KSÜ Tarım ve Doğa Derg 24 (3): 505-514, 2021
KSU J. Agric Nat 24 (3): 505-514, 2021
https://doi.org/10.18016/ksutarimdoga.vi.730477

Plant Growth, Ion Accumulation and Essential Oil Content of *Salvia officinalis* Mill. and *S. tomentosa* L. Grown under Different Salt Stress

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ABSTRACT
This study was conducted to determine the response of *Salvia tomentosa* Mill. and *Salvia officinalis* L. to different salinity levels. The salvia plants were grown in 8 L pots filled with the continuously aerated nutrient solution under different salt levels 1 (control), 2, 3, 4, 5 and 6 dS m^{-1} in fully automated climate chambers. The fresh and dry weight of shoot and root, stem length, leaf area, SPAD, CO_2 fixation, root length, root diameter, root volume, leaf Na^+, K^+, Ca^{++} Cl^- content, and amount of essential oil were determined. The increasing salt level decreased significantly plant growth of both *Salvia* species. However, highly significant differences were found between two *Salvia* species in terms of shoot and root biomass. Generally, *S. tomentosa* showed better plant growth performance in plant growth compared to *S. officinalis.* The Na^+ and Cl^- content of the leaves significantly increased with increasing salt concentration and the increase was higher in *S. tomentosa*. The amount of K^+ in the leaves decreased due to the increasing salt concentration, while the amount of Ca^{++} varied depending on the dose. The study showed that the essential oil contents of the sage leaves could be increased with the moderate salt application. The increase in essential oil due to salt stress was higher in *S. officinalis*. The results showed that *Salvia* species can be cultivated in low and medium saline soils, second class waters can be used for irrigation of sages and essential oil yield of sages can be increased by using salt stress.

Research Article

Article History
Received : 01.05.2020
Accepted : 21.10.2020

Keywords
Salvia species
Salt tolerance
Hydroponic culture
Root morphology
Essential oil

Farklı Tuz Stresleri Altında Yetiştirilen *Salvia officinalis* Mill. ve S. *tomentosa* L. Türlerinde Bitki Büyümesi, İyon Birikimi ve Esansiyel Yağ Miktarı

ÖZET
Bu çalışmada, *Salvia officinalis* L. ve *S. tomentosa* Mill. türlerinin farklı tuz seviyelerine tepkisi belirlenmiştir. Ada çayı bitkileri iklim odasında, su kültüründe altı farklı tuz stresinde [1 (kontrol), 2, 3, 4, 5 ve 6 dS m^{-1}] yetiştirilmişlerdir. Bitki organları taze ve kuru ağırlıkları, gövde uzunluğu, yaprak alanı, SPAD, CO_2 asimilasyonu, kök uzunluğu, kök hacmi, kök kalınlığı, yaprak Na^+, K^+, Ca^{++} Cl^- içerikleri ve esansiyel yağ asidi miktarı belirlenmiştir. Aratan tuz seviyesi iki adaçayı türünü de bitki gelişimi açısından önemli derecede etkilemiştir. Ancak, türler arasında biyomas gelişimi açısından önemli farklılıklar olmuştur. Genellikle, *S. tomentosa* türü *S. officinalis*'e göre daha iyi performans göstermiştir. Artan tuz stresi ile birlikte yaprakların Na^+ ve Cl^- içeriği artmış ve artış *S. tometosa*'da daha yüksek olmuştur. Yaprakların K^+ içeriği tuz stresi ile birlikte sürekli azalırken, Ca^+ içeriği tuz dozlarına karşı farklı tepkiler vermiştir. Çalışma, tuz stresi ile esansiyel yağ asitlerinin miktarının arttırılabileceğini göstermiştir. Yağ miktarındaki oransal artış *S. officinalis*'te daha yüksek olmuştur. Sonuçlar, *Salvia* türlerinin düşük

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 01.05.2020
Kabul Tarihi : 21.10.2020

Anahtar Kelimeler
Ada çayı türleri
Tuz stresi
Su kültürü
Kök morfoloji
Esansiyel yağ

ve orta tuzlu topraklarda yetiştirilebileceğini, adaçayını sulamak için ikinci sınıf suların kullanılabileceğini ve adaçayı esansiyel yağ veriminin tuz stresi kullanılarak arttırılabileceğini göstermiştir.

To Cite : Göçer H, Yetişir H, Ulaş A, Arslan M, Aydın A 2021. Plant Growth, Ion Accumulation and Essential Oil Content of *Salvia officinalis* Mill. and *S. tomentosa* L. Grown under Different Salt Stress. KSU J. Agric Nat 24 (3): 505-514. https://doi.org/10.18016/ksutarimdoga.vi.730477

INTRODUCTION

The members of the *Lamiaceae* family, including the genus *Salvia*, represented by 45 genera and fragrant plants, are generally important in the pharmacology and perfumery industry because they contain volatile and aromatic oils (Mamadalieva et al., 2017). This common use of *Salvia* species arises from monoterpenes and their oxygen derivatives, most of which are present in the essential oil and have a strong antiseptic effect (Tanker et al., 1976). Sage oil is applied in the treatment of various important diseases and has been shown to have antimicrobial, viricidal, cytotoxic, antifungal, and antioxidative activities. In addition to flavoring foods, while the sage essential oils were used as an antioxidant and protective agent against food spoilage, it has found a wide range of applications in aromatherapy and the field of health (Hay et al., 1993).

Plants can encounter many biotic and abiotic stress factors simultaneously or at different times during their lifetime. Stress in crop production can be defined as one or combined biotic or abiotic factors affecting the plant environment, slowing down growth and causing low yield (Mahjan and Tuteja, 2005).

Salinity in soil or water is one of the major abiotic stress factors that adversely affect plant growth (Arzani, 2008). Salinity is a growing problem, especially in arid and semi-arid regions around the world, and according to FAO (2009), the area affected by salt stress has exceeded 800 million hectares. The plant uptake plant nutrients from the dissolved substances in the soil. If these dissolved substances contain salts above a critical threshold for each species, they cause soil salinity and limit plant growth. Salinization in soil occurs as a result of dissolved salts from base rock, salts coming from irrigation water, salts from the groundwater, and incorrect fertilization. Inadequate rainfall, incomplete drainage, and excessive evaporation increase salinity in the soil (Mahjan and Tuteja, 2005).

Adverse effects of salinity on plant growth; (i) inability to absorb water by the roots due to the low water potential in the root zone, (ii) toxic effects of ions such as Na^{+}, Cl^{-} and SO_4^{++}; and (iii) disruption of uptake and transport of plant nutrients (Flowers, 2004). Since it is time-consuming and costly to improve soils affected or likely to be affected by salinity problems, it is of great importance to identify and use species and varieties that are highly tolerant to salt for efficient and sustainable production in such areas (Flowers, 2004; Yetisir and Uygur, 2010). Selecting salt-resistant genotypes from genetic sources, developing them as varieties or using them as parents for new varieties is the most permanent way to overcome salt stress.

Phytochemical contents of medicinal plants are affected by cultural applications such as fertilization and irrigation (Perry et al., 1999), climatic conditions such as lighting and temperature (Máthé et al., 1992), and biotic and abiotic stress conditions (Ben Taarit et al., 2009; Ben Taarit et al., 2010). Annual and perennial sage species used as medicinal purpose or landscaping, are negatively affected by salt stress (Tounekti et al., 2011). Plant growth (61%) and fatty acid content (32%) were decreased in *S. officinalis* grown under salt stress (100 mM). It has been observed that salinity decreased the ratio of unsaturated fatty acids but increased the ratio of saturated fatty acids. Low salt stress (25 mM NaCl) increased the fatty acid amount, while high salinity (50> mM NaCl) decreased fatty acid amount (Ben Taarit et al., 2010; Ben Taarit et al., 2011).

In salt-stressed *S. officinalis*, leaf water content, photosynthetic activity, pigment content, Ca^{++} and K^{+} concentration decreased, however, Na^{+} content increased (Ben Taarit et al., 2011; Tounekti et al., 2012). Escalona et al. (2014) reported that the shoot of sage plants grown under saline conditions accumulated Ca^{++}, Mg^{++}, Cl^{-} and NO_3^{-} ions, while the roots accumulated Na^{+} and K^{+} ions. Phenolics and tocopherols increased with a salt application (Tounekti et al., 2012). While 100 mM salt stress caused increases in 1.8-cineole, camphor, and beta-thujone concentrations, low salt stresses had no significant effect (Tounekti and Khemira, 2015). Kulak (2011) reported that sage productivity was affected differently from different salt applications and the best dry herb weight was obtained with $MgCl_2$ application, and the lowest dry weight was harvested from $CaCl_2$ application. It was suggested that the studies to determine sage species and varieties that can be capable of growing in saline conditions should be carried out. Therefore, this study aimed to determine the response of *S.officinalis* and *S. tomentosa* to salinity stress and determine the change in plant growth alterations, ion concentration and essential oil in two *Salvia* species.

MATERIAL and METHODS

The experimental site and material

This study was carried out in the research area and laboratory of the Department of Horticulture and Soil Science, Agricultural Faculty, Erciyes University. Two *Salvia* species, *Salvia officinalis* and *S. tomentosa,* were tested to determine the effect of salinity on plant growth, ion accumulation, and essential oil content. The cuttings were taken from *Salvia* production plots in Agricultural Research and Training Center of Erciyes University. The cuttings were planted in a mixture of peat and perlite (1:2 v/v) in rooting facilities with high air humidity and shading for 15 days.

Methods

Hydroponic system and salt testing

A continuously aerated hydroponic system was used as plant growth media. The modified Hoagland solution was the basic nutrient solution in the experiment. All chemicals used were of analytical grade, and composition of the nutrient solution was : [$3x10^{-3}$ M $Ca(N0_3)_2$, $1x10^{-3}$ M K_2SO_4, $1x10^{-3}$ M $MgSO_4$, $0.2x10^{-3}$ M KH_2PO_4, $1x10^{-5}$ M H_3BO_3, $1x10^{-6}$ M $MnSO_4$, $1x10^{-7}$ M $CuSO_4$, $1x10^{-8}$ M $(NH_4)_6Mo_7O_{24}$, $1x10^{-6}$M $ZnSO_4$ve $1x10^{-4}$ M Fe EDTA] (Electrical conductivity and pH of the solution were 1.30 dS m^{-1}, 6,5-7, respectively). The NaCl doses were control (1), 2, 3, 4, 5 and 6 dS m^{-1}. The salt application was initiated two days after transplanting, and the full dose was achieved six days after transplanting. The growth solution was changed once a week. The average day/night temperatures were 25/20 °C relative humidity was 65-70% and about 350 µmol m^{-2} s^{-1} photon flux was supplied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber. The experiment was continued for 30 days after salt application.

Measured parameters

The effect of salt on the plant was visually evaluated for 0-5 scale [0: Plants not injured by salt stress (control plants), 1: Local chlorosis and curling of leaves; 2: Chlorosis of leaves and 25% necrosis; 3: 25-50% of leaves show necrosis and start to fall; 4: 50-75% necrosis of leaves and plant deaths; 5: 75-100% necrosis of leaves and/or complete plant death (Figure 1).

The values of chlorophyll were recorded in a mature leaf one day before the termination of the experiment through SPAD (SPAD-502, Minolta Corporation, Ltd., Osaka, Japan). CO_2 fixation was measured on two fully developed leaves every seven days after salt application and the mean values were presented (µmol m^{-2} s^{-1}) (LI-6400XTP Model). At the end of the experiment, the total leaf area of each genotype was determined by LI 3100 C Model Leaf Area meter (cm^2 $plant^{-1}$). Plant heights (cm), plant organs fresh and dry weight (g $plant^{-1}$) were determined. At the end of the experiment, harvested plants were separated into organs and fresh weight was recorded (g $plant^{-1}$). The plant organs were dried at 65 °C for 48 h to determine the dry weight (g $plant^{-1}$). The root length (m $plant^{-1}$) and volume (cm^3 $plant^{-1}$) were determined by using the WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada) after recording the root fresh weight. Sodium, K^+, and Ca^{++} concentration (%) of the leaves were determined by Flame Photometer (Kacar ve Katkat, 2010). Chloride concentration (mg g DW^{-1}) of plant tissue was analyzed by Mohr technique (Johnson and Ulrich, 1959). After harvest, the leaf material of sage species was dried under laboratory conditions, the dried material was weighed and prepared for analysis and the essential oil was extracted by Clavenger device by water distillation method (Kılıç, 2008).

Statistical analysis

The experiment was established according to a completely randomized block design with two factors (salt level and species) and three replications with five plants. The data were subjected to variance analysis in SPSS statistical program. The means were compared by LSD test at 0.05 and 0.01 significance level.

RESULTS

Visual evaluation (0-5), plant height and leaf area (cm^2 $plant^{-1}$)

Visual deterioration was increased due to the increase in salt concentration in both sage species and there were significant reductions in plant growth. At 6 dS m^{-1} salt dose, the plants were extremely damaged or died completely (Figure 1), therefore, no other measurements were done in these plants except for the visual evaluation.

Considerable damage was detected at salt doses of 3, 4 and 5 dS m^{-1}, while no remarkable damage at 1 and 2 dS m^{-1} salt concentrations. Visual damage increased with an increasing salt concentration in both species. Plant height and leaf area per plant were significantly affected by, salt, genotype and salt*genotype interaction (Table 1). Plant height decreased with an increasing salt concentration in both sage species and the longest plants were recorded in control treatments, while the shortest plants were measured in 5dS m^{-1} salt application. As in plant height, salt application caused a gradual reduction in the leaf area. *S. tomentosa* had a larger leaf area than *S. officinalis* under all saline conditions.

CO_2 fixation (µmol m^{-2} s^{-1}) and SPAD value

The results of CO_2 fixation and SPAD values are given in Table 2. CO_2 fixation was significantly influenced by genotype and genotype salt interaction. *S. tomentosa*

assimilated more CO_2 ($\bar{x}$=14 µmol m^{-2} s^{-1}) than *S. officinalis* ($\bar{x}$=12.9 µmol m^{-2} s^{-1}). Both species and salt treatments were significantly effective on leaf chlorophyll content (SPAD). When the species were compared, *S. officinalis* was found to have higher SPAD value in both control plants and salt applications. Decreases in SPAD were observed in both species due to the increase in salt concentration. This decrease was ranged from 2% to 12% in *S. officinalis* and from 3% to 17% in *S. tomentosa* (Table 2).

Figure 1. *Salvia officinalis* and *S. tomentosa* plants grown at different salt doses for 30 days.
Şekil 1. *Farklı tuz streslerinde 30 gün yetiştirilen Salvia officinalis* ve *S. tomentosa bitkileri.*

Table 1. Visual evaluation, plant height and leaf area of *S. officinalis* and *S. tomentosa* 30 days after salt treatment
Çizelge 1. 30 gün tuz stresinden sonra S. officinalis ve S. tomentosa'da görsel değerlendirme, bitki yüksekliği ve yaprak alanı

Salt levels	Visual damage (0-5 scale) *Görsel zarar (0-5 skalası)*		Plant height (cm) *Bitki yüksekliği (cm)*		Leaf area (cm^2 $plant^{-1}$) *Yaprak alanı (cm^2 $bitki^{-1}$)*	
Tuz seviyesi	*So*	*St*	*So*	*St*	*So*	*St*
1dS m^{-1}	0	0	36.78±4.9	37.00±2.2	1105.3±47.9	2889.1±90.4
2dS m^{-1}	1	1	38.56±1.9	26.78±2.9	1193.2±9.3	2070.7±38.5
3dS m^{-1}	2	2	34.21±1.2	20.33±1.2	1091.5±5.3	1239.1±57.7
4dS m^{-1}	3	3	25.33±1.8	13.11±1.3	726.7±0.5	814.7±28.5
5dS m^{-1}	4	4	8.43±0.5	18.78±1.3	475.1±54.8	808.8±36.6
6dS m^{-1}	5	5				
Salt			***		***	
Species			***		***	
Salinity*Species			***		***	

So: *Salvia officinalis*, St: *Salvia tomentosa**: 0.05, **:0.01, ***: 0,001 significance level.

Table 2. CO_2 fixation and SPAD values of *Salvia officinalis* and *Salvia tomentosa* plants grown at different salinity levels

Çizelge 2. Farklı tuz streslerinde yetiştirilen Salvia officinalis ve Salvia tomentosa bitkilerinde CO_2 asimilasyonu ve SPAD değerleri

Salinity levels *Tuz seviyesi*	CO_2 fixation (µmol $m^{-2}s^{-1}$) *CO_2 asimilasyonu (*µmol $m^{-2}s^{-1}$)		SPAD *SPAD*	
	S. officinalis	*S. tomentosa*	*S. officinalis*	*S. tomentosa*
1dS m^{-1}	13.89±0.3	15.13±0.5	43.81±1.5	37.61±1.4
2dS m^{-1}	13.42±1.0	12.04±0.2	43.12±1.2	36.39±0.5
3dS m^{-1}	12.12±0.7	16.11±0.9	41.83±1.0	36.92±1.5
4dS m^{-1}	12.54±0.8	14.26±0.3	40.28±0.7	34.29±0.6
5dS m^{-1}	12.53±0.7	12.60±1.2	38.68±2.9	31.37±0.9
Salinity	ns		**	
Species	*		**	
Salinity*Species	*		ns	

*: 0.05, **:0.01 significance level, ns: non significant.

Plant fresh and dry weight (g $plant^{-1}$)

The fresh and dry weight of plant organs was significantly influenced by genotype, salt treatment and genotype salt interactions (Table 3). While a gradual reduction was observed in shoot fresh and dry weight in both species, root fresh and dry weight of *S. officinalis* increased at low and medium salt stress, then they decreased in high salt stress. *S. tomentosa* produced higher biomass than *S. officinalis* under all conditions. Herbal fresh and dry weight, an economically important part of sage, did not decrease in 2 dS m^{-1} salt stress but started to decrease significantly with 3dS m^{-1} salt stress. A negative correlation was found between plant biomass and Na^+ (*S. officinalis r*= -0.58 and-94) and Cl^- (*S. tomentosa r*= -58 and -83) contents of leaves.

Table 3. Fresh and dry weights of plant organs of *Salvia officinalis* and *Salvia tomentosa* plants grown at different salt concentrations

Çizelge 3. *Farklı tuz streslerinde yetiştirilen Salvia officinalis ve Salvia tomentosa bitkilerinde bitki organlarının taze ve kuru ağırlıkları*

Salinity levels *Tuz seviyesi*	Shoot fresh weight (g $plant^{-1}$) *Gövde taze ağırlığı (g $plant^{-1}$)*		Root fresh weight (g $plant^{-1}$) *Kök taze ağırlığı (g $plant^{-1}$)*		Shoot dry weight (g $plant^{-1}$) *Gövde kuru ağırlığı (g $plant^{-1}$)*		Root dry weight (g $plant^{-1}$) *Kök kuru ağırlığı (g $plant^{-1}$)*	
	So	*St*	*So*	*St*	*So*	*St*	*So*	*St*
1dS m^{-1}	102.33±3.3	195.75±1.0	53.67±0.9	93.50±1.7	63.5±2.3	111.8±9.5	3.73±0.1	8.05±0.2
2dS m^{-1}	102.25±1.6	174.83±5.1	62.75±3.0	84.25±5.3	63.9±2.6	110.3±1.4	4.37±0.4	5.85±0.5
3dS m^{-1}	75.00±6.6	107.00±0.6	88.33±4.2	73.75±0.7	50.0±4.8	76.1±2.5	5.93±0.4	4.24±0.2
4dS m^{-1}	75.17±4.7	80.33±4.5	44.25±3.9	48.75±1.3	46.2±4.1	56.4±3.8	4.58±0.6	3.07±0.1
5dS m^{-1}	33.00±1.2	60.75±3.9	19.67±1.2	44.75±3.3	22.9±0.4	45.0±2.0	1.81±0.1	2.22±0.1
Salinity	***		***		***		***	
Species	***		***		***		**	
Salinity*Species	***		***		***		***	

So: *Salvia officinalis* , *St: Salvia tomentosa* *: 0.05, **:0.01; ***: 0,001 significance level.

Root length (m $plant^{-1}$), volume (cm^3 $plant^{-1}$) and diameter (mm)

Root length was significantly affected by species. *S. tomentosa* produced longer roots at all salt concentrations than *S. officinalis*. As in *S.officinalis* root biomass, the root length increased up to 3 dS m^{-1} salt stress, but 4 and 5 dS m^{-1} salt concentrations decreased root length significantly. In *S. tomentosa*, root length decreased gradually due to increasing salt concentrations. The longest root was measured in the control plant of *S. tomentosa* with 67 m $plant^{-1}$, while the shortest root length was recorded as 12.8 and 27.3 m in *S. officinalis and S. tomentosa,* which were grown under 5 dS m^{-1} salt stress, respectively (Table 4). Salt application and salt genotype interaction significantly affected root volume, however, the genotypic effect was not significant. Similar to root length and root biomass, *S. officinalis* increased root volume in low and moderate salt stress, whereas the root volume of *S. tomentosa* decreased with increasing salt stress. Root diameter differed between species, but salt application

and interaction did not have a significant effect on root diameter (Table 4).

Table 4. Root length, root volume and root diameter results of *S. officinalis* and *S. tomentosa* species grown at different salinity levels

Çizelge 4. Farklı tuz streslerinde yetiştirilen Salvia officinalis ve Salvia tomentosa bitkilerinde kök uzunluğu, kök hacmi ve kök çapı

Salinity levels	Root length (m plant^{-1}) *Kök uzunluğu (m bitki^{-1})*		Root volume (cm^3 plant^{-1}) *Kök hacmi (cm^3 bitki^{-1})*		Root diameter (mm) *Kök çapı (mm)*	
Tuz seviyesi	*S. officinalis*	*S. tomentosa*	*S. officinalis*	*S. tomentosa*	*S. officinalis*	*S. tomentosa*
1dS m^{-1}	35.5±2.1	67.0±1.4	35.05±0.9	58.39±6.8	0.36±0.009	0.33±0.012
2dS m^{-1}	41.6±2.9	62.5±1.0	44.43±1.9	57.08±3.2	0.37±0.008	0.34±0.014
3dS m^{-1}	52.1±2.4	46.4±5.2	65.46±3.9	40.23±8.0	0.40±0.013	0.33±0.017
4dS m^{-1}	31.9±1.7	36.6±5.5	31.09±2.5	30.44±7.8	0.35±0.010	0.33±0.022
5dS m^{-1}	12.7±1.4	27.3±1.2	13.64±6.7	24.71±7.4	0.37±0.003	0.34±0.016
Salinity	***		***		ns	
Genotype	***		ns		**	
Salinity*genotype	***		***		ns	

*: 0.05, **:0.01, ***: 0,001 significance level, ns: non-significant.

Na$^+$, Cl$^-$, K$^+$ and Ca^{++} content of leaves

Sodium, Cl$^-$, K$^+$ and Ca^{++} contents of the leaves of *S. officinalis* and *S. tomentosa* grown under different salt concentrations are given in Table 5. Both sage species showed an increase in Na$^+$ content with the salt application. The increase in Na$^+$ content of *S. tomentosa* was twice that of *S. officinalis* from 2 dS m^{-1} to 4 dS m^{-1}. In 5 dS m^{-1} salt application, Na$^+$ content reached the highest level in both species and the Na$^+$ content of the species was close to each other. While *S. officinalis* tried to exclude Na$^+$ at lower level of salt, *S. tomentosa* has uptaken Na$^+$ and tried to reduce the damage of Na$^+$ in different ways. However, in 5 dS m^{-1} salt stress, Na$^+$ intake increased to the same level in both species.

The chloride content of the leaves of sage grown under salt stress increased with increasing salt stress. A similar trend was observed in both species. In the control, the concentration of Cl$^-$ detected in the range of 16-18 mg g DW^{-1} and increased by approximately 6-fold in *S. officinalis* (85 mg g DW^{-1}) and *S. tomentosa* (88 mg g DW^{-1}) at a dose of 5 dS m^{-1} which was the highest salt application (Table 5).

Table 5. Leaf Na$^+$, Cl$^-$, K$^+$ and Ca^{++} contents of *S. officinalis* and *S. tomentosa* grown at different salinity levels.

Çizelge 5. Farklı tuz streslerinde yetiştirilen Salvia officinalis ve Salvia tomentosa yapraklarının Na$^+$, Cl$^-$, K$^+$ and Ca^{++} içerikleri

Salinity levels	Na$^+$ (%) *Na$^+$ (%)*		Cl$^-$ (mg g DW^{-1}) *Cl$^-$ (mg g KA^{-1})*		K$^+$ (%) *K$^+$ (%)*		Ca^{++} (%) *Ca^{++} (%)*	
Tuz seviyesi	*So*	*St*	*So*	*St*	*So*	*St*	*So*	*St*
1dS m^{-1}	0.25±0.03	0.20±0.03	18.06±0.6	16.58±0.6	3.45±0.3	3.95±0.02	2.24±0.03	1.26±0.04
2dS m^{-1}	0.32±0.04	0.83±0.02	33.45±1.8	43.51±1.7	3.17±0.1	3.80±0.17	1.72±0.03	1.76±0.04
3dS m^{-1}	0.65±0.03	1.31±0.01	43.81±1.7	45.59±1.7	3.04±0.1	3.63±0.03	2.50±0.30	1.28±0.05
4dS m^{-1}	0.89±0.05	1.61±0.02	54.17±2.3	55.35±2.9	2.80±0.2	3.76±0.04	2.25±0.14	1.60±0.11
5dS m^{-1}	1.54±0.02	1.71±0.04	85.25±2,9	88.51±1.7	2.84±0.1	3.79±0.05	1.83±0.05	1.24±0.02
Salinity	***		***		***		***	
Species	***		***		***		***	
Salinity*Species	***		***		ns		***	

DW: Dry weight, *So*: *Salvia officinalis* , *St: Salvia tomentosa* *: 0.05, **:0.01; *** : 0,001 significance level, ns: non-significant.

Potassium concentration decreased in both species with increasing salt concentration. In *S. officinalis*, K$^+$ contents decreased more than that of *S. tomentosa*. While the K$^+$ content in *S. officinalis* showed a continuous decrease with the increasing salt concentration, it showed a decrease in *S. tomentosa* and then increased partially. In *S. tomentosa*, the lowest K$^+$ in leaves value was determined in 3 dS m^{-1} salt application (Table 5). The Ca^{++} contents of the leaves of sage grown under different salt concentrations showed a fluctuation in the rising salt concentration. *S. officinalis* had the lowest values in 2

and 5 dS m^{-1} salt application, and the highest value in 3 dS m^{-1} salt application. In *S. tomentosa*, the rising Ca^{++} content at the 2 and 4 dSm^{-1} salt dose had the lowest value in 5 dS m^{-1} salt application. Both species had the lowest Ca^{++} concentration in 5 dS m^{-1} salt application, while irregular decreases and increases in other doses were observed (Table 5).

Essential oil content (%)

The essential oil yield of the leaves of sage grown under different salt stresses increased with increasing salt stress regardless of sage genotypes. The amount of essential oil detected under control conditions between 0.91% and 1.38% regularly increased in both species and it reached 2.31% and 2.19% in *S. officinalis* and *S. tomentosa* species in 5 dS m^{-1} salt dose, respectively. In *S. officinalis*, the highest essential oil content was recorded at 4 dS m^{-1} salt dose, while, the highest essential oil content in *S. tomentosa* was determined at 3 dS m^{-1} salt dose. While the increase in essential oil content caused by salt stress in *S. officinalis* ranged from 80% to154%, it varied from 83% to 46% in *S. tomentosa*.

Table 6. Essential oil content of *S. officinalis* and *S. tomentosa* grown at different salinity levels (%)
Çizelge 6. Farklı tuz stresi altında yetiştirilen S. officinalis ve S. tomentosa bitkilerinde esansiyel yağ içerikleri (%)

	Essential oil yield (%) *(Esansiyel yağ asidi verimi (%))*			
Salinity levels		% increase over control		% increase over control
Tuz seviyesi	*S. officinalis*	*Kontorle gore % artış*	*S. tomentosa*	*Kontrole gore % artış*
1dS m^{-1}	0.91±0.09	--	1.38±0.08	--
2dS m^{-1}	1.64±0.12	80	1.11±0.03	-20
3dS m^{-1}	2.01±0.03	120	2.52±0.07	83
4dS m^{-1}	2.71±0.03	197	2.01±0.06	46
5dS m^{-1}	2.31±0.06	154	2.19±0.11	59
Salinity	***			
Species	***			
Salinity*Species	***			

*: 0.05, **:0.01, ***: 0,001 significance level.

DISCUSSION

As shown in Figure 1 and Table 1, 2 and 3, a significant reduction in plant biomass was detected following the application of different salt levels. Similar results were reported by Kulak (2011) that plant height, leaf fresh weight, root fresh weight, herbal fresh weight of *S. officinalis* were significantly affected by different salt sources and doses. In consistent with this study, Ben Taarit et al., (2012) and Escalona et al. (2014) reported that salt stress adversely affected plant biomass and plant height in *S. officinalis*. In Kulak's (2011) study, a significant influence of different salt sources and doses on root growth was reported and a proportional decrease in root fresh weight was observed due to an increase in salt concentration. In the current study, the species responded differently to salt stress in terms of root fresh weight. While *S. officinalis* attempted to reduce the effect of salt stress by investing in root volume increase to absorb more water in low and medium salt, this was not observed in *S. tomentosa*. Excessive root formation effort of *S. officinalis* decreased the shoot fresh weight therefore, the root: shoot ratio increased. An increased amount of root under salt stress has been reported in *S. officinalis* (Hendawy, 2005), watermelon (Yetişir and Uygur, 2009), eggplant (Unlukara et al., 2010) and cucumber (Colla et al., 2012). The primary reason for the decrease in plant biomass under salt stress was the poor photosynthesis due to stomatal closure that limits carbon dioxide uptake (Zhu, 2001; Ben Taarit et al., 2010). These well-known phenomena was also confirmed in the current study that the amount of photosynthesis and SPAD decreased with increasing salt stress (Table 2). Results of the present study are in agreement with a study conducted in sage by Tounekti et al. (2015) they reported that the water content, photosynthetic activity and pigment content of the leaf decreased with increased salt stress.

Kulak (2011) reported that *S. officinalis* biomass was not affected to a certain level by different salt sources and doses, but it was negatively affected by high salt concentration. This is in agreement with the results of the present study. While plants can combat low-stress conditions at certain rates, the damage increases significantly after exceeding the stress threshold based on plant genotype (Figure 1). Root biomass and shoot biomass reacted differently to increasing salt stress in both *Salvia* species. Although shoot biomass decreased significantly with increasing salt dose, root biomass increased above the control treatments in moderate salt stress and then decreased (Table 3-4). An increase in root biomass has been reported in plants grown under salt stress conditions at certain levels in *S. officinalis* (Ben Taarit et al., 2009), watermelon

(Yetisir and Uygur, 2009), eggplant (Unlukara, 2010) and cucumber (Colla et al., 2012). This behavior of the plant is attributed to overcoming the reduced water intake problem due to low osmotic pressure in the root zone with root volume and roo surface area (Mahjan and Tuteja, 2005; Munns, 2002).

Sodium and Cl^- content of the leaves of sage species showed a significant increase with increasing salt level. In agreement with the present study, Escalona et al. (2014) reported that Na^+ and Cl^- content of *S. officinalis* leaves increased with increasing salt concentration, aboveground organs of plants accumulated higher Cl^- ions and the roots accumulated more Na^+. Ben Taarit et al. (2011) reported that salt stress reduced the water content of the leaf and increased the Na^+ content. The same investigators have also reported that *S. officinalis* was able to store excess Na^+ in vacuoles actively (Ben Taarit et al., 2012). Similar to the results of the present study, Tounekti et al. (2011) reported that salt stress application in *S. officinalis* disrupted ion regulation and reduced Ca^{++} and K^+ concentrations. Increases in Na^+ and Cl^- concentration of the plant organs due to NaCl stress was reported in cucumber (Colla et al ., 2012), melon (Colla et al., 2006), watermelon (Yetisir and Uygur, 2009), bottle gourd (Chuang et al., 2003) and pumpkins (Balkaya et al., 2016). The high concentration of Na^+ and Cl^- in soil or irrigation water may disrupt ion activities and cause excessive anion and cation rates to form (Grattan and Grieve, 1999). The metabolic toxicity of Na^+ is a consequence of its ability to compete with K^+ for the binding sites, which are substantially critical for cellular function. K^+ activates more than 50 enzymes, and Na^+ competing with potassium binding sites cannot substitute this function (Bhandal and Malik, 1988). The decrease in K^+/Na^+ sodium ratio may cause a reduction in plant growth due to K^+ deficiency. Under different salt stress conditions, a reduced level of NO_3 due to the high concentration of Cl^- in cucumber was reported (Colla et al., 2012). It is a well-known physiological condition that an increase in Cl^- uptake, and accumulation in plants causes a significant reduction in the nitrogen content of plants (Grattan and Grieve, 1999). Calcium content was also significantly affected by the salt application and sage species (Table 5). Ca^{++} content in the leaves showed a fluctuating attitude. Calcium plays an important role in increasing tolerance to salt stress as in many other stresses (Greenway and Munns, 1980). The positive effect of Ca^{++} on the salt-stressed plant is explained by mitigating the ionic impact of salt stress rather than osmotic stress (Rengel, 1992). Studies have shown that Ca^{++} can maintain membrane stability in roots and leaves by limiting the adverse effects of Na^+ ions on the membrane, decrease Na^+ uptake and increase K^+ uptake (Cramer et al., 1985).

Effective eustress (inductive stress) applications such as moderate salinity or nutritional stress, can elicit specific plant responses involving the activation of physiological and molecular mechanisms and the strategic accumulation of biologically active compounds necessary for adaptation to suboptimal environments (Rouphael et al., 2018). The essential oil content of sage leaves changes depending on the season, geographic origin, environmental factors and growth conditions, extraction techniques and plant organ (Santos-Gomes and Fernandes, 2001), sampling methods and developmental stage (Putievsky et al., 1986), and genetic differences (Perry et al., 199). The essential oil content of the leaves increased with salt application in both sage species. The increase in *S. officinalis* was higher than the increase in *S. tomentosa*. The highest increases were 197% (4 dS m^{-1}) and 83% (3 dS m^{-1}) in *S. officinalis* and *S. tomentosa*, respectively. Similar to previous studies (Ben trait et al., 2010; Tounekti et al., 2015), it was observed that moderate salt stress increased the essential oil content and high salt concentration started to decrease the essential oil content. Ben Taarit et al. (2011) reported that the essential oil content increased with 25 mM salt application, while it decreased in 100 mM salt application. In addition to the increase in the amount of essential oil in sage leaf, significant variation in the composition of the essential oil and some other seconder metabolites such as jasmonate and methyl jasmonate were also reported (Ben Taarit et al.,2011; Tounekti et al., 2015). The enhancing effect of moderate and low salt stress on phytochemical content (sugars, organic acids, and amino acids) of some plant species such as tomato (Zushi and Matsuzoe, 2015), pepper (Marin et al., 2009), melon (Rouphael et al., 2012), lettuce (Sakamoto et al., 2014), *Origanum majorana* (Baâtour et al., 2012), *S. coccinea* (Grzeszczuk et al., 2018) and *S. officinalis* (Tounekti et al., 2015) was reported. Therefore, current findings and the results of previous studies showed that the phytochemical content of the plants could be modified by increasing the salinity level of the growth medium or irrigation water by adding NaCl or plant nutrients. Today, advanced soilless culture systems provide the opportunity to realize these modifications in vegetables and medicinal plant species without damaging the environment.

CONCLUSION

S. officinalis and *S. tomentosa* grown under salt stress showed a different response to biomass production. Biomass production in 2 dS m^{-1} salt application, assumed as mild salt stress, was found close to the control treatment. Biomass decreased with increasing salt concentration, but essential oil content increased. According to the results of the study, it is concluded that sage can be cultivated in the soils having 2 dS m^-

^{1} salinity without causing significant yield loss and water with 2 dS m^{-1} salt level can be used for irrigation. *S. tomentosa* was more prominent in biomass production under moderate saline conditions. In areas with moderate salt problems, *S. tomentosa* may be preferred. The study showed that the phytochemical contents of the leaves could be increased with the salt application. In *S. officinalis*, 2 dS m^{-1} salt application showed a significant increase (120%) in essential oil content compared with the control. Considering the buffering properties of the soil, it was thought that the essential oil content of *S. officinalis* and *S. tomentosa* could be increased without significant yield loss by 3-4 dS m^{-1} salinity level in soil or irrigation water under field conditions. Such studies should be performed with more sage genotypes and more salt-tolerant genotypes should be determined. Stress conditions can affect not only the quantity of chemical contents but also their composition. Since the management of salt stress in soilless culture is easy by adding some salts or increasing plant nutrient concentrations, the biofortification of medicinal plants and vegetables has become a possible application. Studies on the effects of different stress conditions on the phytochemical contents and compositions of sage should be conducted with more sage genotypes to elucidate the eustress level for each stress factor.

ACKNOWLEDGMENTS

This study was supported by the Scientific Projects Unit of Erciyes University with Project FLY-2016-6738.

Contribution Rate Declaration

Authors declare that they have contributed equally to the article

Conflict of interest

There is no conflict of interest to declare.

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KSÜ Tarım ve Doğa Derg 24 (3): 505-514, 2021
KSU J. Agric Nat 24 (3): 505-514, 2021
https://doi.org/10.18016/ksutarimdoga.vi.730477