

ORIGINAL ARTICLE

The Effects of Salt Stress on Certain Physiological Parameters in Summer Savory (*Satureja hortensis* L.) Plants

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Savory plants were treated with different concentrations of NaCl. Plants were grown under controlled environment and harvested after 42 days for measurements of biochemical and physiological parameters. The essential oil of dried aerial parts of treated plants were isolated and analyzed with GC/MS. The main essential oil compounds were determined as carvacrol (55.37%) and γ -terpinene (32.92%) in control plants. In NaCl treated plants, with increasing NaCl, carvacrol content increased and γ -terpinene decreased. In all the plants treated with NaCl, growth parameters, pigments contents and photosynthetic rate were decreased, while, proline and soluble sugars contents increased.

Our results indicated that with increasing salinity, carvacrol amount increased which can be considered for medical usages.

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Abbreviations: LA – leaf area; LFM- leaf fresh matter; LWCA- leaf water content area; LDM- leaf dry matter; NAR- net assimilation rate; RDM- root dry matter; RFM- root fresh matter; RGR- relative growth rate; RLGR- relative leaf growth rate; SDM- stem dry matter; SFM- stem fresh matter; SLA- specific leaf area.

In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress (Burbott and Loomis, 1969). Secondary products of plants can be altered by environmental factors and water stress is a major factor affecting the synthesis of natural products (Solinas and Deiana, 1996). Changes in essential oils extracted from aromatic plants and their composition were observed with water stress (Sabih *et al.* 1999). Water stress resulted in significant reduction of fresh

and dry matters, nutrient content, and essential oil yield of Japanese mint plants (Mirsa and Strivastava, 2000). Fresh and dry weights of *Ocimum basilicum* L. were decreased as plant water deficit increased (Simon *et al.* 1992). The linalool and methyl chavicol contents of sweet basil, as percentage of total essential oil, increased as water stress increased (Simon *et al.* 1992). Essential oil and proline contents of sweet basil increased in response to water stress but plant growth was decreased with increasing water

stress. The essential oil yield of basil was increased by subjecting plants to water stress just before harvesting (Baeck *et al.* 2001). Essential oil, total carbohydrate, and proline contents were pronouncedly increased with increasing stress levels of *Salvia officinalis* L. (Sage) plants (Hendawy and Khalid, 2005).

Satureja hortensis is an annual, herbaceous plant belonging to the family Labiatae. It is known as summer savory, native to southern Europe and naturalized in parts of North America. The main constituents of the essential oil of *S.hortensis* are phenols, carvacrol and thymol, as well as p-cymene, β -caryophyllene, linalool and other terpenoids (Sefidkon *et al.* 2006). The essential oil and oleoresin are used in food industries. In addition, the essential oil of *S.hortensis* has been used in the perfume industries, either alone or with other essential oils (Sefidkon *et al.* 2004).

As a medicinal plants, *S.hortensis* has been traditionally used as a stimulant, stomachic, carminative, expectorant and aphrodisiac. The essential oil has demonstrated antimicrobial and antidiarrheic activities because of phenols in the oil (Sefidkon and Jamzad, 2004).

In this study, the effects of salinity on quality and quantity of essential oil and certain physiological parameters in summer savory (*Satureja hortensis* L.) plants were studied.

MATERIALS AND METHODS

Plant materials and treatments. Savory (*Satureja hortensis* L.) seeds were prepared from Agricultural Research Center, Tehran, Iran. Seeds were sterilized in 5% (W/V) sodium hypochlorite (15 min) and washed five times with sterile distilled water. Seeds germinated in pots containing seeds in a growth chamber at $24 (\pm 1) ^\circ\text{C}$ temperature and at a relative humidity of 70%. Germinated seeds were transferred to pots in growth chamber with 17 h light periods and $200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ light intensity, day/ night

temperatures of $25(\pm 1)/18(\pm 1) ^\circ\text{C}$ and irrigated with Hoagland's solution.

Thirty eight days old plants transplanted into the saline nutrient solutions containing 0, 30, 50, 70 and 100 mM sodium chloride, at pH 6.5, and the nutrient solution was renewed every week. The plants were grown under controlled environment (17 h light periods, $200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ light intensity, day/night temperatures of $25(\pm 1)/18(\pm 1) ^\circ\text{C}$) in a greenhouse. After 42 days of experimental period, for essential oil assays and each physiological analysis from each treatment, four plants were harvested.

Isolation procedure. For essential oil assays, the aerial parts of *Satureja hortensis* plants were collected at full flowering stage. They were dried by air and weighed to extract the essential oil. Dry plant material (15 g) from each replicate of all treatments was subjected to steam distillation for 3 h using a Clevenger type apparatus (Clevenger, 1928). The essential oil content was calculated as a percentage.

Gas chromatography. GC analyses were performed, using a Thermoquest Finnigan instrument equipped with a DB-1 fused silica column (60 m \times 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of $1.1 \text{ cm}^3 \text{ min}^{-1}$. The oven temperature was raised from $60 ^\circ\text{C}$ to $250 ^\circ\text{C}$ at a rate of $8 ^\circ\text{C min}^{-1}$ and held for 20 min. The injector and detector (FID) temperatures were kept at $250 ^\circ\text{C}$ and $280 ^\circ\text{C}$ respectively.

Gas chromatography- mass spectroscopy. GC/MS analysis was carried out on a Thermoquest-Finnigan trace GC/MS instrument equipped with a DB-1 fused silica column (60m \times 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from $60 ^\circ\text{C}$ to $250 ^\circ\text{C}$ at a rate of $8 ^\circ\text{C/ min}$ and held for 20 min, transfer line temperature was $250 ^\circ\text{C}$. Helium was used as the carrier gas at a flow rate of $1.1 \text{ cm}^3 \text{ min}^{-1}$ with a split ratio equal to 1/50.

The quadrupole mass spectrometer was scanned over the 35-465 a.m.u. with an ionizing voltage of 70 eV and ionization current of 150 μA .

Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds.

Growth analyses. At the end of the experiment, four plants from each treatments were harvested to determine the leaf area, leaf and stem fresh weights. Dry weights of leaves, stems and roots were determined by oven drying the tissues for 48 h at 65°C. RGR, NAR, RLGR, SLA and LWCA were carried out using the equations (Watson, 1952; Evans and Hughes, 1962).

Pigments assasy. 150 mg of fresh leaf tissue of each treatments was extracted in 15 cm³ 80% acetone and absorbance of extracts was recorded at 470, 646.8 and 663.2 nm (Lichthenthaler, 1987).

Gax exchanges assays. Photosynthetic rate, respiration rate and CO₂ compensation concentration were determined in intact plants, employing an Infrared Gas (CO₂) Analyzer (IRGA) (Khavari-Nejad, 1980; 1986).

Proline assays. 500 mg fresh leaves of mature plants were powdered in liquid nitrogen. 10 cm³ of 3% sulfosalicylic acid was added to each sample and centrifuged at 1300 rpm for 10 min. 2 cm³ of the supernatants from each sample, 2 cm³ of acidified ninhydrin solution and 2 cm³ of 100% acetic acid were mixed and boiled for one hour. To stop further reaction, samples were immediately transferred into ice-water and left to cool down for at least 20 minutes. To each sample, 4 cm³ of toluene was added at room temperature, mixed well, and the absorbance at 520 nm was measured (Bates *et al.* 1973).

Soluble sugars assays. 500 mg of dried leaves of each treatment was extracted with 80% ethanol and after using Ba(OH)₂ and ZnSO₄ for removing pigments from extracts and adding %5 phenol and sulfuric acid, absorbance of extracts was recorded at 540 nm (Hellubust and Craigie, 1978).

Statistical analysis. The research was conducted using completely randomized design with four replications. The significance of salinity treatments was analyzed by analysis of variance (AVOVA) using SAS software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Decreasing effects of NaCl on growth have been reported in different plants (Cordovilla *et al.* 1996; Dua, 1992; Singleton and Bohlool, 1984; Zahran and Sprent, 1986). The results showed that the salinity levels of various solutions had significant effects on the growth parameters examined (Table 1). In treated plants with NaCl, the highest fresh and dry weights were observed in 30 and 50 mM NaCl. Increasing the salinity level of solution reduced the fresh and dry weights.

RGR, NAR and RLGR decreased under the various salt stress levels. Differences among salt stress treatments were significant. LWCA was significantly enhanced in 30, 50 and 70 mM NaCl. As described earlier (Tester and Davenport, 2003), an ability to grow in saline conditions has been attributed to an ability to close stomata. In fact both glycophytes and halophytes tend to show reduced stomatal conductance in higher NaCl concentrations (Ball, 1988; Robinson *et al.* 1997; James *et al.* 2002).

With decreasing growth, a reduction in chlorophylls contents and consequently, a reduction in photosynthetic rate observed (Tables 4 and 5) (Abdul- Hamid *et al.*,1990; Castonguay and Markhart,1991; Nunez-Barrious, 1992; Viera *et al.* 1991). Amongst damages caused by salinity stress in plants, the reduction of photosynthetic processes is one of the most important (Delfine *et al.* 1999). Our results indicated that, in 70 and 100 mM NaCl, both photosynthetic and respiration rates were significantly decreased and CO₂ compensation

Table 1 Effects of NaCl on growth parameters, means(\pm SE) of four replicates, numbers followed by the same are not significantly different ($P>0.05$).

Growth parameters (Mean final values)	NaCl(mM)				
	0	30	50	70	100
LFM(g)	1.2128 \pm 0.0656 a	0.7780 \pm 0.0921 b	0.7446 \pm 0.0905 bc	0.5627 \pm 0.101 c	0.1927 \pm 0.0178 d
SFM(g)	1.6807 \pm 0.234 a	0.7726 \pm 0.1445 b	0.6927 \pm 0.1291 b	0.3992 \pm 0.0783 bc	0.2617 \pm 0.0434 c
RFM(g)	1.5901 \pm 0.186 a	1.0328 \pm 0.1428 b	0.8002 \pm 0.1907 b	0.7312 \pm 0.1899 b	0.2127 \pm 0.055 c
LDM(g)	0.3151 \pm 0.204 a	0.0728 \pm 0.006 a	0.0652 \pm 0.008 a	0.0650 \pm 0.008 a	0.0622 \pm 0.005 a
SDM(g)	0.2010 \pm 0.0247 a	0.1145 \pm 0.0101 b	0.0777 \pm 0.0133 bc	0.0497 \pm 0.0035 cd	0.0326 \pm 0.0015 d
RDM(g)	0.1108 \pm 0.0152 a	0.0624 \pm 0.0104 b	0.0579 \pm 0.0092 b	0.06022 \pm 0.0105 b	0.01686 \pm 0.0025 c
LA(cm ²)	49.800 \pm 4.700 a	32.849 \pm 1.563 ab	26.699 \pm 2.538 bc	16.720 \pm 0.578 bc	9.830 \pm 0.181 c
NAR(g m ⁻² day ⁻¹)	3.294 \pm 0.244 a	2.941 \pm 0.255 ab	2.610 \pm 0.270 ab	2.876 \pm 0.202 ab	2.354 \pm 0.166 b
RGR(g kg ⁻¹ day ⁻¹)	76.45 \pm 2.34 a	63.57 \pm 1.95 b	58.14 \pm 3.33 bc	54.76 \pm 1.68 c	44.89 \pm 1.51 d
RLGR(cm ² m ⁻² day ⁻¹)	468.63 \pm 16.57 a	307.1 \pm 12.29 b	246.4 \pm 21.36 c	136.3 \pm 9.38 d	12.35 \pm 4.42 e
SLA(m ² kg ⁻¹)	59.2 \pm 5.47 a	47.32 \pm 2.04 b	41.66 \pm 2.05 b	27.11 \pm 3.37 c	16.10 \pm 0.99 d
LWCA(g(H ₂ O) m ⁻²)	165.65 \pm 12.88 cd	205.51 \pm 19.36 bc	253.6 \pm 16.92 ab	293.9 \pm 48.74 a	131.7 \pm 11.73 d

Table 2 Effect of salinity on the essential oil content extracted from summer savory (*Satureja hortensis* L.) plants. Means(\pm SE) of four replicates, numbers followed by the same are not significantly different ($P>0.05$).

Treatment (NaCl mM)	Essential Oil (W %)
control	1.4395 \pm 0.0404 a
30	1.3174 \pm 0.0537 a
50	1.4165 \pm 0.1552 a
70	1.2186 \pm 0.1093 a
100	1.0873 \pm 0.1122 a

Table 3 Effects of salt stress treatments on the chemical constituents of essential oil extracted from *Satureja hortensis* L. Plants.

Compounds	NaCl (mM)				
	0	30	50	70	100
α -Thujene	1.05	1.33	0.84	0.19	0.34
α - Pinene	0.62	0.85	0.6	0.13	0.25
β -Pinene	0.27	0.43	0.34	0.1	0.18
Myrcene	1.26	1.79	1.32	0.53	0.76
α -Phellandrene	0.23	0.37	0.25	0.08	0.12
α -Terpinene	5.68	7.21	5.78	-	-
Limonene	0.36	0.58	0.42	0.17	0.25
γ -Terpinene	32.32	26.87	28.81	16.71	18.18
Carvacrol methyl ether	0.11	0.25	0.28	0.84	0.8
Carvacrol	56.32	57.45	58.54	71.86	69.04
α -Farnesene	0.48	0.50	0.84	1.35	1.27
β -Caryophyllene	0.03	.08	0.11	0.54	0.4
Bicyclogermacrene	0.4	0.56	0.42	1.1	0.81
β -Bisabolene	0.7	0.86	1.07	2.41	2.16
Trans- α -Bisabolene	0.05	0.06	0.08	0.2	0.19

Table 4 Effects of NaCl on chlorophylls, chl.a/chl.b ratio and carotenoids contents, means(\pm SE) of four replicates, numbers followed by the same are not significantly different ($P>0.05$).

Pigment contents (mg g ⁻¹ fw)	Chl.a	Chl.b	Chl.a+ Chl.b	Chl.a/Chl.b ratio	Carotenoids
Treatment (NaCl mM)					
Control	1.2220 \pm 0.201 a	0.4259 \pm 0.023 a	1.3972 \pm 0.070 a	2.2863 \pm 0.065 b	0.2234 \pm 0.011 a
30	0.8088 \pm 0.056 b	0.3181 \pm 0.020 b	1.1268 \pm 0.076 b	2.5404 \pm 0.020 a	0.20182 \pm 0.008 ab
50	0.6599 \pm 0.018 b	0.2563 \pm 0.008 c	0.9163 \pm 0.026 cd	2.5759 \pm 0.022 a	0.1596 \pm 0.003 cd
70	0.5544 \pm 0.059 b	0.2232 \pm 0.022 c	0.7777 \pm 0.081 d	2.4789 \pm 0.022 a	0.1443 \pm 0.018 d
100	0.7210 \pm 0.023 b	0.2778 \pm 0.006 bc	0.9988 \pm 0.026 bc	2.5974 \pm 0.084 a	0.1837 \pm 0.011 bc

Table 5 Effects of NaCl on photosynthetic rate, respiration rate, CO₂ compensation concentration, means(\pm SE) of four replicates, numbers followed by the same are not significantly different ($P>0.05$).

Treatment (NaCl mM)	Photosynthetic rate(μ molCO ₂ dm ⁻² S ⁻¹)	Respiration rate (μ molCO ₂ dm ⁻² S ⁻¹)	CO ₂ compensation concentration (μ l l ⁻¹)
control	12.1693 \pm 0.4209 bc	21.697 \pm 1.0716 a	111.90 \pm 9.266 d
30	17.1366 \pm 1.3260 a	16.346 \pm 0.8938 b	178.82 \pm 3.344 c
50	14.5230 \pm 2.2280 ab	14.530 \pm 0.6274 b	202.82 \pm 2.822 b
70	9.68000 \pm 0.3400 c	17.190 \pm 0.6084 b	195.13 \pm 2.362 bc
100	8.14230 \pm 0.3218 c	6.7416 \pm 0.5124 c	239.76 \pm 7.884 a

Table 6 Effects of NaCl on proline content and soluble sugars. Means(\pm SE) of four replicates, numbers followed by the same are not significantly different ($P>0.05$).

Treatment (NaCl mM)	Proline ($\mu\text{g g}^{-1}\text{fw}$)	Soluble sugars (mg g^{-1})
Control	11.022 \pm 0.4861 c	8.879 \pm 0.8010 c
30	12.197 \pm 1.1278 c	10.864 \pm 1.4319 c
50	29.090 \pm 1.4559 c	22.135 \pm 0.8010 b
70	516.60 \pm 31.660 b	33.091 \pm 3.0307 a
100	912.89 \pm 42.566 a	11.945 \pm 0.9638 c

concentration increased as compared to that of control. However, 30 and 50 mM NaCl had a low effects on gas exchanges (Table 5).

As shown in Table 4, decreasing in essential oil percentage in plants grown in different levels of NaCl were not significant. Table 3 shows the effects of salt levels on the chemical composition of essential oil extracted from *Satureja hortensis*.

The main components were found to be γ -terpinene (32.32%) and carvacrol (56.32%).

The lowest components were α -thujene, α -pinene, β -pinene, myrcene, phellandrene, α -terpinene, limonene, carvacrol methyl ether, α -farnesene, β -caryophyllene, bicyclogerma, bisabolene, trans α -bisabolene.

The results showed that by increasing the salinity level, a higher amount of carvacrol (69.04%) and the lowest amount of γ -terpinene (18.18%) were obtained. Also, with increasing the NaCl level in the culture solution, higher amount of carvacrol methyl ether (0.8%), α -farnesene (1.27%), β -caryophyllene (0.4%), bicyclogerma (0.81%), bisabolene (2.16%) and trans α -bisabolene (0.19 %) were obtained, while the constituents of α -thujene, α -pinene, β -pinene, myrcene, α -phellandrene, limonene in 50, 70 and 100 mM NaCl were decreased.

In fact, the effect of salinity on essential oil and its constituents may be due to its effects of enzyme activity and metabolism (Burbott and Loomis, 1969).

Table 6, shows that proline and soluble sugars contents are higher in plants treated with NaCl. Accordingly, it may be concluded that high soluble sugars play an important role in turgor maintenance and proline is regarded as a source of energy, carbon and nitrogen for recovering tissues, so it increased under water stress levels (Blum and Ebercon, 1976). As a result of water stress and salinity, proline accumulation was observed in many organisms. Including bacteria, fungi, algae and plants (Csonka and Hanson, 1991; Delauney and Verma, 1993; Hanson and Hitz, 1982; Yoshiba *et al.* 1995).

These results indicated that summer savory plants are resistant to 30 mM NaCl and in higher concentrations of NaCl, growth parameters reduced, while, levels some of the chemical composition of essential oil for example carvacrol increased.

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