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Review Article

An investigation on the effects of chitosan and salinity on the physiological traits and some chemical compounds of purslane (*Portulaca oleracea* L)

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ABSTRACT

Salinity 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 is a major component of cell wall in many fungal species. This study aimed to evaluate the effects of salinity and chitosan on some physiological and growth parameters of *Portulaca oleracea* L. This experiment was conducted as a factorial based completely randomized design with 3 replications. For this purpose, the plant was exposed to certain concentrations of NaCl (0, 25 and 35 ds m^{-1}) and chitosan (0, 0.2 and 0.4 g L^{-1}). Considering the plant species are salt tolerant, the results showed that the 25ds m^{-1} salinity improved the plant growth; while the higher level of 35 ds m^{-1} reduced it. The chitosan treatment at the 25 ds m^{-1} salinity significantly improved the photosynthetic pigments; while both concentrations of chitosan did not show any significant moderating effect on the plants under 35 ds m^{-1} salinity. The application of 25 ds m^{-1} salinity or 0.4 g L^{-1} chitosan resulted in the highest contents of the K^+, Na^+, and compatible solutes that can be associated to the activation of defense enzymes such as ascorbate peroxidase activity. The analysis of variance showed that the amount of fatty acids such as linoleic acid (omega-6) and uronic acids significantly increased with salinity to mitigate the abnormal growth conditions. Considering the growing salinity of the soil, it is crucial to apply the right elicitors for reducing the adverse effects of salinity stress on *Portulaca oleracea* L.

Keywords: Salinity stress, Compatible solutes, Physiological traits, Fatty acids, Chitosan, *Portulaca oleracea* L

Abbreviations: CHL: Chlorophyll Pigments; GC/MS: Gas Chromatography/Mass Spectrometry; K^+: Potassium ion; Na^+: Sodium ion; PAL: Phenylalanine Ammonia Lyase; PPO: Poly Phenol Oxidase; RL: Root Length; ROS: Reactive Oxygen Species; RWC: Relative Water Content; SAR: Systemic Acquired Resistance; SL: Shoot Length; SOS: Salt Overly Sensitive; HKT: High Affinity K^+ Transporter.

INTRODUCTION

Portulaca oleracea L., commonly called purslane is a member of the portulacaceae family, growing in gardens, crop fields, and road sides across Africa, America, Asia, Australia, and Europe. They are succulent, up to 30 cm high, have small leaves with oval shape and dark green color. *P. oleracea* L. is recorded within the world health organization as one of the foremost utilized therapeutic plants and it has been given the state global panacea. *P. oleracea* contains carbohydrates, proteins, calcium, zinc, potassium, sodium, manganese, iron, phosphorus, selenium, vitamins C, E, fiber, essential amino acids, fatty acids, steroids, and organic cinnamic, caffeic, malic, malic oxalic and citric acid. Linolenic acid, linoleic acid, palmitic acid, oleic acid, and stearic acid are the most important fatty acids in the leaf and seeds of *P. oleracea*. Linoleic acid (or omega-6) and linolenic acid (or omega-3) is the main fatty acids *of P. oleracea*. About 60% of the leaf fatty acids and 40% of the seeds fatty acids are omega-3s. The seeds of purslane are more effective than the herb and are of good use.

To improve the plant production, it is crucial to outwit the biotic and abiotic stresses. Salinity stress is one of the major abiotic

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stresses limiting agricultural production, especially in arid and semi arid regions, typical of Iran. Salinity stress inhibits the plant shoot growth and production. NaCl obstruction of leaf expansion has been linked to reduced leaf turgor, decreased net CO_2 assimilation, toxic ion accumulation, and/or mineral nutrition disruption.

LITERATURE REVIEW

Chitosan is a natural biopolymer and biodegradable polysaccharide that can diminish abiotic stresses such as soil salinity and drought, and consequently improve plant growth. Chitosan is a chitin derivative, a bioactive and biocompatible compound that is totally safe for the environment. This compound is characterized by interesting unique properties such as bioactivity and biocompatibility [1-3]. Chitosan is able to chemically form bonds with fats, cholesterol, proteins, DNA, RNA, and metal ions. Chitosan is soluble in dilute acid solutions at pH <6. Recently, it has been reported that chitosan and its derivatives can be effective in reducing stress in plants. For example, chitosan improved photosynthetic pigments and osmolytes in the *Solanum lycopersicum* L. subjected to salinity stress. The combination of salinity and chitosan increased the contents of soluble sugars (70%) and proline (46%) in *Triticum aestivum* L.

In this study, the salinity tolerance of *Portulaca oleracea* was evaluated with the aim of increasing the plant tolerance to the stress using chemical stimulants such as chitosan, which is a non-toxic substance of natural origin. To this end, the plant response to the salinity stress was examined by measuring the content of soluble sugars, proline, and fatty acids as well as the leaf ions such as Na^+ and K^+. Chitosan was exogenously applied to the culture media to understand its regulatory role on the purslane plants cultivated under saline and normal growth conditions [4]. Different growth parameters, the contents of osmolytes, ions and the photosynthetic pigments were also measured in both control and chitosan treated plants in order to discover the underlying physiological responses and the possible use of chitosan in mediating salinity stress.

MATERIALS AND METHODS

Culture conditions and treatments

The uniform 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 16/8 h light/dark photoperiod. The pots were irrigated with distilled water. For salinity treatment, the plants aged 40 days (the three leaf stage) were irrigated with water containing either one of 0, 25 and 35 ds m^{-1} salinity once per two days for 10 days. Thereafter, the 0, 0.2 and 0.4 g L^{-1} chitosan was sprayed to the plants three times a week. The foliar application avoids the microbial degradation of the chitosan. The leaves of plants aged 57 days old were then harvested in the vegetative growth stage. The harvested samples were washed with double distilled water and then immediately used for further measurement of the Shoot Length (SL), Root Length (RL), and Fresh Weights (FWs) of the shoots and roots. It is good to mention that P. oleracea can complete its life cycle in 2–4 months in both tropical and calm districts.

Determination of Relative Water Content (RWC)

The relative water content was determined according to the method of Yamasaki. Fresh weights (FWs) of the plants were measured by scales sartorius TE313, Germany. The leaves were placed in distilled water in petri dish to complete turgor for 6 h in dark (TW). To determine Dry Weights (DWs), the fresh plants were dried in oven at 70°C and weighed after reaching a constant mass (72 hrs). The RWC was then calculated by the following equation:

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

Determination of photosynthetic pigments

The chlorophyll (chl) and carotenoids (car) of the leaves were extracted by 80% acetone. The absorbance of the extract was then read at 470, 662, and 645 nm by spectrophotometry, model Unico 2100, USA. The contents of the pigments were then estimated using available empirical correlations in literature.

Determination of osmolytes contents

The contents of proline in the plant leaves were measured according to the method of bates [5]. To this end, 0.5 g fresh weight of either sample was frozen in liquid nitrogen and then ground to homogenization with 10 mL 3% (w/v) sulfo salicylic acid. The samples were then centrifuged at 15,000 × g for 30 min. The supernatant was then mixed with ninhydrin reagent and acetic acid at 1:1:1 ratio. The samples were subsequently placed in a boiling water bath (~100ºC) for 1 h. Thereafter, the samples were left in ambient condition and then incubated in an ice bath for 5 min. A 2 mL toluene was added to each specimen and quickly shaken with a vortex device until two distinct phases were formed. The absorbance of the upper phase was measured by a spectrophotometer at 520 nm. The free proline content was calculated by a standard curve method and expressed as μg g^{-1} FW.

The contents of soluble sugar of the plant leaves were measured according to the method of Dubois. The plant leaves were harvested and then dried in an oven at 70ºC for 24 hrs. The 0.5 g of the samples was ground to homogenization with 10 mL 80% (vol) ethanol and the supernatant were separated by centrifugation at 15,000 × g for 30 min. The sample absorbance was measured at 490 nm using a spectrophotometer. The soluble carbohydrate content was then calculated by a standard curve method using glucose as standard and expressed in mg g^{-1} DW.

Extraction of enzyme and total soluble proteins

The protein content was determined according to the method of Bradford using bovine serum albumin as standard. To determine enzyme content and activity, the leaves (500 mg) were homogenized in 50 mM potassium phosphate buffer (pH 7.2). The homogenate was centrifuged at 14,000 × g for 15 min and the supernatant was used for further analysis. All procedures were performed at a temperature of −4°C.

Catalase activity

The reaction mixture comprised of a 50 mM (pH 6.8) phosphate buffer, H_2O_2 (15 mM) and the enzyme extract. The CAT activity was measured by the method of Chance and Maehly. The decomposition of H_2O_2 was followed by measuring the decrease in absorbance at 240 nm. The CAT activity values are expressed in units (U) mg^{-1} $protein^{-1}$ FW.

Ascorbate Peroxidase Activity (APX)

The oxidation of ascorbic acid in the presence of a 50 mM (pH 7) phosphate buffer, H_2O_2 (15 mM), ascorbic acid (5 mM), EDTA (10 mM) and enzyme extraction was measured at 290 nm by spectrophotometry according to Nakano and Asada in 1981 [6]. APX activity was defined as the amount of enzyme that decomposed 1 mM H_2O_2 min^{-1} FW.

Determination of K^+ and Na^+ concentrations

The dried samples (50–100 mg) were first digested in 10 mL HNO_3. The digested materials were filtered and diluted with distilled water and the ion contents were assayed using an atomic absorption spectrometer (Spectra AA 220, Varian, Australia). The concentration of each ion was then calculated from the relative standard curve.

Determination of fatty acids

Oil samples of the purslane leaf were extracted in triplicate according to the method. In brief, the sample (10 g) was mixed with isooctane (5 mL) in 2 min. After two phase separation, the upper phase was trans esterified with KOH solution. The fatty acid components of the final solution were identified by Varian Star 3400 gas chromatography.

Statistical analysis

All the experiments were done in triplicates unless otherwise specified. The experiments were performed in a randomized complete block design with three replicates. The data analysis was done by one way analysis of variance using Excel and SPSS software packages. The set of means were compared using the Duncan test at 95% confidence interval.

RESULTS

Growth parameters

The chitosan and salinity significantly affected the shoot and root lengths of purslane. However, the interaction of chitosan and salinity has no significant effect on the shoot length. The results of analysis of variance showed that the interaction of salinity and chitosan had a significant impact on the RL. An increase in salinity stress to 35 ds m^{-1} reduced the SL from 13.4 to 11.6 cm. The 25 ds m^{-1} salinity increased the RL but the 35 ds m^{-1} salinity had no significant effect on it.

At normal growth conditions, a concentration of 0.2 g L^{-1} chitosan reduced the SL; while a concentration of 0.4 g L^{-1} significantly increased it (~26%), . A 0.2 g L^{-1} chitosan reduced root length (~27%) and the high level of 0.4 g L^{-1} chitosan increased the root length about 25%.

The combination of 25 ds m^{-1} salinity and 0.4 g L^{-1} chitosan had no significant effect on the shoot length, but the 25 ds m^{-1} salinity and 0.2 g L^{-1} chitosan caused a 34% reduction in the shoot length. At salinity of 35 ds m^{-1}, addition of chitosan had no significant moderating effect on the shoot length. Combination of the 25 ds m^{-1} salinity with 0.2 g L^{-1} chitosan decreased the root lengths from 4.45 to 2.95 cm and with 0.4 g L^{-1} chitosan increased the root length about 46%. Interaction of 35 ds m^{-1} salinity with 0.2 g L^{-1} chitosan decreased the root length about 33% and with 0.4 g L^{-1} chitosan imposed no significant effect [7]. Addition of 0.4 g L^{-1} chitosan showed no significant moderating effect on the plants under 35 ds m-^{1} salinity stress; while addition of 0.2 g L^{-1} chitosan reduced the root length from 4.65 to 2.52 cm .

The salinity stress caused a significant reduction in the relative water content. Addition of 0.2 g L^{-1} and 0.4 g L^{-1} chitosan caused 57% and 34% reduction in the relative water content compared to that of the plants at normal growth conditions, respectively. Combination of 0.2 and 0.4 g L^{-1} chitosan significantly improved the effect of 25 ds m^{-1} salinity; while the addition of 0.4 g L^{-1} chitosan to the plants exposed to 35 ds m^{-1} salinity had no significant effect. A 12% increase in the RWC was observed in the plants under 0.2 g L^{-1} chitosan compared to those plants exposed to only 35 ds m^{-1} salinity. The results of ANOVA showed the significant effect of salinity, chitosan, and the interaction of salinity and chitosan on the RWC.

At normal growth conditions, a concentration of 0.2 g L^{-1} chitosan reduced (~28%) the shoot fresh weight; while a concentration of 0.4 g L^{-1} significantly increased (~7%) shoot fresh weight (~8%), The salinity of 25 ds m^{-1} increased (54%) the shoot fresh weight, but the salinity of 35 ds m^{-1} reduced (~12%) it. Combination of 0.2 and 0.4 g L^{-1} chitosan significantly reduced (~ 63% and ~ 36%) shoot fresh weight in 25 ds m^{-1} salinity; while the addition of 0.4 g L^{-1} chitosan to the plants exposed to 35 ds m^{-1} salinity increased (~11%) shoot fresh weight. The ANOVA showed the significant effect of chitosan, salinity, and the interaction of salinity and chitosan on the shoot fresh weight of purslane.

Salinity of 25 ds m^{-1} increased root fresh weight (~70%) and salinity of 35 ds m^{-1} resulted in 2.5 fold increase in the root fresh weight. At normal growth conditions, a concentration of 0.2 and 0.4 g L^{-1} chitosan caused 3.5 fold and 4 fold increase in the root fresh weight. The combination of 25ds m^{-1} salinity and 0.02 and 0.4 g L^{-1} chitosan reduced (~16% and 54%) root fresh weight. Interaction of 25ds m^{-1} salinity and concentration of 0.2 and 0.4 g L^{-1} chitosan reduced (~75% and 25%) root fresh weight [8]. The ANOVA showed the significant effect of chitosan, salinity, and the interaction of salinity and chitosan on root fresh weight.

Salinity of 25 and 35 ds m^{-1} increased (~15% and 50%) shoot dry weight. At normal growth conditions, a concentration of 0.4 g L^{-1} chitosan reduced (~40%) the shoot dry weight; while a concentration of 0.2 g L^{-1} had no significant effect on the shoot dry weight. Combination of 0.2 and 0.4 g L^{-1} chitosan significantly reduced (~16% and ~68%) shoot dry weight in 25 and in 35 ds m^{-1} salinity reduced (~86% and ~60%). The ANOVA showed the significant effect of chitosan, salinity, and the interaction of salinity and chitosan on the shoot dry weight.

Salinity of 25 ds m^{-1} increased (~100%) root dry weight. At normal growth conditions, a concentration of 0.2 and 0.4 g L^{-1} chitosan increased (~90% and 100%) the root dry weight from 0.001 to 0.002 g. Combination of 0.2 g L^{-1} chitosan significantly reduced (~50%) root dry weight in 25 ds m^{-1} salinity; while the addition of 0.4 g L^{-1} chitosan to the plants exposed to 25 ds m^{-1} salinity had no effect on the root dry weight. Interaction of salinity 35 ds m^{-1} and 0.2 g L^{-1} chitosan had no effect on the root dry weight; while the addition of 0.4 g L^{-1} chitosan to the plants exposed to 35 m^{-1} salinity increased (~8%) the root dry weight. The ANOVA showed the significant effect of chitosan, salinity, and the interaction of salinity and chitosan on root dry weight of the purslane.

The contents of photosynthetic pigments

The 25 ds m^{-1} salinity significantly decreased (~28%) the contents of chlorophyll a in the plants, but had no significant effect on the contents of chlorophyll b, carotenoids, and total chlorophyll altogether. Higher salinity of 35 ds m^{-1} had no effect on the chlorophyll a but increased the contents of chlorophyll b (23%), carotenoids (34%) and total chlorophyll (23%). The chitosan treatment of the control plants reduced Chla (19%), but it increased Chl b (33%) and carotenoids (15%). Interaction of the 25 ds m^{-1} salinity and 0.4 g L^{-1} chitosan increased the chlorophyll a (50%), carotenoids (7%) and total chlorophyll (42%), but did not significantly change the Chl b compared to the plants under 25 ds m^{-1} salinity. The contents of chlorophyll a, b, carotenoids and total chlorophyll reduced in the plants subjected to the 35 ds m^{-1} salinity and 0.2 g L^{-1} chitosan. Interaction of the 35 ds m^{-1} salinity and 0.4 g L^{-1} chitosan reduced chlorophyll a (21%), total chlorophyll (19%) and carotenoids (13%) but did not significantly change the chlorophyll b compared to the plants subjected to only 35 ds m^{-1} salinity. The results of ANOVA showed that the chitosan treatment had no significant effect on the photosynthetic pigments, but instead the salinity and the interaction of salinity and chitosan significantly affected the photosynthetic pigments in the plants.

Sodium ion content

The sodium ion content increased more than two folds at salinity conditions. The 0.2 and 0.4 g L^{-1} chitosan decreased (13%) and increased (55%) the sodium ion contents compared to that of the control plants, respectively. The supplementation of 0.2 g L^{-1} chitosan to the plants exposed to the 25 ds m^{-1} salinity increased 50% the sodium ion of the leaf, but the 0.4 g L^{-1} chitosan decreased to half of the sodium ion contents of the plants. The supplementation of 0.2 g L^{-1} chitosan to the plants exposed to the 35 ds m^{-1} salinity had a positive effect on the sodium ion content, but instead the supplementation of 0.4 g L^{-1} chitosan to the plants significantly reduced the sodium ion from 133 to 9 ppm. The ANOVA showed the significant effect of chitosan, salinity, and the interaction of salinity and chitosan on the Na^+ of the purslane.

Potassium ion content

The 25 ds m^{-1} salinity increased potassium ion content; while higher salinity of 35 ds m^{-1} caused 37% reduction in K^+. On the contrary, the low level of 0.2 g L^{-1} chitosan had a negative effect on the potassium ion; while the higher level of 0.4 g L^{-1} chitosan led to an increase in the potassium ion. Supplementation of the 0.2 and 0.4 g L^{-1} chitosan accompanied by a decrease in the potassium ion contents of the plants exposed to 25 ds m^{-1} salinity from 272 to 216 and 159 ppm, respectively. Also, the 0.2 g L^{-1} chitosan-treated plants exposed to the 35 ds m^{-1} salinity showed about two fold increase in the K^+; while the 0.4 g L^{-1} chitosan treated plants showed a sharp reduction in the K^+ (to ~6.0 ppm) compared to that of the plants subjected to 35 ds m^{-1} salinity [9] . The results of ANOVA showed the significant effect of chitosan, salinity, and the interaction of salinity and chitosan on the K^+ of the purslane.

Proline contents

The proline contents of the plants significantly increased after exposure to the 25 ds m^{-1} salinity, but showed no significant change at the 35 ds m^{-1} salinity compared to control. The chitosan treatment of the control plants slightly improved the proline contents compared to the control. Supplementation of 0.2 and 0.4 g L^{-1} chitosan slightly increased the proline contents in the plants subjected to the salinity stress. Overall, the proline contents slightly improved upon all treatment conditions and showed significant relationship with the salinity, chitosan, and interaction of proline and chitosan.

Soluble sugar contents

Soluble sugar contents of the plants increased after exposure to 25 ds m^{-1} salinity (~2%), but there was a decrease (5%) in the sugar content after exposure to the 35 ds m^{-1} salinity. Chitosan treatment of the control plants reduced the soluble sugar content. Supplementation of 0.2 g L^{-1} chitosan to the plants exposed to 25 ds m^{-1} salinity reduced the soluble sugar; while the addition of 0.4 g L^{-1} chitosan made no significant change compared to the control plants [10]. Addition of 0.2 g L^{-1} chitosan to the plants subjected to the 35 ds m^{-1} salinity had no significant moderating effect on the soluble sugar, but the 0.4 g L^{-1} chitosan increased the soluble sugar contents. There were significant relationship between salinity, chitosan, and the interaction of salinity and chitosan with the soluble sugar contents of the purslane.

Protein content

The 35 ds m^{-1} salinity reduced protein content from 8 to 6.7 mg g^{-1} FW. At normal growth conditions, a concentration of 0.2 g L^{-1} chitosan had no effect on the protein content; while the 0.4 g L^{-1} chitosan increased (~2%) it. Combination of 0.2 and 0.4 g L^{-1} chitosan significantly increased (~15% and ~9%) protein content in 25 ds m^{-1} salinity. A 0.2 g L^{-1} chitosan increased protein content in 25 ds m^{-1} salinity (~52%); while the addition of 0.4 g L^{-1} chitosan to the plants exposed to 35ds m^{-1} salinity reduced (~31%) protein content. The results of ANOVA showed the significant effect of chitosan, salinity, and the interaction of salinity and chitosan on the protein content of the purslane.

Catalase activity

Salinity stress of 25 and 35 ds m^{-1}, increased 1.8 and 2.4 fold catalase activity. Chitosan treatment of the control plants 2.2 and 4 fold increased catalase activity [11]. A 0.2 g L^{-1} chitosan increased catalase activity in 25 ds m^{-1} salinity (~23%); while

the addition of 0.4 g L^{-1} chitosan to the plants exposed to 25ds m^{-1} salinity reduced (~88%) catalase activity. Interaction of 35 ds m^{-1} salinity as well as 0.2 and 0.4 g L^{-1} chitosan reduced (~ 67% and 55%) catalase activity. The results of ANOVA showed the significant effect of chitosan, salinity, and the interaction of salinity and chitosan on the catalase activity of the purslane.

Ascorbate peroxidase activity

Ascorbate peroxidase activity had no effect after exposure to 25 ds m^{-1} salinity (~5%), but there was an increase (55%) in ascorbate peroxidase activity after exposure to the 35 ds m^{-1} salinity. At normal growth conditions, a concentration of 0.4 g L^{-1} chitosan had no effect on ascorbate peroxidase activity; while the 0.2 g L^{-1} chitosan increased (~45%) it. Supplementation of 0.2 g L^{-1} chitosan to the plants exposed to 25 ds m^{-1} salinity reduced (~66%) the ascorbate peroxidase activity; while the addition of 0.4 g L^{-1} chitosan increased (~66%) ascorbate peroxidase activity. A 0.2 g L^{-1} chitosan to the plants exposed to 25 ds m-^{1} salinity reduced (35%) the ascorbate peroxidase activity, but addition of 0.4 g L^{-1} chitosan to the plants subjected to the 35 ds m^{-1} salinity had no significant moderating effect on the ascorbate peroxidase activity. The results of ANOVA showed the significant effect of chitosan, salinity, and the interaction of salinity and chitosan on the ascorbate peroxidase activity of the purslane.

Fatty acid composition

The results of analysis variance showed that salinity, chitosan and the interaction of salinity and chitosan had a significant effect on the fatty acids [12]. The step wise increase in the salinity either decreased (the contents of dodecanoic acid, tetradecanoic acid, palmitic acid, linolenic acid, and docosanoic acid of the leaf), increased (the contents of palmitoleic acid, linoleic acid, erucic acid, tetracosanoic acid, and uronic acid), had no significant effect (on the contents of octadecanoic acid), or affected the contents of some fatty acids (paullinic acid, oleic acid, eicosanoic acid, and heptadecanoic acid) in a dose dependent manner.

Similarly, an increase in the concentration of chitosan either decreased (the contents of tetradecanoic acid, palmitic acid, heptadecanoic acid, paullinic acid, and docosanoic acid of the leaf), increased (the contents of oleic acid, eicosanoic acid, and uronic acid), or affected the contents of the fatty acids (dodecanoic acid, palmitoleic acid, octadecanoic acid, linoleic acid, linolenic acid, and tetracosanoic acid) in a dose dependent manner.

Addition of 0.2 g L^{-1} chitosan increased the contents of tetradecanoic acid, palmitic acid, palmitoleic acid, octadecanoic acid, oleic acid, and linoleic acid; while this treatment decreased the contents of dodecanoic acid, heptadecanoic acid, linolenic acid, eicosanoic acid, docosanoic acid, and erucic acid compared to the plants under the 25 ds m^{-1} salinity. The addition of 0.4 g L^{-1} chitosan to the plants under the 25 ds m-^{1} salinity increased the contents of dodecanoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, oleic acid, linolenic acid, and eicosanoic acid; while decreased the contents of tetracosanoic acid and docosanoic acid. It showed no significant effect on tetradecanoic acid and octadecanoic acid.

The addition of 0.2 g L^{-1} chitosan increased the contents of dodecanoic acid, tetradecanoic acid, palmitic acid, heptadecanoic acid, octadecanoic acid, oleic acid, linoleic acid, linolenic acid, and eicosanoic acid in the plants subjected to the 35 ds m^{-1} salinity; while this treatment decreased the contents of palmitoleic acid, docosanoic acid, tetracosanoic acid, erucic acid, and uronic acid. The addition of 0.4 g L^{-1} chitosan to the plants exposed to the 35 ds m^{-1} salinity caused an increase in the contents of all fatty acids except the tetracosanoic acid.

DISCUSSION

Portulaca grows easily in dry and saline soils. *P. oleracea* plants have characteristics for dry and salinity adaptation, including thick cuticles, small pores, special water storage tissues in stems and leaves, small surface to weight ratio, and very branched main roots along with many pointed secondary sprays that spread near the soil surface. Almost all mesophyll in *P. oleracea* are uniformly composed of aqueous tissue and store water. Accordingly, the 25 ds m^{-1} salinity increased the root and shoot lengths and had no significant effects on the total chlorophyll and carotenoids. Salinity of 35ds m^{-1} reduced SDW and SFW but increased RDW and RFW. The shoot response to increasing salinity was minimal in lateral roots and maximum in stems, consistent with other reports in literature.

Consistently, an increase in the contents of free proline and soluble sugars can be attributed to the adaptation of the plant to mild salinity conditions (*i.e.*, 25 ds m^{-1}) or chitosan treatment (0.4 g L^{-1}). The free proline and soluble sugars are non-enzymatic scavengers of free radicals, accumulated under abnormal conditions such as salinity. These compatible solutes protect the membrane, cellular proteins and the entire cells from oxidative damage [13]. These observations are in agreement with similar findings reported in cowpea and tomato. Increased proline levels under salinity in purslane and in barley have been reported due to increased pyrroline-5-carboxylate reductase activity and decreased proline dehydrogenase activity [14]. Soluble sugars protect membranes from dehydration and act as scavengers of free radicals. Elevated sugar levels in plants can be produced by higher rate of photosynthesis and/or decomposition of polysaccharides into soluble sugars, accompanied by a reduction in the shoot lengths.

The higher salinity level of 35 ds m^{-1} reduced the shoot length of the purslane plants possibly because of a reduction in osmotic pressure, nutritional imbalance, ion toxicity, and also oxidative stress. The reduction in the relative water content confirms the possible occurrences of the reduction in osmotic pressure. Consistent with these findings, the reduction of the RWC in plants was reported in other studies as well. The damaging effect of salinity on growth parameters was also reported in *Triticum aestivum* [15].

The photosynthetic pigments increased in the plants subjected to the 35 ds m^{-1} salinity. A similar observation was also reported in *Helianthus annuus* L. They found a reduction in the leaf area and length after exposure to salinity. Consistent with the present findings, it suggests that the increase in chlorophyll content was likely due to smaller cell sizes and a higher concentration of chloroplasts per unit area. The carotenoids increased as these molecules play a key role in photosynthesis and resistance to salinity. They are fundamental molecules

involved in light protection during photosynthesis. They are antioxidants and single oxygen scavengers and prevent lipid peroxidation and membrane destabilization [16]. Less membrane damage is accompanied by higher RWC that is a possible reason for higher RWC in the plants at 35 ds m^{-1} salinity compared to ones at 25 ds m^{-1}. The activation of these secondary metabolites is a possible reason for insignificant changes in the proline contents and slight reduction of soluble sugars at high salinity of 35 ds m^{-1} because the compatible solutes usually protect the plant cells at mild stress conditions. Besides, these solutes might be decomposed by reacting with peroxides.

Low sodium and high potassium concentrations within the cytoplasm are essential to maintain enzymatic processes in the cytoplasm. In numerous plants, salinity stress increased the uptake of sodium and inhibited the uptake of potassium so that plants tried to keep high levels of potassium and low levels of sodium in the cytosol. The plants regulated the activity of sodium and potassium transporters and hydrogen pumps that provide the energy for ions' transport through their Salt Overly Sensitive (SOS) system. The SOS3 system improves the plant tolerance to mild salinity conditions by developing the lateral roots through modulation of auxin gradients and maxima, a possible reason for 22% increase in the root length of the plants subjected to the mild salinity of 25 ds m^{-1}, accompanied by two fold increase in the Na^+ and 28% increase in K^+ contents of the plant. In the present study, the 35 ds m^{-1} salinity increased sodium content and significantly decreased potassium compared to control. It is because of the accumulation of sodium in the media which led to a disturbance of ionic balance and a defect in the absorption of beneficial ions and disruption of plant normal metabolism [17]. High salinity proved that sodium is inactively absorbed in large quantities in *P. oleracea* and transport mechanisms involve Na^+ and/or K^+ transporters and channels as well as non-selective cation channels. Sodium accumulation is part of the osmotic mechanism and osmotic adjustments. On the other hand, potassium under salinity stress decreased. These change in ionic balance damaged the plant both physiologically and biochemically, and it can be considered as one of the main causes of salinity toxicity which agree with the similar observation reported in soybean. At low salinity of 25 ds m^{-1}, the potassium content not only decreased, but also showed about 28% increase consistent with higher shoot and root lengths of the purslane.

The positive effects of chitosan treatment on the plant growth were reported in bean [18]. The chitosan treatment may improve the growth and development by some signaling pathways related to auxin biosynthesis *via* a tryptophan independent pathway. Moreover, chitosan can reduce disease severity in plants, possibly by increasing the activity of PAL and PPO, lignifications resulting from increased biosynthesis of phenolic compounds or induced secondary metabolites and systemic acquired resistance. It also improves plant resistance to reactive oxygen species by activating the enzymatic and non-enzymatic defense systems. The foliar application of chitosan to the edible rape under Cd stress significant increased the acetylsalicylic acid and the antioxidant enzymes of the plants. It is a possible reason for a decrease in the shoot and root lengths of the plants subjected to 0.2 g L^{-1} chitosan because of shifting the plant normal metabolism to the defensive conditions. However, the treatment of the plants at normal growth conditions induced the production of reactive oxygen species and led to stomata closure of the leaf tissues.

Moreover, resistance to disease infections may also involve the closure of stomata by abscisic acid. The chitosan affected different parts of photosynthetic system such as glutamate synthesis, chloroplast gene expression, and gas exchange. In the present work, the low level of 0.2 g L^{-1} chitosan treatment has no significant effect on the photosynthetic pigments; while an increase in carotenoids at higher level of 0.4 g L^{-1} chitosan can be attributed to the same defensive mechanism. The higher content of Chl b and lower level of Chl a in the plants subjected to 0.4 g L^{-1} chitosan can be related to the conversion of the primary metabolized chlorophyll a to chlorophyll b by Chl a oxygenase at moderate stress conditions [19]. The chlorophyll a is the necessary first step in the catabolism of chlorophyll upon mild stress conditions.

Consistently, the potassium ion content was significantly increased in the plants treated with the 0.4 g L^{-1} chitosan. According to some studies, higher intracellular potassium increase chlorophyll content which is in agreement with the similar observation reported in tomato, wheat and strawberry. The consequence of this effect possibly resulted in higher synthesis of photosynthetic pigments accompanied by the higher root and shoot lengths of the plants compared to the control.

However, the combined action of chitosan and salinity significantly reduced the root and shoot lengths compared to the plants only under salinity. These observations might be related to the combination of different contradictory effects mentioned above, highly depending on the plant species and genotypes. As an instance, the interaction of salinity and chitosan was reported to increase the sodium and potassium ions in strawberry while it acted in different way in the present work highly depending on the treatment dosage as well.

One way to understand the ability of plants to withstand environmental stress is to identify stress induced changes in protein content. With the idea that stress adaptation is due to protein synthesis in response to environmental stresses and altered gene expression. One of the important biochemical events in plants under salinity stress is the decrease or increase of protein. In the present study, the amount of protein at a salinity level of 25 ds m^{-1} increased compared to the control and at a salinity of 35 ds m^{-1} showed a significant decrease compared to the control [20]. Reduction of protein can be made of decreased protein synthesis, accelerated degradation or proteolysis, reduction of amino acids, degradation of enzymes in the protein synthesis pathway. Potassium had effect on more than 50 enzymes and some elements that used in protein synthesis pathway, it binds tRNA to ribosomes. A reduction in potassium and an increase in sodium in the plant and in the cytosol under salinity stress will lead to a decrease in protein synthesis and protein content. Leaf soluble protein increases at low salinity and decreases at higher salinity which is in agreement with the similar observation reported in *Paulownia imperialis* and *Paulownia fortunei*. In the present study, a concentration of 0.2 g L^{-1} chitosan increased protein content compared to the control. Chitosan in safflower plant increased protein. It was reported that treatment with low chitosan concentrations in safflower seedlings under stress increased the

concentration of soluble protein, which is in agreement with our findings. This result shows that low concentration chitosan has been able to prevent the accumulation of sodium ions such as SOS1 HKT1 and NHX in the cytosol.

The catalase and peroxidase enzymes break down compounds such as hydrogen peroxide into water and oxygen, thus preventing cell damage. In the present study, the activity of catalase showed a significant decrease in stress conditions compared to the control. This decrease may be due to increased activity of ascorbate peroxidase under salinity stress, which causes the decomposition of hydrogen peroxide. Salinity stress in rice plant and safflower reduced the activity of catalase enzyme and also in the concentration of 0.2 g L^{-1} chitosan, the activity of catalase enzyme is increased. In the present study, salinity of 35 ds m^{-1}, the activity of ascorbate peroxidase increased. The ascorbate peroxidase activity has been reported to decrease with decreasing catalase activity under stress. In confirmation of the findings of the present study, Lee, et al. reported that the activity of ascorbate peroxidase enzyme in tomato plants increased under salinity. In this study, a 0.4 gL^{-1} of chitosan increased ascorbate peroxidase activity and 0.2 g L^{-1} chitosan reduced ascorbate peroxidase activity. Chitosan increased the activity of ascorbate peroxidase in culture of *Silybum marianum* hair roots. It seems that the simultaneous treatment of salinity of 25 and 35 ds m^{-1} and two concentrations of chitosan, the activity of catalase and ascorbate peroxidase acted in such a way that reducing one of them increased the activity of the other enzyme to be able to remove H_2O_2. Increasing ascorbate peroxides activity in low concentration of chitosan had been reported in mungbean.

The aforementioned effects of chitosan and salinity activated different metabolic pathways that led to different concentrations of the fatty acids. For example, the supplementation of 0.4 g L^{-1} chitosan to the plants under 35 ds m^{-1} salinity resulted in the highest linoleic acid possible because of the over expression and enhanced activity of Δ12 desaturase enzyme after chitosan treatment and salinity stress. Similarly, the unsaturated fatty acids significantly increased in the halophytic plants under salinity. Consistently, the reduction in the saturated fatty acids such as palmitic acid, oleic acid, eicosanoic acid, dodecanoic acid, and docosanoic acid with salinity treatment can be related to their conversion to unsaturated fatty acids (palmitoleic acid, linoleic acid, erucic acid, and paullinic acid). About six fold increases in uronic acid in the plants exposed to the 35 ds m^{-1} salinity compared to the plants under normal conditions can be attributed to the high level of reactive oxygen species and the consequent induction of the soluble sugars (as antioxidant osmolytes). According to, the decrease in the soluble sugars of the plants exposed to this 35 ds m^{-1} salinity is possibly because of their conversion to uronic acids. Further metabolic studies should be done in the future to clearly explain the underlying mechanism behind fatty acids after these treatments.

CONCLUSION

The purslane is a salt tolerant plant that easily adapted itself to mild salinity conditions (25 ds m^{-1}) by increasing the shoot and root lengths to supply the required nutrients. This adaptation caused higher absorption of potassium and sodium ions. Step wise increase in salinity disturbed the ionic balance, the absorption of beneficial ions, and finally the plant normal metabolism. It reduced the leaf area and length and increased the contents of chlorophyll (likely due to smaller cell sizes and a higher concentration of chloroplasts per unit area) and carotenoids. The foliar application of chitosan at 0.4 g L^{-1} significantly increased the photosynthetic pigments and potassium ions, consequently improved the plant growth. Carotenoids possibly caused the higher RWC in the plants under 35 ds m^{-1} than the ones under 25 ds m^{-1} salinity. Consistently, the decomposition of the compatible solutes under high salinity decreased their contents in the plants. The stress conditions possibly converted the saturated fatty acids to unsaturated ones such as linoleic acid. The highest increase was observed in uronic acid possibly because of high soluble sugars and reactive oxygen species in the plant after exposure to salinity. An increase in salinity or the combination of salinity and chitosan resulted in different pathways with significant changes in the physiological traits and biochemical components.

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