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Original Article

Effects of Foliar Application of Nano-ZnO on Morpho-physiological Characteristics and Ionic Content of *Salvia leriifolia* Benth. under Salinity Stress

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ABSTRACT

Salinity stress, like many other abiotic stresses, limits the plant growth. Nanotechnology has been globally accepted as a modern, advanced technology that could enhance research in many fields. In order to investigate the effect of Nano-ZnO and salinity stress on morpho-physiological characteristics of *Salvia leriifolia* Benth., a factorial experiment was conducted as completely randomized design with three replications, the treatments were three nano-ZnO concentrations (0, 2, 4 mg/l) and five salinity levels (0, 50, 100, 150, 200 mM NaCl) in the greenhouse of Mashhad Islamic Azad University in 2019. The results showed that salinity stress had a significant effect on morpho-physiological indices as well as mineral nutrients. It was also found that 4 mg/l nano-ZnO concentration at the mild salinity stress (50 and 100 mM) increased leaf and root length, leaf and root soluble protein, and proline content compared to the control. Salinity stress also decreased the concentration of K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, P, and K^+/Na^+ ratio in roots and leaves, while Na^+ content increased significantly during stress in both organs. Nevertheless, the application of nano-ZnO increased the content of Zn^{2+}, Ca^{2+}, K^+, and K^+/Na^+ ratio in leaves and roots.

INTRODUCTION

Salvia leriifolia Benth. (Lamiaceae) is a valuable species in desert rangelands of cold-semi arid to a cold-arid climate in Iran and small parts of Afghanistan [1]. In recent years, the different properties of this plant, such as the attenuation of morphine dependence, hypoglycemic, anti-nociceptive and anti-inflammatory, antioxidant, antiulcer, antibacterial, and anti-mutagenic have been evaluated. The analgesic and sedative activity of *S. leriifolia* leaf extract is comparable to diazepam [2].

Salinity is one of the most important environmental stresses. It affects plant growth by affecting physiological and biochemical processes. Sodium chloride is the most soluble and abundant salt in the world and is a significant contributor to salinity in most areas. Accordingly, much of the salinity research has focused on its effects [3].

Research has shown that salinity damages biological membranes and disrupts cellular and physiological processes, including photosynthesis, nutrient uptake, water uptake, growth, and cellular metabolism, all of which significantly reduce the wet and dry weight of leaves, stems, and roots [4]. In response to salinity and drought stresses, plants enhance the production of osmo-protectants. Osmo-protectants are probably universal and regulate cellular osmotic adjustment, prevent membrane injury, and stabilize proteins and enzymes [5]. Proline is the most important osmolyte and signaling molecule, which accumulates in plants against various stresses. Four reasons have suggested increasing proline accumulation during stress, including stimulation of glutamic acid synthesis, reduction of its transport through the phloem,

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Inhibition of oxidative stress and ultimately prevention of degradation and disruption of protein synthesis [6].

Researches have shown that total soluble protein content is also subject to environmental stresses. In this regard, it was reported that salinity induces different types of them. Proteins may provide a form of nitrogen storage to be reused in the high stresses and involved in osmotic regulation [7].

Salinity also may decrease or increase the concentration of micronutrients in the plant or the concentration of these elements is ineffective. Considering the nutritional status of the plant when exposed to salt stress can lead to a strategy to increase plant tolerance to stress [8]. Salinity may reduce micronutrients uptake due to stronger competition with salt cations at the root surface. In saline and sodic soils, the solubility of micronutrients such as Cu, Mn, Fe, Zn, and Mo is particularly low, and plants growing on such soils may experience deficiencies in these [9].

Zinc contributes in growth and development and play an essential physiological role in plants. Zn is also a very important micronutrient in plants and a key constituent of more than 70 metalloenzymes [10]. Zinc plays a role in chromosome synthesis, carbohydrate and protein metabolism, chlorophyll production, photosynthesis, preservation of biological membranes, tryptophan synthesis, production of growth hormones (Auxin) such as indole acetic acid and gibberellic acid, and in enzymatic and reaction systems [11].

Today, nanotechnology is expanding in all fields of science, including various agricultural sectors. Nanoparticles can interact with plant biological systems and these specific interactions originate mainly from their small size, large surface area, and intrinsic catalytic reactivity [12]. Therefore, this study conducted to investigate the effect of nano-ZnO spraying on the growth and proline, soluble proteins and ionic content of *S. leriifolia* in different soil salinities.

MATERIAL AND METHODS

To investigate the effect of spraying of nano-ZnO on *S. leriifolia* under saline conditions, a factorial experiment conducted in a completely randomized design with three replications in the greenhouse of Mashhad Islamic Azad University in 2019. The treatments were three levels of nano-ZnO spraying solution (0, 2 and 4 mg/l) and five salinity levels (0, 50, 100, 150 and, 200 mM NaCl). The nano-ZnO was manufactured in China by Pishgaman Nanomaterials of Iran and had a purity of 99%, average particle size less than 30 nm, particle specific area greater than 30 m^2 per gram, white and powder.

The seeds were obtained from Khorasan-e-Razavi Agricultural and Natural Resources Research and Education Center. After disinfecting the seeds with 60% carbendazim wet table powder, they were incubated between two disks of Whatman No.1 filter papers in 90 mm Petri dishes and germinate in a refrigerated germinator at a temperature of 15±1°C [13]. After germination, six seedlings with a maximum root length of 10 to 15 mm transferred to plastic pots. Pots of approximately 3.5 to 4 kg soil weight with physicochemical properties of Table 1 were exposed to greenhouse conditions at a temperature of 25±4 °C, a brightness of 5000 lux, and a photoperiod of 16 hours of illumination and 8 hours of darkness. Salinity treatments was applied to pots in the third week after transferring to different solutions (0, 50, 100, 150 and, 200 mM NaCl). For this purpose, a container was placed at the bottom of the pots so that the outlet water of the pot could be added to the pot again in the next irrigation.

Foliar application of nano-ZnO at concentrations of 0, 2, 4 mg/l for eight weeks was performed weekly by aerial spraying of 8cc for each plant. During stress, to minimize the experimental error and the uniform growing conditions for plants, pots per treatment were randomly switched. Nano-ZnO foliar application at concentrations of 0, 2, 4 mg / l for eight weeks with weekly aerial spraying of 8 cc per plant. During stress, pots were randomly replaced in each treatment to minimize experimental error and uniform plant growth conditions.

Table 1 Some characteristics of the soil used in the experiment.

pH	EC	Lime	Clay	Silt	Sand	OC	N	SP	K	P	Zn
	(dS/m)	(%)							(ppm)		
7.4	1.2	12	4	10	86	0.98	0.16	35	160	35	0.54

Morphological Traits

Leaf and root lengths were measured in cm, and leaf and root weights with scales with 0.0001 accuracies were measured in grams. To measure leaf and root dry weight, samples were placed in the oven at 70 °C for 48 hours and then weighed.

Physiological Traits

Proline: 0.5 grams of fresh plant matter (leaf and root) weighed, and proline content was determined by ninhydrin assay at A 520 nm [14].

Soluble proteins: Determination of leaf and root soluble proteins determined by the Bradford method and bovine albumin standard. Initially, 50 mg of a leaves were extracted with 3 ml buffer (phosphate buffer with pH= 6.8) at 4 °C on ice. The samples were then centrifuged for 20 minutes at 15,000 g at 4 °C with a refrigerated centrifuge (Vision Model VS-15000 CFN). 100ml of the resulting vessel added to 5 ml of Bradford reagent, and after complete mixing, the optical absorption of the samples was recorded at 595 nm using a spectrophotometer (Shimadzu UV-1100). Finally, the concentration of soluble proteins was determined using the standard curve [15].

Ion content: In order to investigate the effect of salinity stress on the concentrations of various elements such as sodium, potassium, calcium, phosphorus, zinc, magnesium, copper, and iron and their distribution in root and leaf, some fresh tissue of each organ placed at 80 °C for 48 hours. The dried texture used to make ash. To a certain amount of dry tissue, a concentration of concentrated nitric acid (10 ml of nitric acid per 10 g dry tissue) added. After 48 hours to complete the digestion of plant tissue, the solution is heated slowly to be clear and colorless finally. Then, the volume of the solution in the Jujube balloon distilled to 50 ml. This solution used to measure the elements in plant tissue. Determination of sodium, potassium, and calcium content by a flame photometer (manufactured by Jenway Model PFP7) with sodium, potassium, and calcium filters and to measure other elements by Inductively Coupled Plasma Emission Spectrometry (ICP-Arc 45) used.

Statistical analysis: Data were analyzed using SAS 9.0 statistical software and the means were compared by Multiple Duncan's test at 0.05% significant level.

RESULTS AND DISCUSSION

Morphological Traits

The results of the analysis of variance showed that the effect of salinity and foliar nano-ZnO on root and leaf length and root dry weight were significant at 99% level, while the interaction effect was not significant (Table 2).

Results showed that with increasing NaCl, root length (RL) increased initially, but at 150 and 200 mM, RL significantly decreased. The highest RL obtained in 50 mM stated that the decrease in shoot and root growth could be due to the toxic effects of sodium and chlorine and consequently, a decrease in potassium uptake and imbalance in the absorption of other nutrients by the plant [16]. Therefore, the decrease in growth observed in *S. leriifolia* can also be due to ion toxicity and consequently the accumulation of sodium and chlorine ions, decrease in potassium uptake, and complications due to potassium deficiency and decrease in water uptake due to increased osmotic potential and water deficit ability. Salinity-induced water deficit also reduces leaf cell expansion, which leads to a more rapid concentration of Na^+ due to reduced cell volumes [17].

According to the results, nano-ZnO increased RL, so that the highest RL in 50 and 100 mM NaCl concentration observed in 4 mg/l nano-ZnO treatment, which was 82.3% and 72.41% higher than the control, respectively. The lowest RL was observed at 200 mM NaCl without nano-ZnO, with a decrease of 73.30% compared to the control (Table 3).

The data showed that the effect of salinity, nano-ZnO on root dry weight (RDW) was significant (Table 2). Mean comparison showed that the highest RDW belonged to a salinity level of 50 mM, which was significantly different from other levels. Also, nano-ZnO caused an increase in RDW so that the highest amount of RDW was in 50 mM NaCl and 4 mg/l Nano-ZnO (Table 3).

The effect of salinity on leaves dry weight (LDW) was significant. The LDW was not affected up to 50 mM salinity but decreased significantly with increasing salinity. Application of 4 mg/l Nano-ZnO in all salinity levels improved LDW than its control treatments (Table 2). Similar report has also observed in sage plants. [18].

The plants that grow in saline soils, due to their osmotic properties, also experience water stress in

addition to salinity, which slows down growth, cell division and growth and all plant metabolic reactions [19]. Some reports have attributed the decrease in growth parameters in *S. sclarea* L. due to the transfer of photosynthetic material from growing areas to produce enzymes and antioxidant compounds (secondary metabolites) [18].

Hendawy and Khalid [20] reported the positive effect of 100 mg/l zinc in *S. officinalis* on leaf dry weight gain and plant height under salinity stress. Torabian *et al*., [21] on sunflower plant reported that nano-ZnO spraying under salinity stress increased dry weight, leaf area, photosynthetic parameters, and zinc concentration in leaves. They concluded that spraying nano-ZnO was more active on shoot dry weight than root dry weight, and the use of micronutrients increased photosynthetic activities and consequently increased cell division, leading to increased biomass. The decrease in photosynthesis rate has observed in zinc-deficient plants [22].

Proline content: Analysis of variance showed that the effect of salinity and nano-ZnO on leaf and root proline content was significant at 99% level, and its interaction was significant at 95% level (Table 4). Results indicated that increasing salinity up to 100 mM NaCl significantly increased leaf and root proline content in *S. leriifolia,* but increasing salinity decreased proline content. Application of 2 and 4 mg nano-ZnO in 100 mM NaCL increased leaf proline by 9.8 and 14.3% and root proline by 11.4% and 16%, respectively, compared to control. (Table 5).

Increased proline content in *S. officinalis* under salinity stress has also reported [23]. The higher accumulation of proline could be due to enhanced activities of ornithine amino transferase (OAT), the enzymes involved in proline biosynthesis, or due to inhibition of proline catabolizing enzymes, proline oxidase and proline dehydrogenase (PDH) [24].

Torabian *et al*., [21], by investigating the effect of nano-ZnO foliar application on five sunflower species under salinity stress, attributed the decrease in glutamate conversion to the chlorophyll biosynthesis pathway to the increase in proline content. Glutamate is a precursor to chlorophyll and proline. Increasing the proline content at the salinity level of 100 mM in *S. leriifolia* and then decreasing it at higher levels, it can be concluded that this plant tolerates this salinity level by using the mechanism of increasing the proline content, but cannot tolerate higher salinity levels.

Increasing the amount of nano-ZnO increased proline content in leaves and roots under both stress and non-stress conditions (Table 5). Zinc is a constituent of some non-enzymatic proteins and a co-factor of some enzymes and involved in the metabolism of carbohydrates and proteins such as aldolases, isomerase, trans-phosphorylase, in environmental stress condition [23].

Total Soluble protein: Results indicated that the effect of salinity, nano-ZnO and its interaction on leaves and root soluble protein content were significant (Table 4). Increasing salinity stress increased leaves and root soluble protein content up to 100 mM and decreased with increasing higher salinity levels (Table 5). Leaves soluble protein content was highest in 100 mM salinity and 4mg/l nano-ZnO and decreased with increasing salinity level. Root soluble protein accumulation also raised by increasing the content of nanoparticles. The lowest leaves soluble protein observed in 200 mM NaCl treatment without nanoparticle application. Manaa *et al*., [26] in tomato also found that the soluble protein levels decreased with increasing salinity. Salinity stress causes oxidative stress. Free radicals produced during oxidative stress due to their affinity with proteins destroy nucleic acids and cellular proteins and cellular destruction [27]. Zinc is an essential element for protein synthesis and gene expression in plants [25], so that when zinc level decreases, the protein concentration decreases while the amino acids increase [28]. Nano-fertilizers, by rapidly participating in enzymatic reactions, increase the activity of antioxidant enzymes and stabilize protein bands against oxidative stress [29].

Sodium content: The results showed that the main effects of salinity on the amounts of leaves and root sodium were significant at 99% probability level, while effects of nano-ZnO and its interaction with Salinity were not significant (Table 6). The sodium content of leaves increased significantly with increasing salinity levels. This increase in the salinity level of 200 mM was 173% compared to control. (Table 7). In the case of root, with increasing salinity level, sodium content was increased by 50, 100, 150, and 200 mM as compared to the control with 28, 65, 92, and 103%, respectively (Table 7). Research has shown that salinity increases the sodium ion in the aerial parts of plants, and especially in the root. When salinity stress occurs, the reduction of osmotic potential and toxicity caused accumulation of sodium

ions and leads to a decrease in water potential, a change in the absorption of essential ions and an ionic imbalance and ultimately decreased photosynthesis rate and limited leaf growth [30].

Zn alleviates the NaCl stress injury by inhibiting Na^+ and/or Cl uptake or translocation. Zn has a stabilizing and a protective effect on cell membranes against oxidative and peroxidative damage and loss of plasma membrane integrity [31]. It is possible that Zn deficiency promoted uptake of Na due to its adverse effects on membrane integrity [32]. Zinc application was decreased Na concentration in eggplant [31] and pepper [32].

Potassium content and K^+/Na^+ ratio: Analysis of variance showed that the effect of salinity and ZnO on leaves and root potassium content was significant at 99% level (Table 6). With increasing salinity levels of 50, 100, 150 and 200 mM, the average leaf potassium decreased 11.9, 22.3, 43.3 and 58.6%, respectively, compared to the control [33].

Results indicated that the highest amount of leaf and root potassium obtained in 4 mg/l nano-ZnO in saline-free conditions, and the lowest amount was obtained in salinity of 200 mM NaCl without nano-ZnO. Also, at different levels of salinity, 4 mg/l nano-ZnO concentration had a more additive effect on leaf potassium than other levels. Potassium is one of the most essential elements in the balance of anion and cation within the cell. It also has a significant effect on the uptake of other elements by the root and helps to eliminate of certain nutrients in the soil [4].

The results of Table 6 show that the interaction of salinity and nano-ZnO on the K^+/Na^+ ratio in leaves was significant at 99% level. This ratio decreased significantly with increasing salinity. So that the lowest K^+/Na^+ ratio decreased by 82.3% and 88.2%, at concentrations of 150 and 200 mM, respectively, compared to the control (Table 7). Potassium to sodium ratio in plants is always used as an essential factor in determining plant tolerance to salinity. Sodium has a dual effect on plant potassium uptake, at low concentrations of NaCl (50 and 100 mM) in the medium, potassium uptake increased and with increasing NaCl concentration, potassium uptake decreased [34].

These results indicate the positive effect of nano-ZnO on the reduction of sodium uptake by the plant. Zinc application can reduce the harmful effect of sodium and chlorine in plants and prevent sodium transfer in plants and increase potassium concentration in stem and thus increase potassium to sodium ratio [32].

Calcium content: The results showed that the effect of salinity on leaf and root calcium content was significant at 95% level while were not affected by nano-ZnO and their interaction (Table 6). Means comparison showed that with increasing salinity levels, leaf and root calcium content decreased. Leaf calcium reduction at 5, 100,150, and 200 mM NaCl compared to control was 8.8, 24.8, 39, and 56.7%, respectively (Table 7). Calcium is a particularly important nutrient in plants exposed to NaCl salinity because of its role in reducing Na^+ uptake as well as increasing both K^+ and Ca^{2+} uptake. The results of this experiment showed that with increasing salinity the concentration of calcium in leaves decreased, which could be due to disturbance in the particular properties of root membrane, which does not differentiate between calcium and sodium and absorbs more sodium, which is more concentrated in the environment [35].

The effect of nano-ZnO on leaf and root calcium content showed that nano-ZnO increased the amount of calcium in leaf and root (Table 6). In salinity stress, sodium entry into the apoplastic space and calcium replacement depolarize the cell membrane, causing selective uptake of some ions [36].

Zn content: Analysis of variance showed that salinity, nano-ZnO and its interaction had a significant effect on leaf and root zinc content (Table 8). Increasing levels of salinity decreased the amount of this element in roots and leaves. The mean of main effects of salinity treatments showed that the highest amount of zinc in leaves observed in control treatment, which decreased in 50, 100, 150, and 200 mM NaCl by 14, 34.9, 52.6, and 63%, respectively (Table 9). Research has shown that Zinc affects the capacity for water uptake and transport in plants and reduces the adverse effects of short periods of heat and salt stress. The regulation of the gene expression required for the tolerance of environmental stresses in plants are Zn dependent [37]. Tavallali *et al*. [38] showed that salinity stress decreased zinc concentration in pistachio (*Pistacia vera* L.) seedlings and caused deficiency symptoms. Results showed that the increase of nano-ZnO significantly increased the amount of zinc in the leaf and root of

the *S. leriifolia*. The increase in leaf and root concentration at 4 mg/l compared to control was 156 and 54.4%, respectively. It also found that the highest amount observed in control with 4 mg/l Nano-ZnO concentration, which was significantly different from other levels).

Iron Content: The results of Table 7 show that salinity, nanoparticles, and their interactions on root and leaf iron content were significant at 99% level. Increasing salinity significantly reduced the amount of iron in the root and leaves. The amount of iron element in root and leaf at the concentration of 200 mM decreased by 65.8% and 75.74%, respectively, compared to control (Table 9). Means comparison of nano-ZnO effect on the amount of iron in root and leaf of *S. leriifolia* showed that iron content decreased with increasing nano-ZnO level (Table 9). Decreasing iron content have also reported in Japanese mint (*Mentha arvensis* L.) [39]. It seems that the reason for the decrease in iron content at high concentrations of zinc could be the competition between these two elements in the uptake and inhibition of chelating processes during the uptake and transfer of the iron element from the roots to the shoots [40].

Magnesium content: The results of Table 8 showed that the interaction of salinity and nano-ZnO on root and leaf magnesium content was significant at 99% level. The means comparison showed that magnesium content in roots and leaves decreased with increasing salinity. Also, results showed that the highest amount was observed in the control treatment and 4 mg/l nano-ZnO, and the lowest in 200 mM salinity without nano-ZnO (Table 9). Research has shown that Mg^{+2} is involved in protein synthesis. It also forms the central atom of chlorophyll and is essential in determining the size, structure, and function of chloroplasts [41]. In high salinities, exceptionally high sodium ions and unbalanced concentrations of the elements in the soil, the binding sites are mainly occupied by sodium due to the similarity of magnesium and sodium uptake sites and, consequently, less magnesium is transferred into the root cells [42].

CONCLUSION

In this study, salinity stress reduced the shoot dry matter production in *Salvia leriifolia* with adverse effect on morpho-physiological indices and concentration of K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, and K^+/Na^+ ratio in root and leaves. In contrast, foliar application of 4 mg/l nano-ZnO at 50 and 100 mM NaCl salinity improved morpho-physiological indices such as root and leaf length, dry weight, and increased proline content, soluble proteins, Zn^{2+}, Ca^{2+}, K^+, and K^+/Na^+ ratio compared to control and another nano-ZnO levels. Nano-ZnO significantly reduced sodium content in leaf and root organs. The results showed that the application of nano-ZnO in salinity conditions improved ability of *Salvia leriifolia* Benth. to withstand the harmful effects of salinity.

Table 2 Analysis of variance for the effect of nano-ZnO on growth parameters of *S. leriifolia* Benth. under salt stress

	df	Root length	Leaf length	Root dry weight	Leaf dry weight	Root fresh weight	Leaf fresh weight
Salt	4	271.9 **	94.8 **	0.039 **	0.162 **	1.195 **	17.661 **
Nano-ZnO	2	650.5 **	72.6 **	0.036 **	0.017 ^{ns}	2.563 **	4.262 **
Salt* ZnO	8	10.5$^{n.s}$	2.1$^{n.s}$	0.003 ^{ns}	0.003 ^{ns}	0.058 *	0.381 *
Error	30	16.2	3.3	0.002	0.007	0.022	0.158
C.V.		24.4	17.2	12.7	26.2	10.25	10.79

^{ns}, *, **: Representing non-significant and significant effects at 5 and 1% probability level, respectively.

Table 3 Interaction effect of salinity and Nano-ZnO on length, dry and wet weight of root and shoot of *Salvia leriifolia* Benth.

Salinity (mM)	Nano-ZnO (mg/l)	Root length (cm)	Leaf length (cm)	Root dry weigh(gr)	Leaf dry weigh(gr)	Root fresh weigh (gr)	Leaf fresh weigh(gr)
0	0	15.23 de	12.46 bc	0.388 cd	0.523 a	1.723 bc	5.544 a
	2	19.70 b-d	14.90 ab	0.394 cd	0.462 ab	1.886 b	5.258 ab
	4	23.80 a-c	16.13 a	0.434 bc	0.475 ab	2.364 a	5.475 a
50	0	19.50 b-d	12.06 bc	0.492 ab	0.481 ab	1.101 fg	4.513 bc
	2	17.96 cd	12.13 bc	0.418 cd	0.403 a-c	1.704 bc	4.719 bc
	4	27.76 a	15.23 ab	0.539 a	0.451 ab	2.258 a	5.362 ab
100	0	17.36 cd	8.50 de	0.348 c-f	0.288 cd	1.015 f-h	2.974 fg
	2	15.86 d	9.73 cd	0.422 b-d	0.255 c-e	1.395 de	2.786 fgh
	4	26.26 ab	12.16 bc	0.490 ab	0.320 cd	1.642 bcd	3.989 de
150	0	7.43f g	6.26 ef	0.330 d-f	0.206 de	0.760 hi	2.098 h-j
	2	12.62 def	8.66 de	0.336 d-f	0.221 de	1.266 ef	2.553 g-i
	4	16.93 cd	11.03 cd	0.437 bc	0.294 cd	7.602 cd	3.477 ef
200	0	4.06 fg	3.56 f	0.286 ef	0.173 de	0.631 i	1.466 j
	2	8.10 e-g	5.92 ef	0.263 f	0.103 e	0.964 gh	1.874 ij
	4	15.00 de	10.13 cd	0.365 c-e	0.238 de	1.596 cd	3.178 fg

* Values with the same letter are not significantly different (P ≤0.05) with other treatments

Table 4 Analysis of variance for the effect of Nano-ZnO on proline and soluble proteins of *Salvia leriifolia* Benth. under salt stress

Treatments	df	Proline		Soluble protein	
		Leaves	Root	Leaves	Root
Salt stress	4	5.374 **	3.323 **	4.732 **	1.500 **
Nano-ZnO	2	0.543 **	0.918 **	0.603 **	0.413 ^{ns}
Salt*ZnO	8	0.110 *	0.102 ^{ns}	0.081 *	0.277 ^{ns}
Error	30	0.039	0.063	0.034	0.202
C.V.		15.43	12.90	7.01	7.45

^{ns}, *, **: Representing non-significant and significant effects at 5 and 1% probability level, respectively.

Table 5 Interaction effect of salinity and Nano-ZnO on Proline and Soluble protein content of *Salvia leriifolia* Benth.

Salinity (mM)	Zno Nano (mg/l)	Proline (µm/g FW)		Soluble protein (mg/g FW)	
		Leaves	Root	Leaves	Root
0	0	1.38 d	1.453 e-g	1.607 gh	0.754 d
	2	1.74 c	1.499 e-g	1.740 gh	0.801 cd
	4	2.28 b	1.580 d-f	1.875 fg	0.884 cd
50	0	1.91c	1.788 c-e	2.374 e	1.010 cd
	2	2.25 b	1.919 b-d	2.257 e	1.193 a-d
	4	2.54 ab	2.047 bc	2.759 e	1.284 a-d
100	0	2.32 ab	2.232 ab	3.038 b-d	1.497 a-c
	2	2.55 ab	2.487 a	3.324 b	1.600 a-c
	4	2.65 a	2.590 a	3.647 a	2.003 a
150	0	0.97 e	1.117 gh	2.92 6 cd	1.582 a-c
	2	0.96 e	1.596 d-f	3.242 bc	1.520 a-c
	4	1.02 e	1.991 bc	3.024 b-d	1.907 ab
200	0	0.71 e	0.837 h	1.465 h	0.925 cd
	2	0.72 e	0.863 h	1.783 gh	1.108 b-d
	4	0.72 e	1.356 fg	2.109 ef	1.156 a-d

* Values with the same letter are not significantly different (P ≤ 0.05) with other treatments.

Table 6 Analysis of variance for the effect of zinc oxide nano on Na^+, K^+ and Ca^{+2} contenet of S*alvia leriifolia* Benth. under salinity stress

Treatments	df	Na +		K +		Ca +2		K^+/Na^+	
		Leaves	Root	Leaves	Root	Leaves	Root	Leaves	Root
Salt stress	4	389592 **	141994 *	409820**	409819 **	28185 **	19088 *	14.1**	40.8 ^{ns}
Nano-ZnO	2	61222 ^{ns}	53467 ^{ns}	152419 **	152419 **	2555 ^{ns}	10253 ^{ns}	1.4**	21.8 ^{ns}
Salt*ZnO	8	8149 ^{ns}	7194 ^{ns}	4265 ^{ns}	4265 ^{ns}	371 ^{ns}	49 ^{ns}	0.1$^{n.s}$	17.4 ^{ns}
Error	30	41094	4787	8674	8674	3353	7081	0.2	19.7
C.V.		36.4	47.8	12.6	12.6	31.9	50.5	24.3	29.6

^{ns}, *, **: Representing non-significant and significant effects at 5 and 1% probability level, respectively.

Table 7 Interaction effect of salinity and Nano-ZnO on Na^+, K^+ and Ca^{+2} content of *Salvia leriifolia* Benth.

Salinity (mM)	Zno Nano (mg/l)	Na + (mg/Kg)		K + (mg/Kg)		Ca +2 (mg/Kg)		K +/ Na +	
		Leaves	Root	Leaves	Root	Leaves	Root	Leaves	Root
	0	247.0 d	273.1 a	912.3 bc	1175.1 a-c	246.0 a	194.3 ab	3.73 a	5.95 ab
0	2	273.1 cd	293.2 a	981.3 ab	1234.5 ab	235.9 ab	201.0 ab	3.63 a	6.27 ab
	4	294.1 cd	303.9 a	1098.4 a	1303.8 a	252.4 a	284.9 a	3.96 a	4.95 ab
	0	482.5 a-d	410.4 a	780.9 c-e	987 a-e	226.2 ab	180.6 ab	1.65 cd	3.02 ab
50	2	406.5 b-d	371.0 a	859.5 b-d	967 b-d	205.4 a-c	194.3 ab	2.27 bc	2.64 ab
	4	349.0 b-d	332.5 a	994.4 ab	1116.7 a-c	237.9 ab	228.4 ab	2.87 b	3.74 ab
	0	706.1 ab	572.8 a	758.0 c-e	755.3 a-e	185.8 a-d	153.3 ab	1.18 de	1.67 b
100	2	612.8 a-d	475.9 a	659.3 e-g	733.3 a-e	172.0 a-d	166.9 ab	1.17 de	1.81 b
	4	546.2 a-d	392.4 a	884.9 b-d	945.8 a-e	194.2 a-d	201.1 ab	1.69 cd	11.12 a
	0	832.5 a	636.4 a	529.2 g-i	574.1 c-e	128.7 b-d	119.1 ab	0.66 e	1.04 b
150	2	743.1 ab	549.6 a	560.1 f-h	665.5 b-e	146.8 a-d	139.6 ab	0.87 de	1.46 b
	4	638.4 a-d	479.7 a	716.1 d-f	857.7 a-e	171.9 a-d	166.9 ab	1.22 de	1.78 b
	0	855.6 a	694.0 a	366.3 i	345.9 e	85.3 d	87.2 b	0.44 e	0.44 b
200	2	705.7 ab	595.1 a	432.0 hi	422.2 de	106.3 cd	91.8 ab	0.68 e	0.59 b
	4	661.5 a-c	481.2 a	589.4 f-h	653.0 b-e	125.8 b-d	132.8 ab	0.99 de	1.28 b

* Values with the same letter are not significantly different ($P \leq 0.05$) with other treatments.

Table 8 Analysis of variance for the effect of Nano-ZnO on Zn + 2, Cu 2, Fe +2, Mg + 2 and P content of *Salvia leriifolia* Benth. under salt stress

Treatments	df	Zn + 2		Fe + 2		Mg + 2	
		Leaves	Root	Leaves	Root	Leaves	Root
Salt stress	4	1125.93 **	0.256 **	984.94 **	78.05 **	1413.36 **	77.07 **
Nano-ZnO	2	2407.81 **	0.255 **	46.65 **	34.62 **	1247.62 **	167.61 **
Salt*ZnO	8	19.98*	0.008 ^{ns}	19.76 **	3.43 **	90.77 **	15.59 **
Error	30	7.08	0.009	2.81	0.93	19.17	2.23
C.V.		9.28	16.56	8.32	12.23	6.51	4.71

^{ns}, *, **: Representing non-significant and significant effects at 5 and 1% probability level, respectively.

Table 9 Interaction effect of salinity and Nano-ZnO on Zn+2, Cu +2, Fe+2, Mg+2 and P content of *Salvia leriifolia* Benth.

Salinity (mM)	Nano- ZnO (mg/l)	Zn+2 (ppm)		Fe+2 (ppm)		Mg+2 (ppm)	
		Leaves	Root	Leaves	Root	Leaves	Root
0	0	28.16 ef	0.633 bcd	34.92 a	13.21 a	83.54 ab	39.30 a
	2	43.17 c	0.762 ab	29.67 bc	10.28 cd	77.77 bc	37.33 ab
	4	56.90 a	0.901 a	25.82 d	9.53 de	87.43 a	31.11 ef
50	0	23.38 f	0.541 def	31.95 b	12.68 ab	82.80 cd	36.23 bc
	2	36.40 d	0.750 ab	30.91 b	8.91 def	67.74 ef	35.76 bc
	4	50.40 b	0.888 a	27.90 cd	7.43 fghi	85.24 ab	32.73 de
100	0	13.49 g	0.412 efg	25.48 d	11.44 bc	66.91d e	37.97 ab
	2	28.18 ef	0.566 cde	19.55 e	7.78 efg	54.00 gh	26.82 gh
	4	41.74 c	0.726 abc	18.61 e	7.61 fgh	78.88 bc	29.23 fg
150	0	12.82 g	0.376 fg	12.106 f	6.32 ghi	61.90 ef	34.26 cd
	2	17.18 g	0.469 def	10.617 f	5.70 ij	57.33 fg	30.77 ef
	4	30.79 e	0.483 def	12.524 f	5.98 hij	72.58 cd	28.68 fg
200	0	3.10 h	0.276 g	6.117 h	4.41 jk	37.00 i	32.40 de
	2	16.47 g	0.409 efg	7.151 h	3.65 k	47.25 h	28.53 fg
	4	27.77 ef	0.460 def	8.753 gh	3.23 k	62.91 ef	26.82 h

* Values with the same letter are not significantly different ($P \leq 0.05$) with other treatments

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