83 ± 0.10df	5.53 ± 0.40b	0.43 ± 0.03d	18.33 ± 0.94ef	14.33 ± 2.47f
	Salicylic acid	10.37 ± 0.62e	0.93 ± 0.09ce	4.53 ± 0.54de	0.26 ± 0.02de	20.90 ± 1.04de	17.33 ± 0.94de
	CeO <sub>2</sub> -nanoparticles	11.17 ± 0.62de	0.96 ± 0.11cd	4.30 ± 0.63de	0.22 ± 0.01ef	23.73 ± 0.61c	18.33 ± 0.67cd
	CeO <sub>2</sub> :SA-nanoparticles	13.67 ± 0.85bc	1.23 ± 0.04b	3.37 ± 0.59f	0.17 ± 0.02f	22.73 ± 1.25cd	20.33 ± 0.92c
100	No spray	6.03 ± 0.49h	0.46 ± 0.06g	8.37 ± 0.41a	0.81 ± 0.08a	13.33 ± 1.24g	10.00 ± 1.63g
	Salicylic acid	9.60 ± 0.57ef	0.63 ± 0.07fg	5.40 ± 0.49bc	0.39 ± 0.08cd	17.67 ± 0.47f	13.67 ± 0.94f
	CeO <sub>2</sub> -nanoparticles	10.97 ± 0.84de	0.81 ± 0.02df	4.57 ± 0.24cd	0.28 ± 0.04d	16.50 ± 1.22f	15.13 ± 0.94ef
	CeO <sub>2</sub> :SA-nanoparticles	12.30 ± 1.12cd	0.86 ± 0.05ce	3.70 ± 0.43ef	0.19 ± 0.01ef	18.27 ± 0.95ef	17.27 ± 1.26de

Means ± SD (n = 3) with different letters within the same column are statistically different according to the LSD test ( $p < 0.05$ ).



### 2.18. Magnesium Content

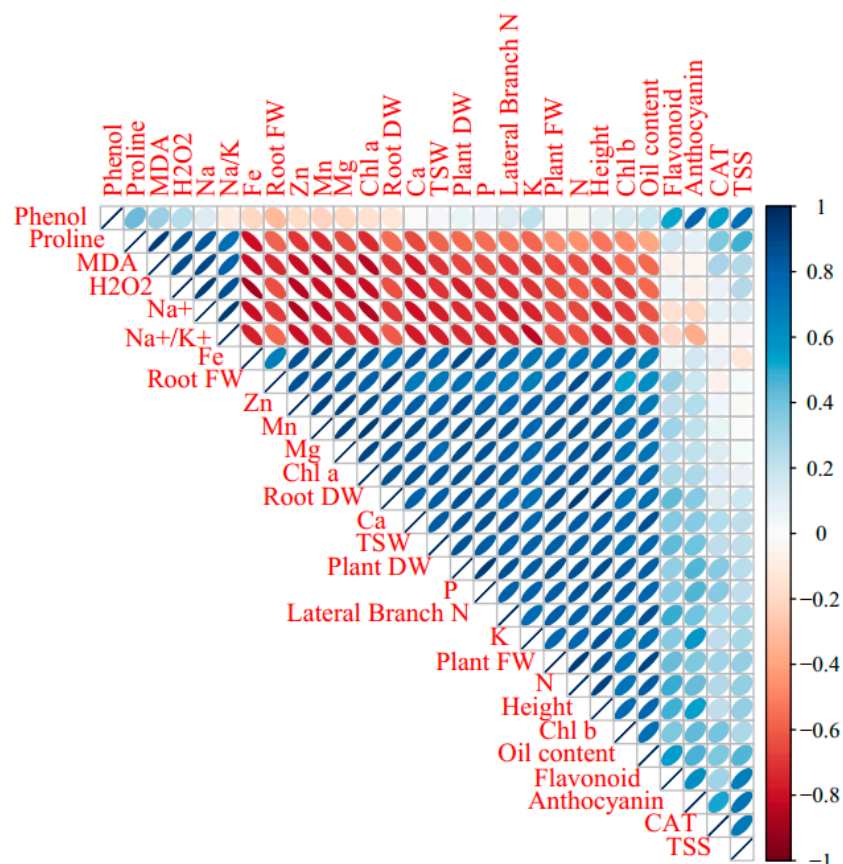
The Mg content was affected by the salinity stress and foliar applications (Table 8). The 100 mM salinity decreased the Mg content, whereas the foliar application of salicylic acid and CeO<sub>2</sub>-nanoparticles increased the Mg amount in the plants, compared to the control. The highest Mg content was observed in the treatment with CeO<sub>2</sub>:SA-nanoparticles, which showed a 43% increase compared to the control (Table 11).

### 2.19. Manganese Content

The lowest Mn content was observed in the 100 mM salinity stress variant without foliar applications. All foliar treatments improved the Mn content at all levels of salinity stress. The highest Mn content was observed in the plants exposed to CeO<sub>2</sub>:SA-nanoparticles, in which the Mn content was enhanced by 96% compared to the control (Table 11).

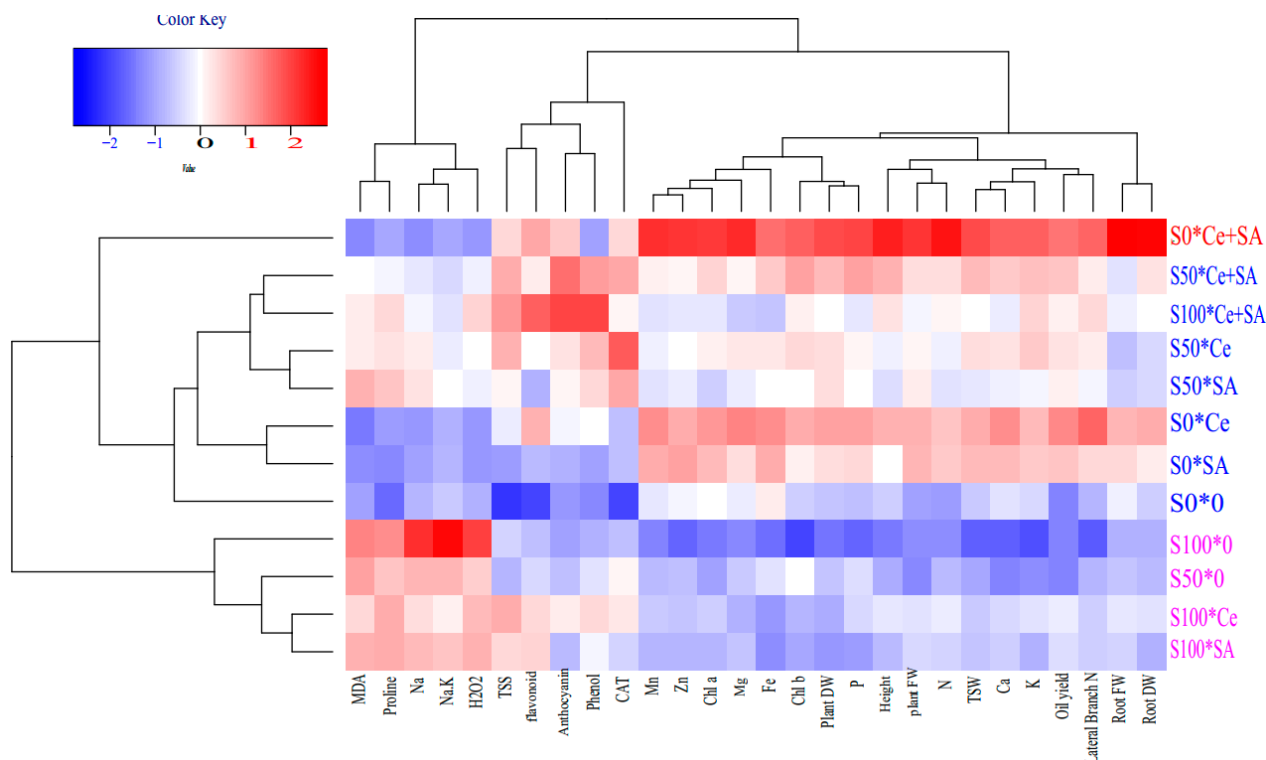
### 2.20. Multivariate Analysis of NaCl Salinity × Foliar Use of Salicylic Acid, Cerium Oxide-NPs, and CeO<sub>2</sub>:SA-NPs Effects on Growth Responses in *Portulaca Oleracea* Plants

The Pearson's correlation heat map of the morphological and biochemical responses, nutrient elements, and antioxidant attributes is presented in Figure 1. The results indicated a significant positive correlation among Na, Na/K, H<sub>2</sub>O<sub>2</sub>, MDA, and proline. In turn, Na, Na/K, H<sub>2</sub>O<sub>2</sub>, MDA, and proline negatively correlated with Fe, Zn, Mn, Mg, Ca, P, K, and N content and with root fresh and dry weight, plant fresh and dry weight, branch number, plant height, oil yield, photosynthetic pigment content, and 1000-seed weight (TSW). The total soluble solid content was correlated with CAT activity and the content of anthocyanins, total phenolics, and flavonoids.



**Figure 1.** Pearson's correlation heat map of NaCl salinity × foliar applications (distilled water, salicylic acid, cerium oxide nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles) on the growth, physiological responses, and elemental content in *Portulaca oleracea* plants. FW & DW refers to fresh and dry weight, respectively.

The heat maps (Figure 2) showed that the MDA, proline, Na, Na/K, and H<sub>2</sub>O<sub>2</sub> had positive compliance with salinity. In turn, the salinity stress adversely affected such traits as the Fe, Zn, Mn, Mg, Ca, P, K, and N content, root fresh and dry weight, plant fresh and dry weight, branch number, plant height, oil yield, photosynthetic pigment content, and TSW. The heat maps revealed that salicylic acid and cerium oxide NPs modulated the harmful effect of salinity stress by improving some of the morphological and biochemical responses and content of nutritional elements.



**Figure 2.** Heat map of the cluster analysis dendrogram for the effects of NaCl salinity and foliar applications (distilled water, salicylic acid, cerium oxide nanoparticles, and CeO<sub>2</sub>:SA-nanoparticles) on the growth, physiological responses, and elemental content in *Portulaca oleracea* plants. FW & DW refers to fresh and dry weight, respectively.

The cluster analysis and dendrograms in the heat map revealed three main groups in the evaluated characteristics of *Portulaca oleracea* under salinity stress × foliar treatments. Group 1 contained the Fe, Zn, Mn, Mg, Ca, P, K, and N content, root fresh and dry weight, plant fresh and dry weight, branch number, plant height, oil yield, photosynthetic pigment content, and TSW. Group 2 contained the flavonoid content, phenolic content, CAT activity, and anthocyanin content. Group 3 contained proline, MDA, H<sub>2</sub>O<sub>2</sub>, Na, and Na/K. Groups 1 and 3 exhibited a negative correlation with each other. In general, the cluster analysis of the heat maps for the treatments showed three main groups. Group 1 contained the use of Ce + SA without salinity stress, and group 2 included Ce, SA, and Ce + SA under the 50 mM NaCl treatment, Ce and SA under the 0 mM NaCl treatment, the control, and, finally, Ce + SA with the 100 mM NaCl treatment. Group 3 included 50 and 100 mM NaCl and Ce + SA under 100 mM NaCl.

### 3. Discussion

The results showed the damaging effects of salinity stress on the growth and yield of *Portulaca oleracea* plants. He et al. [10] reported that the highest purslane yield (as a halophyte plant) was obtained under 100 mM salinity. In a study on two purslane genotypes, salinity adversely influenced the number of leaves due to salt accumulation in the aerial parts of plants [24]. It seems that the yield of purslane plants in a salinity environment is

dependent on other growth stimuli, e.g., plant ecotype or cultivar, temperature, and light intensity [25]. In bean plants, the application of cerium oxide nanoparticles ( $250 \text{ mg L}^{-1}$ ) increased plant yields due to the enhanced pollen grain genesis, ovary development, and enhanced grain protein content [26]. This was related to the reduction in oxidative damage to the ovaries and an improved photosynthesis rate [26–28]. Appropriate concentrations of cerium oxide intensify photosynthesis and plant growth by improving chlorophyll content [27,29]. Salicylic acid is a growth regulator essential in protecting the plant against biotic and abiotic stress factors. Foliar application of salicylic acid under salinity reduced SOD and catalase activity, improved potassium content, and increased rice yields [30]. In a study on sunflowers, salicylic acid under 200 mM salinity stress greatly improved plant growth. The increased plant growth induced by the application of salicylic acid is related to enhanced levels of antioxidants and metabolic activities, which increase resilience to stress [31].

The high specific surface area of nanoparticles and their small size facilitate their penetration into the cell and thus help to improve plant growth [32,33]. Considering the positive effect of  $\text{CeO}_2$  nanoparticles/SA on the fresh and dry weight and height of plants, it seems that their combined influence on several growth-related traits improves the yield and productivity of plants. Even though the most significant effect of the co-treatments was recorded in no-salinity conditions, the application of the nanoparticles under 50 and 100 mM salinity improved the plant yield compared to similar salinity treatments without foliar applications. The chlorophyll content may be considered a biochemical marker of salinity tolerance in plants; its variations reflect the plant's responses to salinity conditions [24]. Numerous studies on different plants revealed that salinity stress alters the morphological and physiological responses by changing the sodium and potassium ion ratio, water relations, nutrient uptake, and stomatal closure, and hence considerably decreases the photosynthetic potential and yield of the plant [8]. More possibly, cerium oxide nanoparticles have functions in chlorophyll biosynthesis and the protection of chloroplast structure against salinity-induced defects [19,34,35]. A study on sweet pepper revealed that SA application (0.2 mM) under 60 mM salinity increased leaf area index, photosynthesis, chlorophyll content, plant dry weight, and SOD activity. SA activates the antioxidant defense mechanism, improves ion absorption, concomitantly reduces ethylene production inside plants, improves stomatal conductance, and helps the plants survive in stressful environments [36].

Salinity stress causes hyper-generation of oxygen free radicals, which have a destructive effect on the integrity of the cell membrane and the structure of phospholipids. Biological macromolecules face turbulence and sudden damage due to the excessive production of superoxide and hydroxyl radicals caused by salinity stress. Our results showed increased  $\text{H}_2\text{O}_2$  and MDA contents under 100 mM salinity stress without foliar application. Similar results were reported by Kim et al. [37] in rice. Under salinity, the enhanced production of SA, ethylene, and jasmonic acid triggers signaling cascades as defense mechanisms versus stress factors that control several molecular, biochemical, and physiological pathways in plants, mitigates the effects of the stressors, and gives plants high endurance in extremely stressful environments [37]. Similar to our results, in *Brassica napus* L.,  $\text{CeO}_2$  nanoparticles treatment reduced the MDA and  $\text{H}_2\text{O}_2$  content in the plants [38].

Plants are equipped with enzymatic and non-enzymatic defense systems to maintain the homeostasis of free radicals. The pioneer enzymatic defense system includes the activity of SOD and CAT enzymes. SOD is in the front line of defense against free radicals and manages the elimination of superoxide radicals [39]. The SA effect in regulating the antioxidant defense system depends on the plant genotype and the time of SA consumption [40]. The results of studies on *Brassica carinata* [41], *Dianthus superbus* [42], and *Sorghum bicolor* [43] showed that SA increased catalase activity in the plants, which indicates the positive role of SA in modulating cellular redox balance and protecting the plant against oxidative damage.

Proline, phenolics, and soluble solids play a predominant action in scavenging oxygen free radicals and protection against stress factors [44]. Treatment of plants with cerium oxide

nanoparticles in flax [18] and rapeseed [32] improved plant growth, increased the content of phenolic compounds, chlorophyll, and plant yield, and reduced malondialdehyde and H<sub>2</sub>O<sub>2</sub> content. Similar results, i.e., an increase in the content of phenolic compounds due to salinity stress, have been reported in safflower [31] and *P. oleraceae* [10]. A study in lettuce showed that cerium oxide nanoparticles increased the content of gallic acid and vanillic acid, clearly representing the positive effect of cerium oxide on the biosynthesis and accumulation of phenolic compounds [44]. Application of salicylic acid under 100 mM salinity stress increased plant yield, phenolic content, chlorophyll biosynthesis, and antioxidant properties of *Salvia coccineae* [45].

Furthermore, salicylic acid (100 µM) under salinity stress did not affect the anthocyanin content in strawberries [34]. Still, the stress increased the content of total phenolics and soluble solids in the plant. It seems that the increase in the content and activity of compatible solutes in response to foliar application of salicylic acid establishes metabolic balance, modulates free radical levels in the plant, maintains photosynthetic capacity, prevents membrane degradation, and maintains chloroplast structure in stress conditions, which are crucially important in protection in stressful environments [20,35,46,47].

Significant correlations were determined between salinity and proline content. Salinity stimulates the expression of genes related to proline biosynthesis [47–49]. In the current study, salinity enhanced the proline content, which was in agreement with results reported by Sdouga et al. [46]. In stressful environments, proline functions as an energy source, a compatible osmolyte, and an antioxidant to protect the cells and tissues against stressors' side effects. In stress conditions, proline accumulation contributes to osmotic adjustment in the cell and protects membrane integrity [42].

Purslane plants are rich in nutrients, e.g., K, Ca, Mg, and Fe [10]. The foliar treatments used in the present study reduced the ratio of sodium to potassium, which clearly showed their effectiveness in reducing the sodium content in the plants. The salinity stress reduced the K, Ca, Fe, and Zn content in the plants. In another study, high salinity levels decreased the content of K, Ca, and Mg in purslane. However, at high salinity levels (300 mM), the Fe content of plants was increased [10]. A possible cause of the reduced mineral uptake in the salinity conditions may be the particular root system architecture of purslane plants which produce small roots with low expansion rates [10]. Maintenance of a balanced Na and K homeostasis is critical for cell survival in stress conditions. Potassium activates various enzymes and regulates cytosolic pH, protein biosynthesis, cellular activity, and stomatal behavior. In stress conditions, plants lose more than 50% of their potassium [8,17,44]. Cerium oxide nanoparticle treatment under salinity increased the potassium content and decreased the cytosolic sodium content. Furthermore, a decrease in the activity of root apoplastic carriers mediating sodium ion absorption in response to cerium oxide treatment has been reported to enhance plant tolerance to stress factors [50]. However, the treatment of plants with salicylic acid improved plant growth, fresh and dry weight, TSS content, and potassium and calcium content [22,43]. There is a positive correlation between internal salicylic acid levels and tolerance to salinity stress in halophyte plants. In stress conditions, salicylic acid acts as a signaling molecule and plays an essential role in activating plant defense mechanisms [22,25].

Nanoparticles mitigate the effect of free radicals and have a crucial function in establishing free radical homeostasis and improving plant responses under stress [17]. Treatment with cerium oxide nanoparticles modulates the activity of ROS-activated NSCC channels (non-selective cation channels) and KOR (K<sup>+</sup> outward rectifying channel) to maintain sufficient amounts of K<sup>+</sup> in plants under salinity stress [17,47,48]. In stress conditions, an increased K/Na ratio improves plant resistance to stress. In flaxseed plants growing under salinity, cerium oxide nanoparticles treatment caused upstream regulation of the HKT1 gene, which played an important role in excluding sodium ions from the cell [17]. Some studies have shown that, following the elimination of free radicals by cerium oxide nanoparticles in stress conditions, this substance can regulate the activity of protein channels associated with potassium efflux, thereby helping to maintain sufficient amounts

of potassium in the cell [47,48]. The foliar application of cerium oxide nanoparticles under salinity stress in *Brassica napus* increased potassium content by 29% and the K/Na ratio by 37%, compared to control plants [32]. Under stress, appropriate nutrient uptake via regulating photosynthetic processes, the antioxidant defense system, and enzymatic activity improves cell and plant function, contributing to efficient survival in stress conditions [8,24,49].

In general, nearly all the growth-related traits such as plant fresh and dry weight, root fresh and dry weight, number of branches, physiological attributes, antioxidant enzyme activities, and mineral content were influenced by the salinity stress. The mitigating function of the foliar applications and especially the ameliorative effects of the combined foliar treatment were promising in compensating salinity defects. The positive roles of the foliar treatments may be ascribed to the tiny size of the nanoparticles, facilitating their absorption, translocation, function, and metabolism. Indeed, nanoparticles have frequently evidenced their efficacy in stressful environments. SA is also a predominant signaling molecule whose stress-induced growth-promoting functions are clearly evident. Therefore, the combined application of SA and cerium oxide nanoparticles will intensify their positive effect. The results from the current study indicate the relevance of the joint application of these compounds and maybe their counterparts to combat the stress impact effectively and to ensure the production of valuable plants in harsh environments.

#### 4. Materials and Methods

This experiment was conducted during the spring and summer of 2020 at the Research Greenhouse of Azarbaijan Shahid Madani University, Tabriz, Iran. The greenhouse environmental conditions were as follows: lightening period: 16:8 day and night, temperature regime: 30:25 day and night, and approx. 70% relative humidity.

##### 4.1. Plant Material and Experimental Setup

*Portulaca oleracea* seeds were surface sterilized with sodium hypochlorite (10%) for 10 min, followed by washing with distilled water 3 times. The seeds (local clone supplied by Pakan Bazr Seed Company, Esfahan, Iran) were planted in 5-L pots containing medium-sized perlite. During the early growth stages and acclimation, the plants were nourished with half-strength Hoagland's nutrient solution. When the plants had three real leaves, salinity treatment was imposed. The salinity levels were 0, 50, and 100 mM of NaCl. The lowest salinity level was 25 mM, which gradually increased to reach the final level within ten days [9]. The optimal pH of the nutrient solution (NS) was 5.8 and was recorded every day and adjusted accordingly using H<sub>2</sub>SO<sub>4</sub> (5% v/v). Following the salinity application, the EC of the nutrient solution was 2.1 mS cm<sup>-1</sup> (0 mM NaCl), 6.0 mS cm<sup>-1</sup> (50 mM NaCl), and 12.0 mS cm<sup>-1</sup> (100 mM NaCl). The pots were regularly washed with tap water once every week to avoid salt deposition on the pot surfaces. Foliar treatments were applied as sprays, and four solutions were used: dH<sub>2</sub>O, 100 μM salicylic acid, 50 mg L<sup>-1</sup> cerium oxide nanoparticles, and cerium oxide: salicylic acid nanoparticles (CeO<sub>2</sub>:SA-NPs; 100 μM + 50 mg L<sup>-1</sup>) [50]. Foliar applications were applied twice. The first application was performed just after salinity exposure (3rd-leaf stage), and the second foliar spray was applied two weeks later. The plant samples were further analyzed two months after the second foliar treatment. The experiment consisted of 12 treatment combinations and 3 replications. Each experimental unit had 4 pots. Each pot contained 4 plants. In total, the experiment was composed of 144 pots with 576 plants. The pots were spaced 50 cm between rows and 20 cm within rows. The pots were manually irrigated with 250 mL of the nutrient solution every 5 days.

##### 4.2. Synthesis of CeO<sub>2</sub> Nanoparticles

The CeO<sub>2</sub> nanoparticles were synthesized via a facile sonochemical procedure. The precursor materials used in this research were Ce(NO<sub>3</sub>)<sub>3</sub> hexahydrate and urea. A total of 0.05 M of (Ce(NO<sub>3</sub>)<sub>3</sub>,6H<sub>2</sub>O) was dissolved in 17 mL of water and ultra-sonicated for

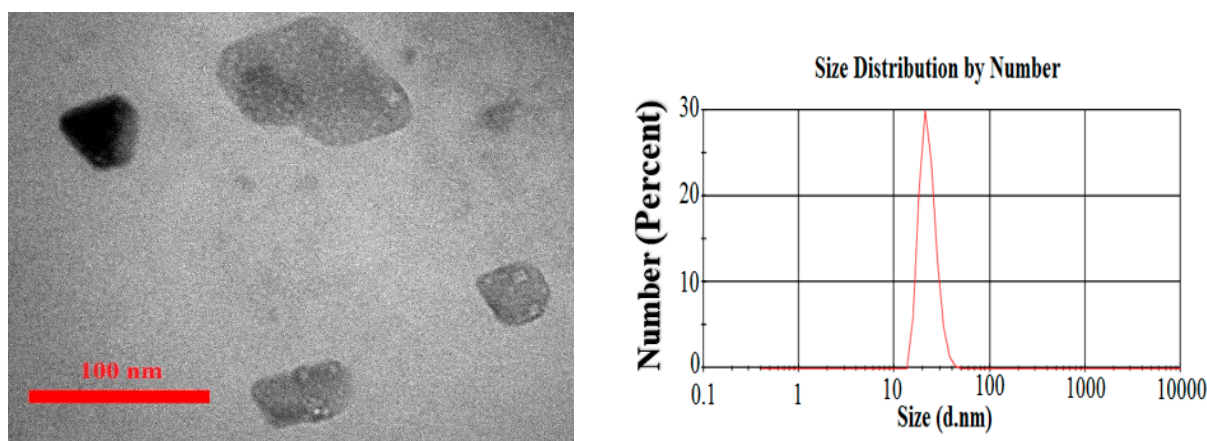
20 min. After that, 20 g urea was added to the solution and allowed to sonicate for 2 h. Subsequently, the solution was centrifuged (8000 rpm/15 min) and washed with water and ethanol to remove the unreacted materials. Finally, the precipitate material was dried at 50 °C overnight. Later, the dried powder was calcined at 850 °C for 5 h.

#### 4.3. Preparation of CeO<sub>2</sub>:SA-Nanoparticles

CeO<sub>2</sub>-NPs were mixed with salicylic acid by molar ratios (1:0 1:1, 1:2, 2:1, and 0:1) with 0, 50, and 100 μm. For example, the 500 mL solution of CeO<sub>2</sub> nanoparticles (50 μm) and salicylic acid (50 μm) (1:1) was fabricated by a sonication bath at 60 °C for 1 hour and then continued by pulse probe sonication for 1 more hour to form a good clear solution without any suspended particles.

#### 4.4. Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) Analysis

Figure 3 (left) represents the TEM image of fabricated CeO<sub>2</sub>: SA-NPs. The successful octahedron nanoparticles are around 30–80 nm, which agrees with the DLS data presented in Figure 3 (right).



**Figure 3.** TEM image of sonochemical synthesis of CeO<sub>2</sub>-SA nanoparticles (left), DLS analysis of CeO<sub>2</sub>-SA nanoparticles (right).

#### 4.5. Fresh and Dry Weight of Plants

The fresh and dry biomass of plant organs was recorded using a digital scale (BB141, Boeco, Germany). After harvesting, the above- and underground plant parts were separated and air-dried until reaching constant weight.

#### 4.6. Chlorophyll Content

Chlorophylls a and b were quantified as in Prochazkova et al. [51] at 645 and 665 nm. Leaf samples (0.5 g for each replication) were extracted using dimethyl sulfoxide (DMSO, Sigma Aldrich, Germany) in the dark for 4 h at 65 °C

#### 4.7. Total Soluble Solid Content (°Brix)

The content of Total Soluble Solids (TSS) in the leaves (1 g for each replication) was quantified with a hand refractometer (Erma, Tokyo, Japan), and the data are presented as °Brix.

#### 4.8. Oil Extraction

The fatty oil yield in seeds was quantified using a diethyl-ether (DEE) solution. A total of 1 gram of ground, dried (60 °C for 24 h) seed from each replication was poured into glass vials. Then, 10 mL of DEE was added and vortexed to acquire a homogenous solution. The solution was transferred to 50 mL falcons and centrifuged for 10 min at 10,000 rpm. The supernatant, i.e., diethyl ether and dissolved fatty oils, were collected and transferred to

another pre-weighed vial. The vials were placed in an oven at 30 °C for 24 h to remove the solvent. The remaining was pure oil, and the oil yield was calculated correspondingly.

#### 4.9. Content of Phenolics and Flavonoids

A methanolic extract of plant tissue (0.5 g dry methanolic extract) was used to quantify the phenolic content by Folin–Ciocalteu reagent at 755 nm, according to Kim et al. [52]. The results were expressed as equivalents of gallic acid (Scharlau, Barcelona, Spain) per g of plant dry weight (mg of GAE/g dry weight).

Total flavonoids were assayed with the aluminum chloride colorimetric method at 510 nm, as described by Kim et al. [52]. The results of total phenolic compounds were expressed as equivalent of gallic acid (Scharlau, Barcelona, Spain) per g of plant dry weight (mg of GAE/g dry weight), and total flavonoids were expressed as rutin equivalents (mg Rutin g<sup>-1</sup> dry weight).

#### 4.10. Anthocyanin Content

Total anthocyanins were determined in fresh leaf (0.5 g for each replication) tissues. The leaves were homogenized in acidified (HCl) methanol. A spectrophotometer measured the absorption of anthocyanins at 550 nm, as in Wanger [53].

#### 4.11. Proline Content

A total of 5 mL of 3% homogenized sulfosalicylic acid was added to 0.2 g of leaf sample. The extract was centrifuged at 6000 rpm for 7 min. Then, 1 mL of the supernatant was mixed with the same volume of ninhydrin acid and 1 mL of glacial acetic acid. Later, the samples were incubated in a 100 °C water bath and then in an ice bath for 30 s. After 30 min, the red phase formed above the sample was used for the proline content measurements at 520 nm, based on the method proposed by Fedina et al. [54]. The proline content was computed using a standard curve of proline, and the results were expressed as micromol of proline per gram of fresh plant weight.

#### 4.12. Hydrogen Peroxide Content and Lipid Peroxidation

The content of H<sub>2</sub>O<sub>2</sub> was assessed according to Alexieva et al. [55]. A total of 0.2 g of leaf tissue was powdered in liquid N<sub>2</sub> and then ground in ice-cold 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 15 min. An aliquot (0.5 mL) of the supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH = 7.5) and 1 mL of 1M potassium iodide. The H<sub>2</sub>O<sub>2</sub> content was evaluated using standards of 5 to 1000 μM of H<sub>2</sub>O<sub>2</sub>, and the calibration curve was plotted accordingly. The absorbance of samples and standards was measured at 390 nm, and results were expressed as μmol H<sub>2</sub>O<sub>2</sub> g<sup>-1</sup> fresh weight.

Lipid peroxidation was determined regarding the malondialdehyde (MDA) content, as described by Sahu and Sabat [56]. Leaf tissue (0.2 g) was homogenized in 0.1% TCA, and the extract was centrifuged at 12,000 × g for 15 min. The reaction mixture of 0.5 mL of the extract and 1.5 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was incubated at 95 °C for 30 min and then cooled in an ice bath. The absorbance was determined at 520 nm and corrected for non-specific absorbance at 600 nm. The MDA amount was determined using the extinction coefficient of 155 mM cm<sup>-1</sup>. The results were expressed as nmol of MDA g<sup>-1</sup> fresh weight.

#### 4.13. Catalase Activity

A total of 0.5 g of purslane leaf samples were homogenized with 0.1 M cold potassium phosphate buffer (pH: 7.5) with 0.5 mM EDTA based on the method developed by Luhova et al. [57]. From the resulting supernatant, 0.05 mL was added to 1.5 mL of 0.1 mM phosphate buffer (pH: 7) and 1.45 mL of double-distilled water. The reaction was started by adding 0.5 mL of 75 mM hydrogen peroxide, and a decrease in absorption was recorded at 240 nm for 1 min [57].

#### 4.14. Elemental Composition

The leaves (3 replications/treatment) were dried at 75 °C for a day, weighted, and ground in a Wiley mill to particles less than 0.42 mm. Subsamples (0.2–0.3 g) were acid digested (2 N HCl) and analyzed for nutrient content as described by Chrysargyris et al. [9]. The content of Na and K was quantified with the flame photometric method (Corning, 410, England). The amounts of Mn, Mg, Zn, Ca, and Fe were measured by atomic absorption spectroscopy (Shimadzu, AA6300, Tokyo, Japan) as previously described by Honarjoo et al. (2013). Phosphorus was determined with the Vanadate Molybdate method [58], and the N content was assessed with the Kjeldahl method [58].

#### 4.15. Experimental Design and Data Analysis

The experiment was conducted as a factorial based on RCBD with three replications. Analysis of variance (ANOVA) was performed by MSTAT-C ver 2.1. Moreover, the significant differences among the means were evaluated with the Least Significance Difference test (LSD) at  $p < 0.05$  and  $p < 0.01$ . Standard deviations ( $n = 3$ ) were evaluated for the traits. Pearson's correlation and cluster dendrogram heat maps were depicted in R software (R foundation for statistical computing, version 4.1.2).

### 5. Conclusions

Salinity stress reduced the yield, chlorophyll content, 1000-seed weight, catalase activity, and elemental content in *Portulaca oleracea*. The highest fresh and dry weight of plants was recorded in the no-salinity  $\times$  CeO<sub>2</sub>:SA-nanoparticles foliar application variant. The maximum number of sub-branches was observed in the foliar treatment with CeO<sub>2</sub>-nanoparticles and CeO<sub>2</sub>:SA-nanoparticles without salinity stress. The TSS content, oil yield, pigment content, antioxidant enzyme activity, and elemental content were responsive to the salinity and foliar applications. CeO<sub>2</sub>:SA-nanoparticles effectively mitigated the adverse salinity effect and partially enhanced the growth and physiological attributes of purslane plants.

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