Journal of Plant Process and Function, Vol. 14, No. 67, Year 2025 **page: 133-141**
https://doi.org/10.22034/14.67.133

Research Article

Investigation of the effect of chitosan and salinity stress on physiological traits and some chemical compounds in purslane (*Portulaca oleracea* L.)

Elahe Khosravi, Azam Salimi* and Maryam Chavoushi Rizi

Department of Plant Sciences, Faculty of Biological Sciences, Kharazmi University, P.O. Box: 15719-14911, Tehran, Iran

(Received: 2023/12/28-Accepted: 2024/08/20)

Abstract

***Portulaca oleracea* L. is a medicinal plant of the *Portulacaceae* family, known for its protective effects against liver damage, viral hepatitis, diabetes, and cancer. The main purpose of this study was to evaluate the effects of salinity stress and chitosan on the growth and physiological traits of the *Portulaca oleracea* L. Salinity stress is one of the most important factors limiting plant growth and development, especially in arid and semi-arid regions. Chitosan is a biological elicitor and a major component of the cell wall of many fungal species. This study was conducted by a completely randomized factorial design. For this purpose, the plant was exposed to different concentrations of NaCl (0, 25 and 35 ds/m) and chitosan (0, 0.2 and 0.4 g/l), and subsequently harvested and assayed for shoot length, root length, relative water content, photosynthetic pigments, soluble sugar, proline, K^+, Na^+, and fatty acids. The results of two-way analysis of variance showed that different concentrations of salt, chitosan and their interaction significantly affected most of the said parameters. The 25 ds/m salinity caused a favorable growth; while 35 ds/m salinity retarded plant growth. Increasing salinity monotonically decreased the shoot length and RWC and increased Na^+ and carotenoids; however, the root length, proline, Chl *a*, K^+, and soluble sugars showed a hormetic response. A 0.4 g/l chitosan acted as an improving agent under the stress conditions, mainly due to positive changes in the physiological traits. Also, this treatment significantly increased the major fatty acids, linolenic acid (omega-3) and linoleic acid (omega-6). With growing soil salinity, using appropriate dose of chitosan can alleviate the adverse effects of salinity on *Portulaca oleracea* L.**

Keywords: Salinity stress, Chitosan, Growth and physiological traits, *Portulaca oleracea* L.

Introduction

In recent years, increasing soil salinization, a shortage of sufficient water supplies for agriculture, and a rising population have made it critical to utilize water resources and saline soil more efficiently. To move forward with plant efficiency, it is of vital significance to outwit the biotic and abiotic stresses. Among these, salinity stress is one of the most damaging ones, especially in arid and semi-arid regions, typical of Iran.

Portulaca grows easily under drought stress and has the capacity to withstand up to 240 mM salt stress (Chen *et al.*, 2023). *Portulaca oleracea* L. is a member of the *Portulacaceae* family. It is usually identified as purslane (USA and Australia), pigweed (England), pourpier (France), and andulam (Malaysia) (Chugh *et al.*, 2019), and also in Iran as "Piper" (Cui *et al.*, 2005) and "khorfe" (Rad *et al.*, 2017). *Portulaca oleracea* L. is recorded within the World Health Organization as one of the foremost utilized therapeutic plants, and it has been given the status 'Global Panacea (Chen *et al.*, 2023). *Portulaca oleraceacan* can complete its life cycle in 2–4 months in both tropical and calm districts. The purslane contains numerous compounds such as alkaloids, polysaccharides, coumarins, flavonoids, and cardiac glycosides. Their contents include abundant nutrition such as proteins, carbohydrates, calcium (Ca^{2+}), potassium (K^+), zinc (Zn^{2+}), and sodium (Na^+) (El-Sayed, 2011), manganese (Mn^{2+}), iron (Fe^{2+}), phosphorus (P_4), selenium (Se), vitamins C and E, fiber, essential amino acids, and carbohydrates. The seeds contain a fixed oil (~17.4%) containing beta-sitosterol. The most important fatty acids in the leaf and seeds of *Portulaca oleracea* are linolenic acid, linoleic acid, palmetic acid, oleic acid, and stearic acid (Chen *et al.*, 2023).

Salinity is a major abiotic stress limiting plant production (Navarro-Torre *et al.*, 2023). Salinity can lead to toxic ion aggregation and/or mineral nutrition disruption (Okon, 2019), reduced leaf turgor, and decreased net CO_2 assimilation (Leiva-Ampuero *et al.*, 2020). The tomato plants under salinity stress showed a reduction of relative water content (RWC) (Mozafari *et al.*, 2023) and chlorophyll (Chl) pigments (Wang *et al.*, 2023). On the other hand, there were significant

*Corresponding Author, Email: Salimi@khu.ac.ir

[DOI: DOI: 10.22034/14.67.133]

increases in fatty acids of the plants (Almutairi *et al.*, 2020). The adverse effects of abiotic stresses were successfully managed by using protective agents such as chitosan. To mention a few reports as an instance, 3 ml/l chitosan and 2 g/l humic acid significantly increased the yield of wheat compared to the control in both non-stress and drought stress conditions (Jahanbani *et al.*, 2023). The application of about 0.5 g/l chitosan and chitosan nanoparticles (of 40 nm in size) to *Phaseolus vulgaris* L. under different levels of salinity (25, 50, 100, and 200 mM NaCl) significantly improved the plant growth and development because of positive changes in the contents of pigments, carbohydrates, ions, osmolytes, and antioxidant system (Alenazi *et al.*, 2024). In another study, it was proved that chitosan treatment enhanced the photosynthesis of rice cultivars by increasing the contents of Chl *a* and Chl *b* and by mediating oxidative stress through higher content of proline and the activity of catalase and peroxidase (THUY *et al.*, 2024).

Chitosan is an organic and naturally occurring amino polysaccharide with several derivatives that can diminish natural stresses (such as soil salinity and drought) and improve plant growth. Chitosan is a chitin derivative, a biodegradable compound that is completely safe for the environment. This compound is characterized by interesting unique properties, such as bioactivity and biocompatibility (Demehin *et al.*, 2024). In addition, chitosan's versatility renders it the ability to chemically bond with fats, cholesterol, proteins, DNA, RNA, and metal ions. Also, chitosan is soluble in dilute acid solutions at pH < 6 (Tajik *et al.*, 2008).

In this study, we aim to demonstrate the positive effects of chitosan at the appropriate dosage in alleviating salinity stress in *Portulaca oleracea* L. To achieve this, we exogenously applied chitosan to the culture media and examined plant growth and physiology under both normal and salinity stress conditions. Specifically, we measured the shoot length, root length, and RWC. We also assessed the contents of photosynthetic pigments, soluble sugars, proline, leaf ions (Na^+ and K^+), and fatty acids. By observing these morphological and physiological responses, we gained insights into the role of chitosan and mechanisms underlying the salinity stress tolerance in the plant.

Materials and methods

Culture conditions and treatments: The uniformly sterilized seeds of purslane were germinated in petri dishes containing double distilled water. The germinated seedlings were transferred to pots containing sand, clay, and humus (1:1:1) in growth chambers with day/night temperatures of 26/18°C under a 16/8 h photoperiod. The pots were irrigated with distilled water. The plants aged 40 days were exposed to salinity (0, 25, and 35 ds/m) every two days for 10 days, followed by chitosan foliar spray (0, 0.2, and 0.4 g/l) three times a day in between. These salinity levels and chitosan concentrations were selected based on a literature review, preliminary laboratory tests, and the typical salinity levels found in soils of arid and semi-arid regions. The plants aged 57 days old were then harvested in the vegetative growth stage. The harvested samples were washed with double-distilled water and used immediately for further analysis.

Growth and physiological assays: The relative water content was determined according to the method of Yamasaki and Dillenburg (1999). The leaf chlorophyll and carotenoids were extracted in cold 80% acetone, and the absorbance was read at 470, 662, and 645 nm (Lichtenthaler, 1987) by a UV/Vis spectrophotometer. The contents of soluble sugars and proline were measured according to the methods of Bates *et al.* (1973) and Dubois (1956). The contents of Na^+ and K^+ were determined using atomic absorption spectrophotometry (Zheljazkov and Nielsen, 1996). The oils of purslane leaf were extracted from the samples according to the method described by Abbasi *et al.* (2008).

Statistical analysis: The experiments were done in a randomized complete block design with three replications for each test. The data analysis was performed by two-way analysis of variance (two-way ANOVA). For comparing the significant differences of the set of means, the Duncan test was applied at $P < 0.05$ using the SPSS software version No. 19. (SPSS Inc., Chicago, IL).

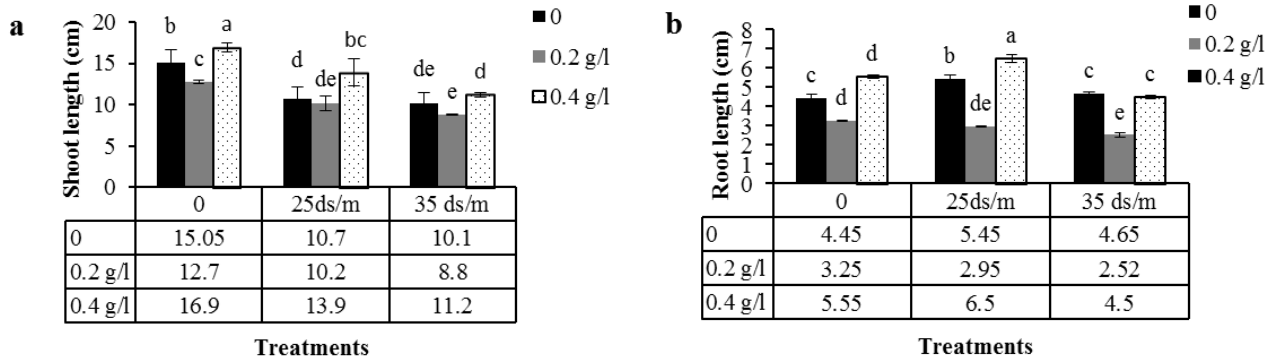
Results

Growth parameters: Both chitosan and salinity had a significant effect on the shoot length (Figure 1a). The salinity stress caused a significant reduction in the shoot length (from 15.05 to 10.10 cm). In non-stress conditions, the chitosan concentration of 0.2 g/l reduced and a concentration of 0.4 g/l increased the shoot length. The chitosan concentration of 0.2 g/l at 25 ds/m salinity had no significant effect on the shoot length, but the concentration of 0.4 g/l led to a significant increase in the shoot length. Exposure to both chitosan concentrations of 0.2 and 0.4 g/l had no significant effect on the shoot length at 35 ds/m salinity (Figure 1).

The salinity, chitosan, and the interaction of salinity and chitosan had a significant effect on the root length (Figure 1b). The 25 ds/m salinity increased the root length (~18%), but the 35 ds/m salinity had no significant effect on the root length. The 0.2 g/l chitosan reduced the root length in non-stress and under both salinity (25 and 35 ds/m) conditions, but the 0.4 g/l chitosan increased the root length in normal and salinity stress (Figure 1b).

The RWC of the plants consistently decreased with increasing salinity. The chitosan concentrations of 0.2 g/l and 0.4 g/l reduced the RWC in non-stress conditions. The interaction of salinity (25 ds/m) and chitosan (0.2 and 0.4 g/l) increased the RWC by about 53% and 97%, respectively. The interaction of 35 ds/m

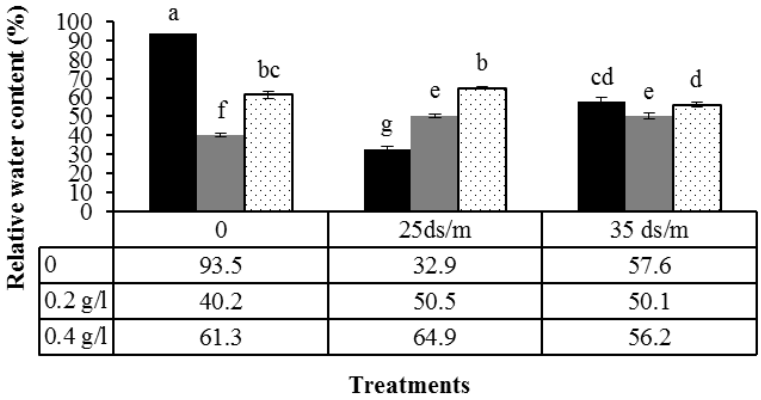
[DOI: DOI: 10.22034/14.67.133]



	0	25ds/m	35 ds/m
0	15.05	10.7	10.1
0.2 g/l	12.7	10.2	8.8
0.4 g/l	16.9	13.9	11.2

	0	25ds/m	35 ds/m
0	4.45	5.45	4.65
0.2 g/l	3.25	2.95	2.52
0.4 g/l	5.55	6.5	4.5

Figure 1. Effect of salinity and chitosan on the shoot length (a) and the root length (b) of purslane. The similar letters on every column show no significant difference at P≤0.05 based on the Duncan test.



	0	25ds/m	35 ds/m
0	93.5	32.9	57.6
0.2 g/l	40.2	50.5	50.1
0.4 g/l	61.3	64.9	56.2

Figure 2. Effect of salinity and chitosan on the relative water content of purslane. The dissimilar letters indicate a significant difference between the set of means at P≤0.05 using the Duncan test.

salinity with 0.2 g/l chitosan decreased the RWC (~13%), and with 0.4 g/l chitosan had no effect on the RWC (Figure 2).

The content of photosynthetic pigments: The 25 ds/m salinity significantly decreased the Chl *a* but had no significant effect on the contents of Chl *b*, total chlorophyll, and carotenoids (Figure 3). The 35 ds/m salinity had no effect on the Chl *a* but increased the contents of Chl *b* and carotenoids. The chitosan treatment of the non-stressed plants reduced Chl *a*, but it increased Chl *b* and carotenoids. The content of photosynthetic pigments increased upon exposure to the 25 ds/m salinity and chitosan (0.2 and 0.4 g/l). The contents of Chl *a*, total chlorophyll, and carotenoids reduced upon exposure to the 35 ds/m salinity and chitosan (0.2 and 0.4 g/l), but Chl *b* showed a decrease under this salinity (35 ds/m) and 0.2 g/l chitosan and no significant change with 0.4 g/l chitosan (Figure 3).

Sodium and potassium ion contents: The sodium ion content was increased more than 2-fold under both salinity stresses (Figure 4a). The 0.2 g/l chitosan treatment of the plants subjected to the salinity stress (25 and 35 ds/m) increased the sodium ion of the leaves, but the 0.4 g/l chitosan reduced it (Figure 4a). The 25 ds/m salinity increased potassium ion content, but the 35 ds/m salinity reduced it significantly (Figure 4b). The interaction of salinity (25 and 35 ds/m) and 0.2 g/l chitosan increased and decreased potassium ion content from 272 ppm to 216 ppm and 282 ppm, respectively (Figure 4b). The 0.4 g/l chitosan treatment of the plants under salinity (25 and 35 ds/m) resulted in significantly lower potassium ion content (Figure 4b).

Proline contents: The proline content significantly increased under the 25 ds/m salinity compared to the control (from 5.57 to 5.64 mg g^{-1} FW), but the 35 ds/m salinity had no significant effect on the proline content. Combined application of salinity and chitosan increased the proline contents compared to the control. Application of the 0.2 g/l chitosan to the plants under 25 ds/m salinity reduced the proline content, while the 0.4 g/l chitosan treatment increased it. Simultaneous application of the 35 ds/m salinity and chitosan (0.2 and 0.4 g/l) increased the proline content as well (Figure 5a).

Soluble sugars: The soluble sugar content increased under the 25 ds/m salinity from 6.08 to 6.21 mg g^{-1} FW but significantly reduced upon exposure to the 35 ds/m salinity. The soluble sugar contents reduced upon combined application of chitosan (0.2 and 0.4 g/l) and the 25 ds/m salinity but increased with the 35 ds/m salinity and the 0.4 g/l chitosan (Figure 5b).

Fatty acids: The salinity, chitosan, and the interaction of salinity and chitosan had a significant effect on the fatty acids (Table 1). Comparison of the

[DOI: DOI: 10.22034/14.67.133]

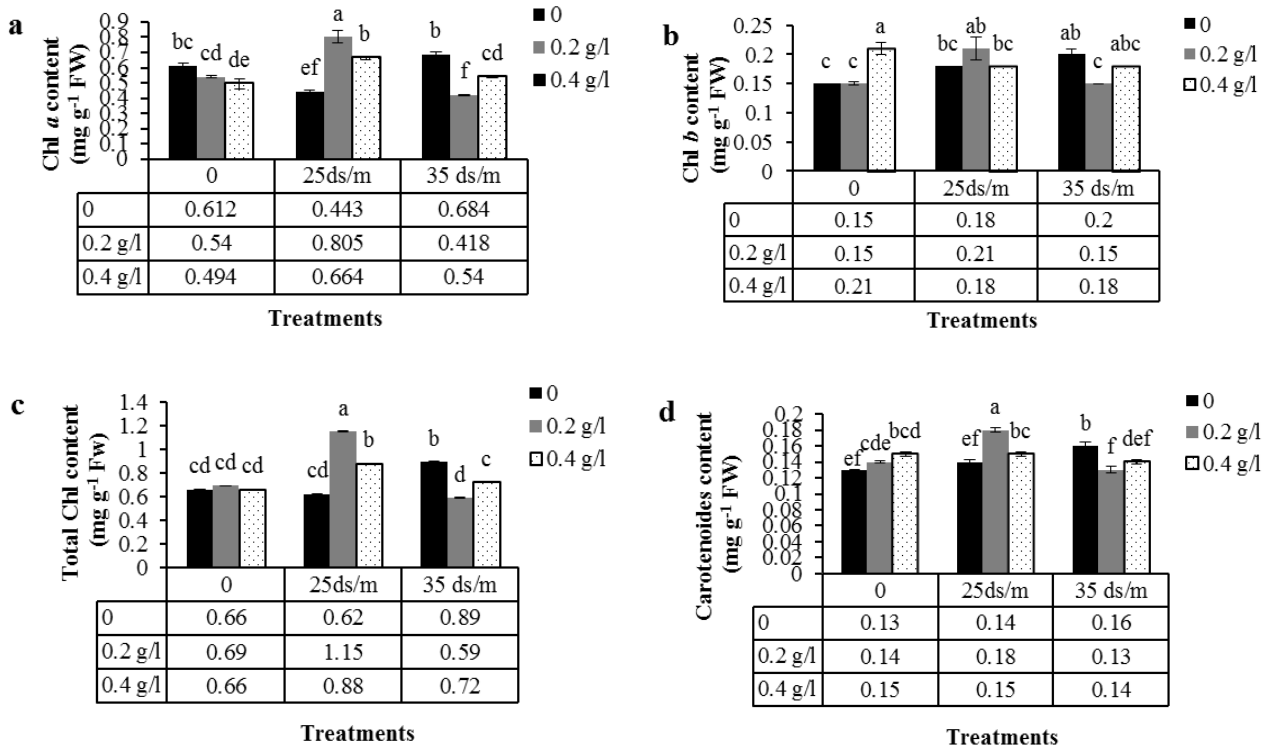


Figure 3. Effect of salinity and chitosan on photosynthesis pigments of purslane, including chl *a* (a), chl *b* (b), total chl (c), and carotenoids (d). The means of three replicates in each column, shown by similar letters, are not significantly different at P ≤ 0.05 using the Duncan test. Please note that "chl" stands for chlorophyll.

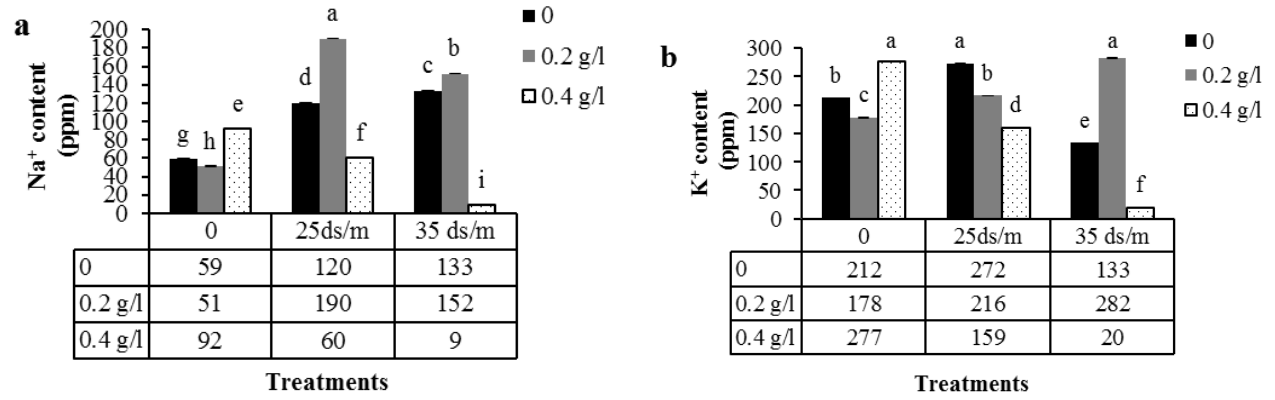


Figure 4. Effect of salinity and chitosan treatments on the contents of Na^+ (a) and K^+ (b) in the purslane. The dissimilar letters indicate a significant difference between the set of means at P ≤0.05 using the Duncan test.

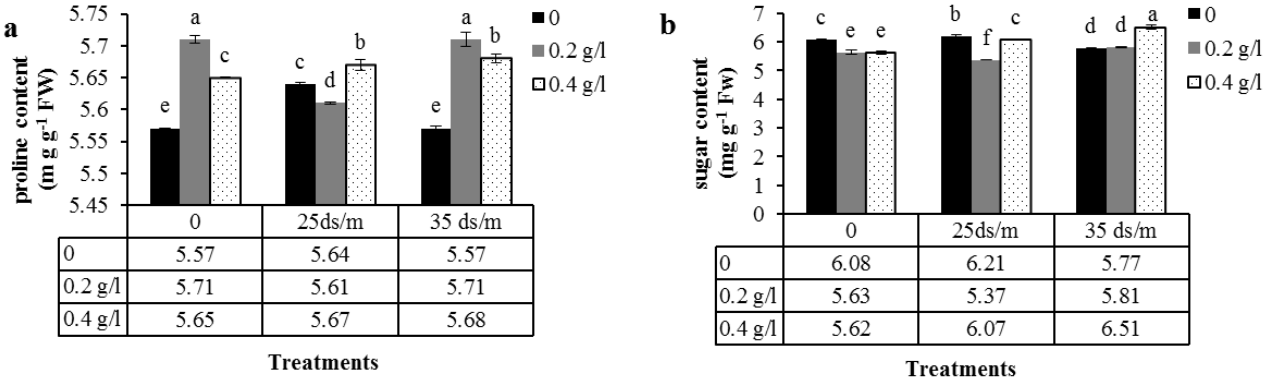


Figure 5. Effect of salinity and chitosan on the proline (a) and soluble sugars (b) of purslane. The dissimilar letters indicate a significant difference between the set of means at P ≤0.05 using the Duncan test.

[DOI: DOI: 10.22034/14.67.133]

Table 1. Effect of salinity and chitosan on the fatty acid profile of the leaves.

Fatty acid	control	25 ds/m	35 ds/m	0.2 g/l	25 ds/m+ 0.2 g/l	35 ds/m + 0.2 g/l	0.4 g/l	25 ds/m + 0.4 g/l	35 ds/m + 0.4 g/l
Dodecanoic acid	0.484 ^{c}	0.408 ^{f}	0.221 ^{h}	0.707 ^{a}	0.203 ^{i}	0.392 ^{g}	0.446 ^{e}	0.468 ^{d}	0.536 ^{b}
Tetradecanoic acid	0.705 ^{a}	0.53 ^{e}	0.347 ^{h}	0.631 ^{b}	0.564 d	0.489 ^{g}	0.566 ^{c}	0.52 ^{ef}	0.513 ^{f}
Myristic acid	0.144 ^{a}	ND ^{c}	ND ^{c}	ND ^{c}	0.133 b	ND ^{c}	ND ^{c}	ND ^{c}	ND ^{c}
Palmitic acid	21.546^{a}	17.93 ^{f}	16 ^{g}	19.735^{c}	18.449 ^{e}	19.03 ^{d}	20.906 ^{b}	18.451 ^{e}	19.703 ^{c}
Palmitoleic acid	1.547 ^{f}	1.693 ^{e}	1.984 ^{b}	1.614 ^{f}	1.949 ^{b}	1.786 ^{d}	1.057 ^{g}	1.868 ^{c}	2.318 ^{a}
Heptadecanoic acid	0.522 ^{c}	0.557 ^{b}	0.327 ^{f}	0.388 ^{e}	0.295 ^{g}	0.604 ^{a}	0.445 ^{d}	0.613 ^{a}	0.389 ^{e}
Octadecanoic acid	2.294 ^{b}	2.45 ^{b}	2.433 ^{b}	2.338 ^{b}	3.191 ^{a}	2.518 ^{b}	2.036 ^{c}	2.42 ^{b}	2.526 ^{b}
Oleic acid	2.719 ^{cd}	2.411 ^{e}	2.694 ^{d}	2.67 ^{c}	19.511 ^{a}	2.992 ^{c}	3.405 ^{b}	2.86 ^{cd}	3.519 ^{b}
Linoleic acid	11.23 ^{g}	11.22 ^{g}	12.545^{d}	10.903^{h}	12.723 ^{c}	13.708 ^{b}	11.326 ^{f}	11.447 ^{e}	13.94 ^{a}
Linolenic acid	45.33 ^{e}	43.405^{f}	40.047^{d}	43.844^{g}	35.088 ^{i}	46.533 ^{a}	46.37 ^{h}	48.59 ^{b}	46.39 ^{c}
Eicosanoic acid	8.905 ^{d}	9.531 ^{c}	7.501 ^{e}	12.203^{a}	5.326 ^{f}	9.381 ^{c}	12.042 ^{a}	11.365 ^{b}	9.438 ^{c}
Paullinic acid	0.73 ^{b}	0.61^{c}	0.847 ^{a}	0.283 ^{d}	ND ^{e}	ND ^{e}	0.27 ^{d}	ND ^{e}	ND ^{e}
Docosanoic acid	1.134 ^{a}	0.891 ^{bc}	0.919 ^{b}	0.836 ^{b}	0.331 ^{e}	0.522 ^{e}	0.562 ^{e}	0.784 ^{cd}	ND ^{f}
Erucic acid	ND ^{f}	3.641 ^{b}	5.192 ^{a}	1.315 ^{d}	2.237 ^{c}	0.549 ^{e}	ND ^{f}	ND ^{f}	ND ^{f}
Tetracosanoic acid	1.058 ^{c}	1.163 ^{c}	1.68 ^{a}	1.2 ^{b}	ND ^{g}	0.75 ^{d}	0.573 ^{f}	0.614 ^{d}	0.685 ^{d}
Uronic acids	1.137 ^{cd}	3.787 ^{b}	6.551 ^{a}	1.326 ^{cd}	ND ^{f}	0.616 ^{e}	ND ^{f}	ND ^{f}	ND ^{f}

Dissimilar letters indicate a significant difference at P ≤0.05 using the Duncan test.

means showed that salinity stress caused a reduction in the dodecanoic acid, docosanoic acid, tetradecanoic acid, myristic acid, and palmitic acid of the leaves. The salinity stress increased erucic acid, palmitoleic acid, and uronic acid. The 25 ds/m salinity reduced the paullinic acid, and the 35 ds/m salinity increased it. Oleic acid was reduced in the plants under the 25 ds/m salinity but showed no significant change in the plants exposed to the 35 ds/m salinity. Octadecanoic acid remained steady under salinity stress. The 25 ds/m salinity had no effects on the linoleic acid, while the 35 ds/m salinity increased it (Table 1). The 25 ds/m salinity decreased the linolenic acid from 45.3 to 43.8, and the 35 ds/m salinity increased it from 45.3 to 46.3 (Table 1).

Combined treatment of the 25 ds/m salinity and the 0.2 g/l chitosan reduced docosanoic acid, docosanoic acid, heptadecanoic acid, linolenic acid, eicosanoic acid, paullinic acid, docosanoic acid, tetradecanoic acid, and uronic acid in the plants but increased linolenic acid, oleic acid, octadecanoic acid, palmitoleic acid, palmitic acid, myristic acid, and tetradecanoic acid. The 25 ds/m salinity and the 0.4 g/l chitosan reduced tetradecanoic acid and uronic acid, paullinic acid, oleic acid, octadecanoic acid, and myristic acid, but increased dodecanoic acid, palmitic acid, heptadecanoic acid, linolenic acid, linolenic acid, palmitoleic acid, and eicosanoic acid. The interaction of the 35 ds/m salinity and the 0.2 g/l chitosan increased all fatty acids except palmitoleic acid, docosanoic acid, tetradecanoic acid, and uronic acid. Combined treatment of the 35 ds/m salinity and the 0.4 g/l chitosan increased all fatty acids except linolenic acid, docosanoic acid, tetradecanoic acid, erucic acid, and uronic acid. These treatments had no effects on octadecanoic acid.

Interaction of the 25 ds/m salinity and the 0.2 g/l chitosan reduced linolenic acid from 43.9 to 35 and increased linoleic acid from 11 to 12.7. The interaction of the 25 ds/m salinity and the 0.4 g/l chitosan increased linolenic acid from 43 to 46 and linoleic acid from 11 to 11.5. The interaction of the 35 ds/m salinity and chitosan (0.2 and 0.4 g/l) increased linolenic acid and linoleic acid (Table 1).

Discussion

The salinity stress negatively affected the shoot length and RWC. Lower shoot length can be attributed to a reduction in osmotic pressure, nutritional imbalance, ion toxicity, depletion of photosynthetic pigments, and also oxidative stress (Shaki *et al.*, 2018). Consistent with these observations, the damaging effect of salinity on growth parameters was reported in *Festuca arundinacea* Shreb (Sharavdorj *et al.*, 2024). In this study, the exogenous application of the 0.4 g/l chitosan improved plant growth in terms of the shoot and root lengths as well as the RWC, which is consistent with the similar observations reported in bean (Sheikha and Al-Malki, 2011) and *Plantago ovata* (Mahdavi, 2013). Chitosan may improve plant growth and development by some signaling pathways related to auxin biosynthesis via a tryptophan-independent pathway (Iglesias *et al.*, 2019). It has effects on the RWC, which is connected with the cell volume, and might be due to its relation with water supply to the plants. The RWC has the ability to protect plant growth and yields from osmotic stress (Hassnain *et al.*, 2020). In addition, chitosan had positive effects on the growth and development of mature plants. For example, chitosan can reduce disease severity in orchids, possibly by increasing the activity of PAL and PPO, lignification resulting from increased biosynthesis of phenolic compounds or induced secondary metabolites, and sodium absorption ratio (SAR). Also, increased resistance of plants may be mediated in part via an increase in the concentrations of jasmonic acid. Moreover, resistance may also involve the closure of stomata by abscisic acid (Uthairatanakij *et al.*, 2007). In this study, according to Figure 2, the salinity reduced the RWC from 93% to 32.9% and 37.6% in 25 ds/m and 35 ds/m salinity, respectively. Consistent with our findings, the reduction of RWC in plants under salinity was reported in other studies as well. However, treating

[DOI: DOI: 10.22034/14.67.133]

the plants under the 25 ds/m salinity with 0.4 g/l chitosan led to higher RWC, which is in agreement with the similar observation reported in tomatoes under osmotic stress (Demehin *et al.*, 2024).

In this study, the photosynthetic pigments, such as chlorophyll *a*, were decreased by the 25 ds/m salinity stress, while the 35 ds/m salinity stress increased chlorophyll *b*, carotenoids, and total chlorophyll. The leaf area and length were reduced by salinity, which suggests that the increase in chlorophyll content was likely due to smaller cell sizes and a higher concentration of chloroplasts per unit area (Rivelli *et al.*, 2010). Some studies reported that osmotic stress decreases or increases photosynthetic pigments such as chlorophyll. This disparity could be because of the plant type and experimental procedure used for analysis (Demehin *et al.*, 2024). Salinity stress increased the chlorophyllase enzyme activity, changed chlorophyll structure, increased ethylene and abscisic acid, and increased the chlorophyllase enzyme activity, then reduced chlorophyll content (Bakhoum *et al.*, 2020). In this study, carotenoid increased in salinity stress, which is in agreement with the similar observation reported in tomato leaves (Leiva-Ampuero *et al.*, 2020). Carotenoids play an essential part in photosynthesis and resistance to salinity stress. Some genes in the carotenoid synthase pathway are also affected by salinity (Leiva-Ampuero *et al.*, 2020). They are fundamental molecules involved in light protection during photosynthesis. They are antioxidants and single oxygen scavengers and prevent lipid peroxidation and stabilize membranes (Ren *et al.*, 2021). In the present study, interaction of the 25 ds/m salinity and both chitosan levels increased chlorophyll *a*, carotenoids, and total chlorophyll, but combined application of the 35 ds/m salinity and both chitosan levels reduced chlorophyll *a*, total chlorophyll, and carotenoids. A similar observation has also been reported in tomatoes because chitosan increased the photosynthetic pattern under osmotic stress (Demehin *et al.*, 2024). In the present study, the interaction of salinity and chitosan increased the shoot length and root length because the higher photosynthetic rate of those treated with chitosan may be associated with the higher carboxylation efficiency exhibited by these plants, which would corroborate the findings of Avila *et al.* (2023). Chitosan increased nitrogen, phosphate, and potassium uptake, as well as improved nitrogen transport to the leaves, which may be responsible for the correlation between chitosan concentration and leaf chlorophyll content. Moreover, chitosan scavenges reactive oxygen species (ROS), protecting chlorophyll content and photosynthetic parameters and improving photosystem functioning (Ullah *et al.*, 2020). When chlorophyll content increased under chitosan treatment in the plants under salinity stress, then chitinase and phytoalexin increased and reduced the effect of damage from salinity stress, which agrees with the similar observation reported in tomato (Demehin *et al.*, 2024). Moreover, chitosan triggers a signaling cascade, which is a fine response system involving important signaling molecules.such as Ca^{2+}, nitric oxide, H_2O_2, and ethylene that play roles in regulating stomatal opening and closure during periods of water deficit. As a result, it has effects on photosynthesis and growth (Avila *et al.*, 2023).

Low sodium and high potassium concentrations within the cytoplasm are basic for maintaining enzymatic processes in the cytoplasm (Sharavdorj *et al.*, 2024). In numerous plants, salinity stress increased the uptake of sodium and inhibited the uptake of potassium, so plants try to keep high levels of potassium and low levels of sodium in the cytosol. They regulated the activity of sodium and potassium transporters and hydrogen pumps that provide the energy to transport ions. They are performed using the SOS system (Taiz *et al.*, 2015). In other words, in control plants, the cytoplasm of plants contains a lot of K^+ and not too many Na^+ ions, but in salinity stress, the Na^+ concentration increases in the roots, which affects K^+ absorption and throws off the K^+/Na^+ balance (Alenazi *et al.*, 2024). Of course, in the present study, the 35 ds/m salinity increased sodium content and significantly decreased the potassium compared to the control. In plants grown under salt stress, the accumulation of sodium increases, which leads to a disturbance of ionic balance and a defect in the absorption of beneficial ions and disruption of plant metabolism and ultimately a decrease in growth (Okon, 2019). Increased sodium during salt treatment proved that sodium is inactively absorbed in large quantities in *Portulaca oleracea*. Of course, sodium accumulation can be part of the osmotic mechanism and osmotic adjustments regulated, which is water potential reduced soluble potential (Alenazi *et al.*, 2024). On the other hand, potassium under salinity stress decreased and damaged the cell both physiologically and biochemically, and can be considered as one of the main causes of salinity toxicity (Hirich and Bhargava, 2024) which agree with the similar observation reported in *Phaseolus vulgaris* L. (Alenazi *et al.*, 2024). In this study, the interaction of salinity (25 and 35 ds/m) and chitosan (0.2 g/l) increased sodium in purslane, which is in agreement with the similar observation reported in strawberry (Abdel-Mawgoud *et al.*, 2010). Interaction of salinity (35 ds/m) and chitosan (0.4 g/l) reduced sodium content and potassium, which is in agreement with the similar observation reported in *Moringa oleifera* (Elkarmout *et al.*, 2022) because high salinity damaged cell membranes, and consequently ion leakage increased.

The free proline is a non-enzymatic scavenger of free radicals that protects the membrane and protective proteins and thus cells (Zayed and Elamry, 2006). In this study, salinity increased proline content, which is in agreement with the similar observation reported in cowpea (Hirich and Bhargava, 2024). Increased proline levels under salinity stress in barley have been reported due to increased pyrroline-5-carboxylate reductase activity and decreased proline dehydrogenase activity in

[DOI: DOI: 10.22034/14.67.133]

wheat (Goharrizi *et al.*, 2020). Salinity (35 dS/m) and chitosan (0.2 g/l) increased proline content, which is in agreement with the similar observation reported in *Zea mays* (Rabelo *et al.*, 2019). Therefore, the effects of chitosan on the proline content in *Portulaca oleracea* leaves can regulate cell osmosis potential and reduce the adverse effects of salinity.

In this study, salinity stress increased osmolytes, like proline, which are linked to antioxidant defense, stress signaling, osmotic adjustment, and energy metabolism during stress (Li *et al.*, 2017). Sugars protect membranes from dehydration and act as scavengers of ROS under low-temperature stress (Tarkowski and Van den Ende, 2015). Elevated sugar levels in plants can be caused by the decomposition of polysaccharides into soluble carbohydrates; Therefore, increased levels of soluble sugars (Bakhoum *et al.*, 2020) under stress conditions. Interaction of salinity and chitosan (0.2 g/l and 0.4 g/l) reduced and increased sugar content, respectively, which depends on the effects of chitosan on upregulation and downregulation of sucrose (Demehin *et al.*, 2024).

Among the most important fatty acids of the purslane, we can mention linolenic acid and linoleic acid. Factors such as variety, soil, and weather conditions affect fatty acids. The most important factor affecting fatty acids is the genotype of the plant; but environmental factors can also affect the percentage of oil and fatty acid during seed filling. In this research, 16 different types of fatty acids were identified in the analysis of the composition of purslane fatty acids. The change of fatty acids in the purslane plant is related to the effect of salt and chitosan on the enzymes of the fatty acid biosynthetic pathway. Results revealed that the 25 ds/m salinity reduced linolenic acid, but the 35 ds/m salinity increased linolenic acid and linoleic acid compared to normal irrigation and interaction of salinity and chitosan increased them. Chitosan seems to play a role in relieving stress and increasing the amount of fatty acids which is in agreement with other reports (Rezaeizadeh *et al.*, 2019). No reports on this subject have been published to date on other fatty acids such as dodecanoic acid, tetradecanoic acid, etc.

Conclusion

The salinity stress negatively affected the purslane growth as a result of sodium ion accumulation, Na^+/K^+ imbalance, and water content reduction. The chlorophyll contents were also increased, mainly because of water loss and cell size reduction, which led to a higher concentration of chloroplasts per unit area. To retain cell water and alleviate salinity stress, the compatible solutes increased in the plant leaves. However, under severe salinity, while the sugar content decreased, the cells managed to maintain a suitable level of proline, making the conditions for the cells similar to non-saline conditions. Salinity has diverse effects on fatty acids, with a significant increase in the proportion of the major fatty acids (linolenic acid and linoleic acid). The exogenous application of chitosan stimulated more mechanisms in the plant. The appropriate dose of 0.4 g/l chitosan alleviated the adverse effects of moderate salinity on the growth, possibly because of higher relative water content, compatible solutes, and photosynthetic pigments. However, with rising salinity, chitosan could not be effective because its interaction with ions led to higher ionic leakage and ionic imbalance, and so significant changes in normal cell function.

Conflict of interest disclosure

The authors declare no potential conflict of interest regarding the authorship and/or the publication of this work.

References

Abbasi, H., Rezaei, K., & Rashidi, L. (2008). Extraction of essential oils from the seeds of pomegranate using organic solvents and supercritical CO_2. *Journal of the American Oil Chemists' Society, 85*(1), 83-89. https://doi.org/10.1007/s11746-007-1158-x

Abdel-Mawgoud, A., Tantawy, A., El-Nemr, M., & .Sassine, Y. (2010). Growth and yield responses of strawberry plants to chitosan application. *European Journal of Scientific Research, 39*(1), 170-177.

Alenazi, M. M., El-Ebidy, A. M., El-Shehaby, O. A., Seleiman, M. F., Aldhuwaib, K. J., & Abdel-Aziz, H. M. (2024) . Chitosan and chitosan nanoparticles differentially alleviate salinity stress in *Phaseolus vulgaris* L. plants. *Plants, 13*(3), 398-432. https://doi.org/10.3390/plants 13030398

Almutairi, A., El-Sayed, A., & Reda, M. (2020). Combined effect of salinity and pH on lipid content and fatty acid composition of *Tisochrysis lutea*. *Saudi Journal of Biological Sciences, 27*, 3553-3558. https://doi.org/10.1016/j.sjbs.2020.07.027

Avila, R. G., Magalhaes, P. C., Vitorino, L. C., Bessa, L. A., de Souza, K. R. D., Queiroz, R. B., Jakelaitis, A., & Teixeira, M. B. (2023). Chitosan induces sorghum tolerance to water deficits by positively regulating photosynthesis and the production of primary metabolites, osmoregulators, and antioxidants. *Journal of Soil Science and Plant Nutrition, 23*(1), 1156-1172. https://doi.org/10.1007/s42729-022-01111-4

Bakhoum, G. S., Sadak, M. S., & Badr, E. A. E. M. (2020). Mitigation of adverse effects of salinity stress on sunflower plant (*Helianthus annuus* L.) by exogenous application of chitosan. *Bulletin of the National Research Centre, 44*, 1-11. https://doi.org/10.1186/s42269-020-00343-7

[DOI: DOI: 10.22034/14.67.133]

Bates, L., Waldren, R., & Teare, I. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil 39*(1), 205-207.

Chen, M., Li, D., Meng, X., Sun, Y., Liu, R., & Sun, T. (2023). Review of isolation, purification, structural characteristics and bioactivities of polysaccharides from *Portulaca oleracea* L. *International Journal of Biological Macromolecules*, 128565. https://doi.org/10.1016 /j.ijbiomac.2023.128565

Chugh, V., Mishra, V., Dwivedi, S., & Sharma, K. (2019). Purslane (*Portulaca oleracea* L.): An underutilized wonder plant with potential pharmacological value. *The Pharma Innovation Journal, 8*(6), 236-246.

Cui, M., Liu, H., & Li, C. (2005). Changes of blood glucose in diabetic rats and the interventional effect of purslane. *Chinese Journal of Clinical Rehabilitation*, *27*, 92-93. https://doi.org/ 10.3390/ su141811300

Demehin, O., Attjioui, M., Goni, O., & O'Connell, S. (2024). Chitosan from mushroom improves drought stress tolerance in tomatoes. *Plants, 13*(7), 1038. https://doi.org/1010.3390/plants 13071038

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances *Analytical Chemistry, 28*, 350-356.

El-Sayed, M. I. K. (2011). Effects of *Portulaca oleracea* L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. *Journal of Ethnopharmacology, 137*(1), 643-651. https://doi.org/10.1016/j.jep.2011.06.020

Elkarmout, A. F., Yang, M., & Hassan, F. A. (2022). Chitosan treatment effectively alleviates the adverse effects of salinity in *Moringa oleifera* Lam via enhancing antioxidant system and nutrient homeostasis. *Agronomy, 12*(10), 2513-2531. https://doi.org/10.3390/agronomy 12102513

Goharrizi, K. J., Baghizadeh, A., Afroushteh, M., Amirmahani, F., & Kermani, S. G. (2020). Effects of salinity stress on proline content and expression of Δ1-pyrroline-5-carboxylate synthase and vacuolar-type H^+ subunit E genes in wheat. *Plant Genetic Resources, 18*(5), 334-342.

Hassnain, M. A., Basit, A., Alam, M., Ahmad, I., Ullah, I., Alam, N., Ullah, I., Khalid, M. A., & Shair, M. (2020). Efficacy of chitosan on performance of tomato (*Lycopersicon esculentum* L.) plant under water stress condition. *Pakistan Journal of Agricultural Research, 33*(1), 27-41. http://dx.doi.org/10.17582/journal.pjar/2020/33.1.27.41

Hirich, A, & Bhargava, A. (2024). Advances in alternative crop production and valorization in salt-affected areas. *Frontiers Plant Science, 15*, 1384030-1384033. https://doi.org/10.3389/fpls .2024.1384030

Iglesias, M. J., Colman, S. L., Terrile, M. C., Paris, R., Martin-Saldana, S., Chevalier, A. A., Alvarez, V. A., & Casalongue, C. A. (2019). Enhanced properties of chitosan microparticles over bulk chitosan on the modulation of the auxin signaling pathway with beneficial impacts on root architecture in plants. *Journal of Agricultural and Food Chemistry, 67*(25), 6911-692.

Jahanbani, A., Asghari Zakaria, R., Ashrafi, V., Ghasemi Kalkhoran, M., & Shahriary, R. (2023). Effect of foliar application of chitosan and humic acid on yield and yield components of bread wheat under end-season drought stress. *Environmental Stresses in Crop Sciences, 16*(4), 905-918. https://doi.org/10.22077/escs.2023.5127.2111

Leiva-Ampuero, A., Agurto, M., Matus, J. T., Hoppe, G., Huidobro, C., Inostroza-Blancheteau, C., Reyes-Diaz, M., Stange, C., Canessa, P., & Vega, A. (2020). Salinity impairs photosynthetic capacity and enhances carotenoid-related gene expression and biosynthesis in tomato (*Solanum lycopersicum* L. cv. Micro-Tom). *PeerJ, 8*, e9742. 10.7717/peerj.9742

Li, Z., Zhang, Y., Zhang, X., Merewitz, E., Peng, Y., Ma, X., Huang, L., & Yan, Y. (2017). Metabolic pathways regulated by chitosan contributing to drought resistance in white clover. *Journal of Proteome Research, 16*(8), 3039-3052.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology, 148*, 350-382.

Mahdavi, B. (2013). Seed germination and growth responses of Isabgol (*Plantago ovata* Forsk) to chitosan and salinity. *International Journal of Agriculture and Crop Sciences, 5*(10), 1084.

Mozafari, H., Hejabi, M., Salari, H., & Oloumi, H. (2023). Effect of iron oxide and zinc oxide nanoparticles on growth improvement and tolerance to salinity stress in tomato plants. *Journal of Ethno-Pharmaceutical Products, 3*(1), 31-45. 10.48306/epp.2023.2011012.1062

Navarro-Torre, S., Garcia-Caparros, P., Nogales, A., Abreu, M. M., Santos, E., Cortinhas, A. L., & Caperta, A. D. (2023). Sustainable agricultural management of saline soils in arid and semi-arid Mediterranean regions through halophytes, microbial and soil-based technologies. *Environmental and Experimental Botany, 212*, 105397. https://doi.org/10.1016/S0168-9452(00)00272-7

Okon, O. G. (2019). Effect of salinity on physiological processes in plants. In: Microorganisms in Saline Environments: Strategies and Functions (eds. Giri, B. and Varma, A.) Pp. 237-262. Springer International Publishing, Cham. doi: 10.1007/978-3-030-18975-4_10

Rabelo, V. M., Magalhaes, P. C., Bressanin, L. A., Carvalho, D. T., Reis, C. O. d., Karam, D., Doriguetto, A. C., Santos, M. H. d., Santos Filho, P. R. d. S., & Souza, T. C. d. (2019). The foliar application of a mixture of semisynthetic chitosan derivatives induces tolerance to water deficit in maize, improving the antioxidant system and increasing photosynthesis and grain yield. *Scientific Reports, 9*(1), 8164.

[DOI: DOI: 10.22034/14.67.133]

Rad, M. A., Sajedi, S., & Domina, G. (2017). First data on the taxonomic diversity of the *Portulaca oleracea* aggregate (Portulacaceae) in Iran. *Turkish Journal of Botany, 41*(5), 535-541. 10.3906/bot-1611-43

Ren, Y., Sun, H., Deng, J., Huang, J., & Chen, F. (2021). Carotenoid production from microalgae: Biosynthesis, salinity responses and novel biotechnologies. *Marine Drugs, 19*(12), 713-734. https://doi.org/10.3390/md19120713

Rezaeizadeh, M., Sayfzadeh, S., Shirani Rad, A. H., Valadabadi, S. A., & Hadidi Masouleh, E. (2019). Influence of drought stress and Chitosan on fatty acid compounds of rapeseed varieties. *Plant Physiology, 9*(3), 2819-2825. 10.30495/ijpp.2019.667143

Rivelli, A., De Maria, S., Pizza, S., & Gherbin, P. (2010). Growth and physiological response of hydroponically-grown sunflower as affected by salinity and magnesium levels. *Journal of Plant Nutrition, 33*(9), 1307-1323. https://doi.org/10.3390/plants10020243

Shaki, F., Maboud, H. E., & Niknam, V. (2018). Growth enhancement and salt tolerance of Safflower (*Carthamus tinctorius* L.), by salicylic acid. *Current Plant Biology, 13*, 16-22. https://doi. org/10.1016/j.cpb.2018.04.001

Sharavdorj, K., Byambadorj, S. O., Jang, Y., Ahn, Y., & Cho, J. W. (2024). Evaluating the effects of long-Term salinity stress on the growth and physiology of mono and mixed crops. *Agronomy, 14*(2), 287. https://doi.org/10.3390/agronomy14020287

Sheikha, S, & Al-Malki, F. (2011). Growth and chlorophyll responses of bean plants to the chitosan applications. *European Journal of Scientific Research, 50*(1), 124-134.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant physiology and development. 6th Ed. Sinauer Associates, Sunderland, CT.

Tajik, H., Moradi, M., Rohani, S. M. R., Erfani, A. M., & Jalali, F. S. S. (2008). Preparation of chitosan from brine shrimp (*Artemia urmiana*) cyst shells and effects of different chemical processing sequences on the physicochemical and functional properties of the product. *Molecules, 13*(6), 1263-1274.

Tarkowski, L. P, & Van den Ende, W. (2015). Cold tolerance triggered by soluble sugars: A multifaceted countermeasure. *Frontiers in Plant Science, 6*, 203-210. https://doi.org/10.3389/fpls.2015.00203

Thuy, N. P., Ngan, V., Trai, N. N., Phong, V. T., Ai, T. N., Thi, Q. V. C., & Dotan, K. (2024). Potentioal effects of nanochitosan on rice under salinity. *Pakistan Journal of Botany, 56*(1), 19-26. DOI: http://dx.doi.org/10.30848/PJB2024-1(40)

Ullah, N., Basit, A., Ahmad, I., Ullah, I., Shah, S. T., Mohamed, H. I., & Javed, S. (2020). Mitigation the adverse effect of salinity stress on the performance of the tomato crop by exogenous application of chitosan. *Bulletin of the National Research Centre, 44*, 1-11.

Uthairatanakij, A., Teixeira da Silva, J., & Obsuwan, K. (2007). Chitosan for improving orchid production and quality. *Orchid Science and Biotechnology, 1*(1), 1-5.

Wang, Y., Ma, W., Fu, H., Li, L., Ruan, X., & Zhang, X. (2023). Effects of salinity stress on growth and physiological parameters and related gene expression in different ecotypes of *Sesuvium portulacastrum* on Hainan Island. *Genes, 14*(7), 1336.

Wang, Y., Ma, W., Fu, H., Li, L., Ruan, X., & Zhang, X. (2023). Effects of salinity stress on growth and physiological parameters and related gene expression in different ecotypes of *Sesuvium portulacastrum* on Hainan Island. *Genes, 14*(7), 1336-1358. https://doi.org/10.3390/genes 1407133

Yamasaki, S, & Dillenburg, L. R. (1999). Measurements of leaf relative water content in *Araucaria angustifolia*. *Revista Brasilleira de Fisiologia Vegetal, 11*(2), 69-75.

Zayed, A, & Elamry, I. (2006). Effect of salinity, mineral ions and organic solutes contents on *Vicia faba* (L.). *Pakistan Journal of Biological Sciences, 9*, 1406-1410. https://doi. org/10.1556/abot.48.2006.1-2.19

Zheljazkov, V. D, & Nielsen, N. E. (1996). Effect of heavy metals on peppermint and cornmint. *Plant and Soil*, 178(1), 59-66.

[DOI: DOI: 10.22034/14.67.133]