Effects of ascorbate foliar application on morphological traits, relative water content and extract yield of Purslane (*Portulaca oleracea* L.) under salinity stress

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Abstract

Ascorbate is a strong antioxidant which has remarkable biological effects on plants growth, including an improvement in plants' tolerance under salinity stress conditions. In the present study, the effect of salinity stress and its interaction with ascorbic acid was investigated on some morphological traits, cell membrane stability, leaf relative water content, and extract yield of purslane (*Portulaca oleracea* L.) under greenhouse conditions. This research was down as factorial based on completely randomized design with 4 replications. The plants were treated in different concentrations of sodium chloride (0, 70, 140, and 210 mM) and ascorbic acid (0, 10, and 20 mM) for 4 weeks after germination. In the treated plants with salt, increases in sodium chloride concentration reduced growth parameters such as roots and shoots fresh and dry weights, roots and shoots lengths, extract yield and relative water content (RWC). However, cell membrane stability and extract percent increased. Under the same salt stress conditions, increase in ascorbic acid concentrations improved all the studied characters, so that spraying 20 mM ascorbate conducted to the maximum amount of extract percentage (0.0054 %) and extract yield reduced (0.037 g/plant). The study also revealed that *purslane* is a medicinal plant with a high resistance to salinity stress and can generally be cultivated in saline soils.

Keywords: ascorbic acid; salinity stress; Purslane (*Portulaca oleracea* L.); extract; morphological traits

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Introduction

Purslane (*Portulaca oleracea* L.) is an annual grassy plant with fleshy stalks, opposite leaves and small yellow flowers. Belonging to C4 plants that easily grow in acid or salty soils, purslane is a halophyte (Kumamoto et al., 2000). There is a long history of using this plant as food and medicine even to the point that the UN World Health Organization considers it as a "cure all" (Samy et al., 2005). It is also an excellent source of antioxidants such as vitamins C, A, E,

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and beta-carotene with the ability to counteract free radicals and prevent cardiovascular disease, cancer and infections. The stalks are rich with omega-3 fatty acids, α-Tocopherol, ascorbic acid, beta-carotene, and glutathione (Simopoulos et al., 2002).

Nowadays medicinal plants are highly valued in promoting public health. The diversity in climatic conditions of Iran and the long tradition of using these plants for remediation purposes have attracted many researchers and research institutes to make proper use of these herbs.

Vast saline soils and limitations posed by salinity on development of agricultural and medicinal crops are among serious problems encountered by farmers. Presently, a general increase is observed in soil salinity and at the same time available fresh water is limited due to its excessive and wasteful use (Khan and Weber, 2006). Munn (2002) reported that more than 800 million hectares of the earth's land surface is under influence of salinity and it is estimated that 20 million hectares of these lands are negatively affected by salinity.

One of the biochemical changes that result from abiotic stresses including salt stress are production of reactive oxygen species which can cause major damage to the membrane, essential oils, proteins, and nucleic acids (Garratt et al., 2002). Plant cells are equipped with a free radicals scavenging system to protect the plant against oxidative damages. Production of ascorbic acid by the plant is part of this protection system.

Ascorbic acid is a very strong antioxidant that controls free radicals through reduction of them (Fecht-Christoffers et al., 2003). Its small water soluble molecules act as primary substrate in the hydrogen peroxide enzymatic detoxification cycle (Beltagi, 2008). Ascorbate exists in cytosol, vacuoles, mitochondria, and plant cell walls. Together with some other compounds such as α-Tocopherol, carotenoids, and phenols, ascorbic acid makes up a non-enzymatic antioxidant system in the plant (Smirnoff, 2001). The damage caused by ROS is mitigated through antioxidants activities (Muhammad et al., 2010).

During their growth, plants are subjected to various abiotic conditions such as salinity stress. Studies have shown that production of medically valuable compounds in the medicinal plants is influenced by plant genotype and abiotic conditions (Filippo et al., 2002). The plant's ability to survive and continue to grow under abiotic stresses depends on its genetic potential which manifests itself in the form of physiological and molecular responses.

Under stress conditions, plants experience physiological and biochemical changes. These include accumulation of ABA, closing up the pores, and reduction in leaf surface area (Uzma and Asghari, 2007). Many studies have reported the negative effect of salinity stress on plant growth parameters, e.g., in sugar beet (Ghoulam et al., 2002) and peas (Beltagi, 2008).

Comprehensive studies and proper exploitation of medicinal plants is necessary, especially when using these plants in medicine, cosmetics, and food industry is on the increase (Mohammad et al., 2010). Because of the importance of *Portulaca oleracea* L. the present study aimed at investigating the effects of salinity stress on this medicinal plant and its interaction with protective effect of ascorbic acid against oxidative stress caused by NaCl.

Materials and Methods

In order to study the effect of salinity stress and its interaction with ascorbic acid on morphological traits, leaf relative water content, stability of cytoplasmic membranes, and

Table 1
Analysis of the soil used in the pots

Sand %	Silt %	Clay %	OC %	Na. mg/kg	K mg/kg	P mg/kg	EC in the saturated essence Ds/M	pH in the saturated essence	FC %
13	78	9	0.27	112	289.9	44	1.4	8.1	29.77

hydroalcoholic extract of purslane (*Portulaca oleracea* L.), a factorial experiment was conducted in a completely randomized design with 4 replications. Forty eight pots, 18 cm in diameter and 15 cm in height were filled with sandy – loamy soil. The pots were bedded with coarse sands (4 cm in height) for draining and each pot was supplied with 4 kg soil (See Table 1 for the analysis of the soil). Several seeds were sown in late February and the grown plants were then thinned to 7 plants in each pot.

The experimental factors included salinity stress in 4 levels (0, 70, 140, and 210 mM) and ascorbate in 3 levels (0, 10, and 20mM). Plants were harvested 4 weeks after sowing. Roots and shoots were separated and washed with water. Their lengths were measured using a ruler with millimeter scale. Fresh and dry weights of roots and shoots were measured using a digital scale (error of measurement = 0.0001). To measure RWC (relative water content), 10 fully matured leaves were selected from the same heights and their fresh weights were recorded. Then the leaves were soaked into distilled water under low lighting conditions for 24 h to measure their saturated weight. After recording turgescence weight, leaves were dried at 75 °C for 48 h and their dry weights were measured. RWC was calculated using the following formula:

RWC = [(fresh weight) – (dry weight)] / (bulge weight) – (dry weight)] × 100

Seven fully mature leaves were selected from each treatment for recording stability of cytoplasmic membranes. These were then weighed and kept for 24 h in 10 ml manitol (-2 Bar water potential) at 20 °C. Electrical conductivity of the solution was used as an indicator of cell membrane stability. Extraction from dried purslane samples was carried out using alcohol with ultrasonic equipment (CP3007).

Data Analysis

The obtained data were subjected to Analysis of Variance using Duncan Multiple Range test (P≤0.05) using SAS software. The figures were drawn using Excel.

Results

Root length

The findings (Table 2) showed a significant difference between various salinity levels and ascorbate regarding the root length (P≤0.01). Also interaction of effects of salinity and ascorbate on the root length did not show significant difference (Table 2). Comparison of the means showed that salinity at 0 mM resulted in the maximum root length of 19.06 cm (Table 3). Application of 20 mM ascorbate increased root length to 17.56 cm (Table 3).

Shoot length

There was a significant difference (P≤01) in stem length between various levels of salinity (Table 2). The maximum shoot length (27.44 cm) was observed under 0 mM salinity and the minimum shoot length (17.00) was recorded under 210 mM salinity (Table 3). Application of

Table 2
Variance Analysis of salinity and ascorbate effects on experimented traits

Source of Variability	DF	Stem Length	Roots Length	Shoot Fresh Weight	Shoot Dry Weight	Root Fresh Weight	Root Dry Weight	Membrane Leakage	RWC	Extract percentage	Extract Yield
Salinity	3	178.44**	129.92**	10.41*	0.09*	1.76**	0.02*	0.18*	6.07**	108.73**	275.89**
Ascorbate	2	143.60**	150.91**	53.58*	0.09*	1.25*	0.06**	1.03**	9.10**	10.10**	49.50**
Salinity × Ascorbate	6	32.19**	11.34^{ns}	1.51^{ns}	0.009**	0.19^{ns}	0.0009^{ns}	0.01^{ns}	194.13**	17.59**	39.61**
Error	33	2.8	9.08	2.24	0.001	0.26	0.08	0.04	7.2	0.00001	0.00001
CV %		7.47	10.43	13.75	3.48	14.74	13.26	13.57	3.61	10.10	15.90

ns: non-significant; *: significant at P≤0.05; **: significant at P≤0.01

ascorbate had a meaningful effect (P≤0.01) on stem (Table 2), so that 20 mM and 0 mM ascorbate resulted in maximum (23.17 cm) and minimum (21.13 cm) stem length, respectively (Table 3). The interaction of effects of the factors on the stem length was significant at p≤0.01 (Table 2). Lack of salinity and foliar spraying of 20 mM ascorbate resulted in the maximum stem length 31.38 cm (Fig. II).

Root fresh weight

The results of the study showed meaningful difference (p≤0.01) in the root fresh weight (Table 2). Maximum and minimum root fresh weights were recorded under control condition (2.21 g) and severe salinity (1.44 g), respectively (Table 2). Also application of ascorbate had a significant effect (p≤0.01) on root fresh weight (Table 2). Spraying 20 mM ascorbate had maximum effect on this character (15.44 g) while in plants with no ascorbate treatment, minimum fresh weight (10.86 g) was recorded (Table 3). Interaction of two experimental factors did not show significant difference in root fresh weight (Table 2).

Shoot fresh weight

Shoot fresh weights were meaningfully different (p≤0.01) under various salinity levels (Table 2). Maximum shoot fresh weight (16.69 g) and minimum shoot fresh weight (6.18 g) were recorded under control and severe salinity stress

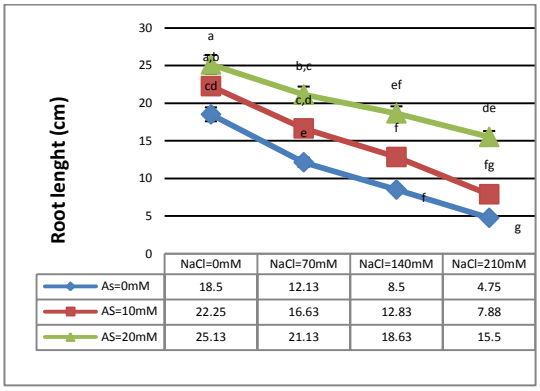

Fig. I. Interaction of effects of salinity and ascorbate on root length

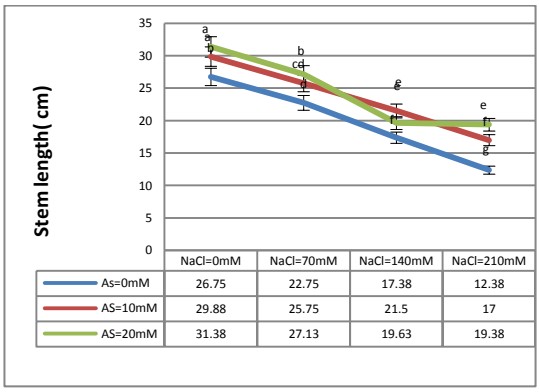

Fig. II. Interaction of effects of salinity and ascorbate on stem length

condition (210 mM), respectively (Table 3). Ascorbate application also had significant effect (p≤0.01) on shoot fresh weight (Table 2) and 20 mM ascorbate had the highest (15.44 g) and no ascorbate application had the lowest (10.86 g) shoot fresh weight (Table 3). Interaction effects of salinity and ascorbate on shoot fresh weight

Table 3
Mean comparison of salinity and ascorbate interaction effects on experimented traits

Source		Stem Length (cm)	Roots Length (cm)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)	Membrane Leakage (µs/cm)	RWC (%)	Extract (%)	Extract yield
Salinity (mM)	0	27.44^{a}	19.06^{a}	16.96^{a}	7.68^{a}	2.21^{a}	1.06^{a}	1.32^{c}	75.97^{a}	0.002^{d}	0.005^{d}
	70	25.75^{a}	17.63^{a}	13.24^{a}	4.99^{b}	2.12^{b}	0.68^{a}	1.51^{b}	75.50^{a}	0.003^{c}	0.011^{c}
	140	18.50^{b}	13.56^{b}	10.79^{ab}	3.70^{c}	1.95^{b}	0.56^{ab}	1.53^{b}	74.84^{a}	0.004^{b}	0.022^{b}
	210	17.00^{b}	7.56^{b}	7.18^{b}	2.58^{d}	1.44^{b}	0.19^{b}	1.62^{a}	71.67^{b}	0.005^{a}	0.039^{a}
Ascorbate (mM)	0	21.13^{b}	12.94^{b}	10.86^{b}	4.11^{c}	1.44^{b}	0.48^{c}	1.71^{a}	73.03^{b}	0.004^{b}	0.02^{b}
	10	23.00^{a}	14.50^{ab}	11.13^{b}	4.38^{b}	1.88^{ab}	0.70^{b}	1.52^{ab}	73.35^{b}	0.004^{a}	0.02^{b}
	20	23.17^{a}	17.56^{a}	15.44^{a}	5.66^{a}	2.46^{a}	0.90^{a}	1.25^{b}	76.44^{a}	0.005^{a}	0.03^{a}

Similar letters in each column show no significant difference based on Duncan test (p≤0.05).

was not significant (Table 2). Comparison of the means under salinity and ascorbic acid treatments (Table 4) showed that under non salinity stress conditions and 20 mM ascorbate foliar application, the maximum shoot fresh weight (71.84 g) was achieved.

Root dry weight

Root dry weights were meaningfully different (P≤0.05) under various salinity levels (Table 2). Maximum root dry weight (1.06 g) was recorded under no salinity conditions, while minimum root dry weight (0.19 g) was observed under 210 mM salinity stress (Table 3). Single effect of ascorbate had significant effect (P≤0.01) on root dry weight of purslane (Table 2) so that application of 20 mM ascorbate resulted in the highest (0.90 g) and no ascorbate treatment resulted in the lowest (0.48 g) dry root weight (Table 3). Interaction of effects of salinity and ascorbic acid on root dry weight was not statistically significant (Table 3).

Shoot dry weight

Shoot dry weight in the study showed significant variation (p≤0.05) through different levels of salinity (Table 2). Maximum (7.68 g) and minimum (2.58) shoot dry weights were observed under control and 210 mM salinity conditions, respectively (Table 3). Application of ascorbate had a significant effect (p≤0.01) on dry shoot weight (Table 2). As Table (3) shows, foliar application of 20 mM ascorbate resulted in the highest shoot dry weight while no application of ascorbic acid led to the lowest shoot dry weight (4.11 g). Interaction of effects of salinity and ascorbate treatments on shoot dry weight was significant at p≤0.01 (Table 3). Mean comparison of no salt and foliar spraying of 20 mM ascorbic acid resulted in the maximum shoot dry weight (9.55 g) in plants under study (Fig. VI).

Extract percentage

Extract percentage at various salinity levels of the study showed significant (p≤0.01) difference (Table 2). Maximum (0.005%) and minimum (0.002%) extract contents were recorded under 210 mM salinity and no salt

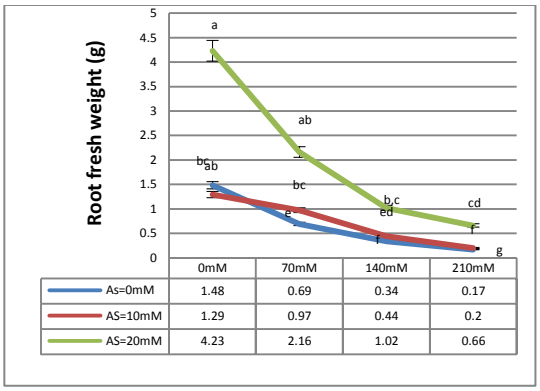

Fig. III. Interaction effects of salinity and ascorbate on root fresh weight

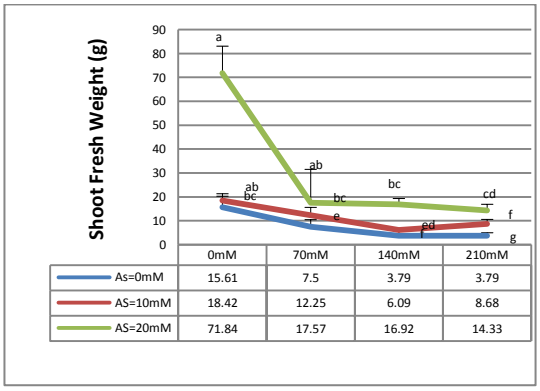

Fig. IV. Interaction effects of salinity and ascorbate on shoot fresh weight

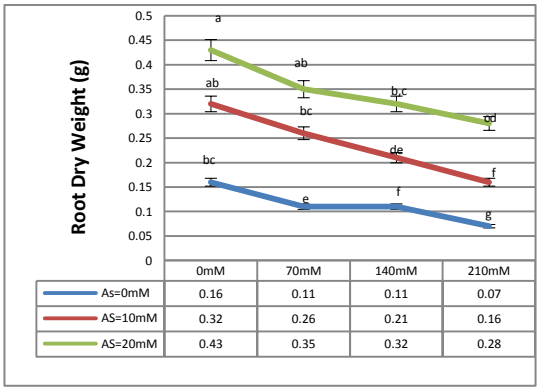

Fig. V. Interaction effects of salinity and ascorbate on root dry weight

conditions, respectively (Table 3). Ascorbate had also a significant effect (p≤0.01) on extract content (Table 2) so that spraying of 20 mM ascorbate and no ascorbate application led to the highest (0.005%) and lowest (0.004 %) levels of extract content, respectively (Table 3). On the other hand, interaction of effects of salinity and

ascorbate on extract percentage was not significant (Table 2). Mean comparison of salinity and ascorbate application (Fig. VII) showed that under salinity stress (210 mM) and foliar spraying of 20 mM ascorbate, maximum extract content (0.0054 %) was achieved.

Extract yield

Extract yield of each plant under various salinity treatments showed significant difference at p≤0.01 (Table 2). Maximum (0.039 g/plant) and minimum (0.005 g/plant) extract yield were observed under no salinity and 210 mM salinity levels, respectively (Table 3). Ascorbate alone had a significant effect on extract yield of each plant under study at p≤0.01 (Table 2). In fact, treatments with 20 mM and 0 mM ascorbate had the highest (0.03 g/plant) and lowest (0.02 g/plant) extract yield, respectively (Table 3). Moreover, interaction of effects of salinity and ascorbate treatments on the extract yield was also meaningful at p≤0.01 (Table 3). Mean comparison of interaction effect of experimental factors (Fig. VI) showed spraying 20 mM ascorbate combined with no salt stress resulted in the maximum extract yield (0.044 g/plant).

Leaf relative water content (RWC)

Various salinity levels in the study showed significant difference (p≤0.01) in RWC (Table 2). Maximum and minimum RWC were recorded as 75.9% for control and 71.67% under 210 mM severe salinity conditions, respectively (Table 3). Ascorbate alone had a significant effect (p≤0.01) on RWC (Table 2). While application of

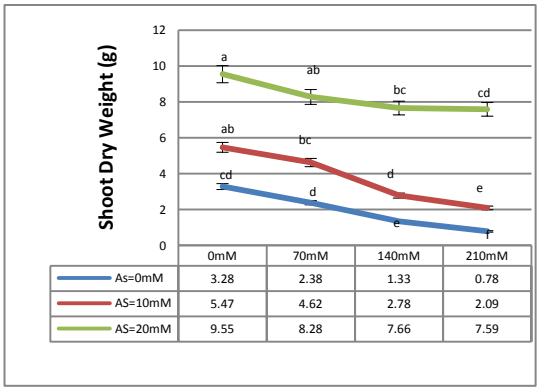

Fig. VI. Interaction of effects of salinity and ascorbate on dry shoot weight

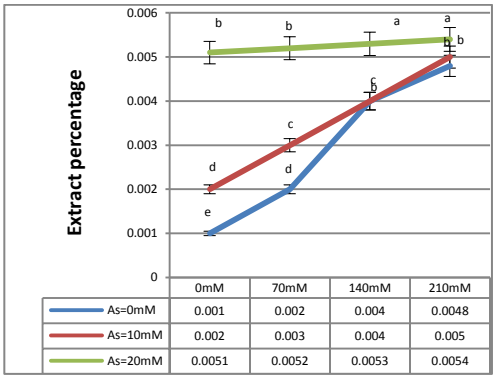

Fig. VII. Interaction of effects of salinity and ascorbate on extract percentage

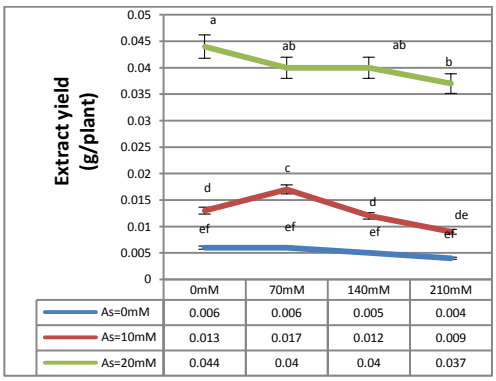

Fig. VIII. Interaction of effects of salinity and ascorbate on extract yield

20 mM ascorbate led to the highest RWC (76.44%), in plants receiving no ascorbate the lowest RWC (73.03%) was recorded (Table 3). Ascorbate and salinity stress had significant interaction effect on RWC at p≤0.05 (Table 2). Mean comparison of this interaction (Fig. X) showed that application of water without sodium chloride and foliar spraying of 20 mM ascorbate resulted in the highest RWC (93.56%).

Cytoplasmic membrane stability

Electrical conductivity of the cytoplasmic membrane leakage of the leaves was used as the criterion for its stability. The results suggested that cytoplasmic membrane leakage was significantly different (p≤0.05) at various salinity levels (Table 2). Maximum (1.62 µs/cm) and minimum (1.32 µs/cm) cytoplasmic membrane stability were recorded under 20 mM salinity stress and control (Table 3). Ascorbate had a

significant ($p \leq 0.01$) effect on cytoplasmic membrane stability (Table 2) so that application of 20 mM and 0 mM ascorbate resulted in the lowest (1.25 µs/cm) and highest (1.71 µs/cm) cell membrane leakage (Table 3). Interaction of effects of salinity and ascorbate on cytoplasmic membrane leakage was not significant (Table 2). Finally, mean comparison of the two factors in the study showed that application of 20 mM ascorbate with no salinity stress resulted in 4.97 µs/cm cytoplasmic membrane leakage (Fig. IX).

Discussion

As a result of salinity, different species of active oxygen are produced. In the mean time, the electrons released from the transportation chain of electron can react with molecular oxygen and produce active oxygen species. These species are toxic to plant cells damaging normal metabolism of lipids and proteins and causing peroxidation of membrane lipids (Smiroff, 2001). Reactive oxygen species control photosynthesis, ATP generation, lipids peroxidation, and damage DNA molecules. Stopping the growth in plants' root and shoot lengths and reducing production of plant matter are the common symptoms of oxidative stress (Ruley et al., 2004). Salinity stress leads to drought stress in plants. Therefore, the plant tightens its stomata and reduces its absorption of CO_2 and as a result photosynthesis and production of substances in the plant are reduced. Salinity stress reduces plant growth and performance. Many studies have reported a reduction in stem and root length under salinity stress conditions, e.g., in pea and broad bean (Alqurainy, 2007). Salinity reduces plant cell division and cell size. This results in the reduction of root and stems weight and leaf area.

Ascorbic acid causes an increase in plant length as it affects cellular life cycle, cell division, and length (Pignocchi and Foyer, 2003). Pastrori et al. (2003) maintain that with an increase in ascorbate, abscisic acid is reduced to control its preventive effect on cellular growth. Compounds with antioxidant features such as ascorbate (Miguel et al., 2006) and salicylic acid (Avacini et al., 2003), reduce oxidative stress damages in plants through increasing their antioxidant potentials. Antioxidant stress in cell membrane is a barrier for generation of reactive oxygen species. Ascorbate increases fresh and dry shoot weights in plants (Baghizadeh et al., 2009). It is also reported that ascorbate increases cellular division, leaf area, fresh and dry weights of plants, and antioxidant effects to compensate for the damages of free oxygen molecules produced as a result of salinity stress (Miguel, 2006).

Abiotic stresses remarkably increase secondary metabolites, extracts, and essences in the plant. Due to chemical reactions and metabolic changes in the effective compounds of the medicinal plants, lack of moisture which is a consequence of salinity stress is particularly harmful. Reduction of water in plant organs may lead to physiological disorders such as reduction of photosynthesis and secretion of hormones (Sarker et al., 2005). In case of aromatic plants, this may make some changes in the signals for properties and chemical components of the essences and extracts. (Said - Al Ahl and Abdou, 2009) argued that ascorbic acid produced in plants affects their growth and metabolism and play an important role in electron transmission system and glucose metabolism. In fact, ascorbic acid as a growth regulating agent affects biologic activities in the plant (Misra and Srivastava, 2000). Water is an environmental factor that has an important role in plant growth and their effective compounds. Drought stress in basil is reported to reduce its essence and extract content (Omidbaigi et al., 2003).

Plants are equipped with different strategies to control thermodynamic and chemical imbalance between the inside of the cells and the outside environment. These include protective measures and tolerance to avoid stress, and capacity of plant protoplasm to put up with stress. Controlling water content under salinity stress is part of these protective measures as water and salt content jointly determine imbibition pressure. It is important to note that both water and salinity contents were reported to correlate with salinity level (Karimi et al., 2005).

Under oxidative stresses, superoxide causes oxidation of cytoplasmic membrane lipids. A product of the oxidation of lipids is malondialdehyde (Borsni et al., 2010) which causes problems for electron pumps and ATP at

membrane regions as lipid peroxidation damages proteins (Sairam et al., 2008). Plants have some antioxidant enzymes and non-enzymatic antioxidant compounds to adapt to abiotic stresses and prevent oxidative damages. Non-enzymatic antioxidants include ascorbate glutathione, tocopherol, zazantyn, and antrazantyn (Wise and Naylor, 2007). The plasmalemma is both a bridge and a barrier between the cytoplasm and the outside world. It is a dynamic interface that perceives and transmits information concerning changes in the environment to the nucleus to modify gene expression. In plants, ascorbate is an essential part of this dialogue. The leaf apoplast contains millimolar amounts of ascorbate that protect the plasmalemma against oxidative damage (Horemans et al., 2000). Ascorbic acid is an abundant small molecule in plants and is a key substance in the network of antioxidants that include ascorbate, glutathione, a-tocopherol, and a series of antioxidant enzymes. Ascorbic acid has also been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion, and other developmental processes (Conklin, 2001). Ascorbate is oxidized by oxygen free radicals and dehydroascorbate is generated (Noctor and Foyer, 1998).

The results of our research showed that salt stress significantly increased cell membrane leakage and consequently, reduced RWC, morphological traits and biomass production. It seems that, ascorbate via neutralizing or scavenging ROS leads to resistance increase. According to these findings, it can be suggested that application of ascorbate can reduce the adverse effects of ROS and improve purslane resistance, especially in severe salinity. Also it was revealed that purslane is a medicinal plant with a high resistance to salinity stress and can generally be cultivated in saline soils using ascorbate foliar application. On the other hand, at 210 mM salinity level (extreme salinity), 20 mM exogenous ascorbate helped to achieve acceptable extract yield.

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