

## Determination of Germination Biology of Some Sage (*Salvia* spp.) Species Under Salinity Stress

Tuzluluk Stresinin Bazı Adaçayı (*Salvia* spp.) Türlerinde Çimlenme Biyolojisinin Belirlenmesi

Banu KADIOĞLU

### Abstract

Soil salinity is one of the most important abiotic stress factors affecting agricultural productivity in arid and semi-arid regions. Salinity negatively affects the soil as well as causes negativity in the plants grown on it. In plants exposed to salt stress, processes such as germination, emergence and sprout are affected, as well as many morphological and physiological negativities and the plant develops mechanisms that can tolerate these negativities. In addition, such plants gain importance in terms of the evaluation of soils with salinity problems. It is necessary to know and make use of the species and varieties that can make use of such soils in conditions where salinity removal is difficult and uneconomical for different reasons. Lamiaceae (Labiatae) family grows in a wide area and different species of salvia and sideritis genus belonging to this family are known as sage. Stress factors in plants cause the growth and development periods of plants to slow down and their metabolic functions to deteriorate, thus causing death in plants. The response of different plants or even different species of a plant to salt concentrations may be different. Therefore, this research was conducted to determine the effects of salt stress on seed germination in some sage species. Seven different (0, 25, 50, 75, 100, 125, 150 mM) of three sage species (*S. nemorosa* L., *S. verticillata* L., *S. officinalis* L.) salt concentration applied. In the study, germination rate, germination speed, average daily germination, peak value and germination values were examined. When the properties investigated were evaluated, it was determined that the woodland sage species, whose germination rate and germination rate decreased as the salt concentration increased, gave the best results. It was determined that lilac sage type gave the best results for the peak value and germination values where the average daily germination value was obtained from 25 mM salt application in three species.

**Keywords:** Salt, Tolerance, Concentration, Effects, Emergence, Seed

<sup>1</sup>\*Sorumlu Yazar/Corresponding Author: Banu Kadioğlu, Eastern Anatolia Agricultural Research Institute. Erzurum. Turkey. E-mail: banu250@hotmail.com

 ORCID: 0000-0002-9041-5992

**Atıf/Citation:** KADIOĞLU, B. Determination of Germination Biology Some Sage (*Salvia* spp.) Species Under Salinity Stress. *Tekirdağ Ziraat Fakültesi Dergisi*, 18 (2), 359-367.

## Özet

Kurak ve yarı kurak bölgelerde toprak tuzluluğu tarımsal verimliliği etkileyen en önemli abiyotik stress faktörlerinden birisidir. Tuzluluk toprağı olumsuz etkilediğı gibi üzerinde yetişen bitkilerde de olumsuzluklara neden olmaktadır. Tuz stresine maruz kalmış bitkilerde çimlenme, çıkış, sürme gibi süreçlerinin etkilenmesi yanı sıra morfolojik ve fizyolojik birçok olumsuzluklar görülmekte ve bitki bu olumsuzlukları tolere edebilecek mekanizmalar geliştirmektedir. Ayrıca tuzluluk problemi olan toprakların değerlendirilmesi açısından da bu tür bitkiler önem kazanmaktadır. Tuzluluğun giderilmesinin farklı nedenlerle zor ve ekonomik olmadığı koşullarda bu tür toprakları değerlendirebilecek tür ve çeşitlerin bilinmesi ve yararlanılması gerekmektedir. Lamiaceae (Labiatae) familyası geniş bir alanda yetişmekte ve bu familyaya ait salvia ve sideritis cinslerinin farklı türleri adaçayı olarak bilinmektedir. Bitkilerde stres faktörleri, bitkilerin büyüme ve gelişme dönemlerinin yavaşlamasına ve metabolik fonksiyonlarının bozulmasına bu nedenle de bitkilerde ölümlere sebep olmaktadır. Farklı bitkilerin hatta bir bitkinin farklı türlerinin tuz konsantrasyonlarına cevabı farklı olabilmektedir. Bu nedenle bazı adaçayı türlerinde tohum çimlenmesi üzerine tuz stresinin etkilerini belirlemek amacı ile bu araştırma yapılmıştır. Tesadüf parselleri deneme desenine göre on tekrarlı olarak yürütülen çalışmada üç adaçayı türüne (*S. nemorosa* L., *S. verticillata* L., *S. officinalis* L.) yedi farklı (0, 25, 50, 75, 100, 125, 150 mM) tuz konsantrasyonu uygulanmıştır. Çalışmada çimlenme oranı, çimlenme hızı, ortalama günlük çimlenme, tepe değeri ve çimlenme değerleri incelenmiştir. İncelenen özellikler değerlendirildiğinde çimlenme oranı ve çimlenme hızının tuz konsantrasyonu arttıkça azaldığı woodland sage (*S. nemorosa*) türünün en iyi sonuçları verdiği belirlenmiştir. Ortalama günlük çimlenme değerinin üç tür içinde 25 mM tuz uygulamasından elde edildiğı tepe değeri ve çimlenme değerleri için en iyi sonuçları lilac sage (*S. verticillata*) türünün verdiği saptanmıştır.

**Anahtar Kelimeler:** Tuz, Tolerans, Konsantrasyon, Etki, Çıkış, Tohum

## 1. Introduction

Soil salinization, usually occurring in arid and semi-arid areas, is a major problem in our country as well as in the world. Salinity, one of the abiotic stress factors, impacts the soil's structural structure and causes productivity to decline. Whereas the water at the optimum level in the environment ensures plant growth, soil salinity affects plant water intake and reduces plant yield and quality. The response of the plants to salt stress varies depending on the form and amount of salt being applied and the plant type and variety (Yilmaz et al., 2011); (Shavrukov, 2013); (Arslan and Aydinoglu, 2018); (Tiryaki, 2018). Reaction to salt applications varies depending on the plant (Pastori and Foyer, 2002); (Bartels and Sunkar, 2005). These variations refer not only to different species but to varieties of the same species as well (Munns, 2002); (Turhan and Seniz, 2010); (Onal Asci and Uney, 2011).

Since improving salty soils is difficult and not economical growing salt-resistant plants is very important in the assessment of salty soils (Turhan and Seniz, 2010); (Onal Asci and Uney, 2011). Salinity which is one of the abiotic stress factors restricting the growth and development of plants that define the geographical distribution of medicinal and aromatic plant species (Vriezen et al., 2007), affects medicinal and aromatic plants in various physiological periods such as germination, seedling and maturation (Mondal and Kaur, 2017). Camlica and Yaldiz (2017) found in their study that as salt concentrations increased basil germination time, shoot and root length decreased. In other studies investigating the effect of salt stress it has been stated that different salt concentrations negatively affect the wet and dry weight of the plant, stem and shoot development (Hosseini and Rezvani Moghadam, 2006); (Akbari et al., 2007); (Mahdavi et al., 2007); (Hamidi and Safarnejad, 2010).

To date, there have been several studies conducted to determine the impact of salt stress and it has been concluded that salinity affects germination. The cultivation of medicinal and aromatic plants with an economic value in troublesome soils such as salinity would both ensure soil productivity and increase farmers' earnings. In the study conducted with this assumption the goal was to determine the response of various sage species in germination biology parameters at different salt concentrations.

## 2. Materials and Methods

In the study carried out in 2020 under controlled conditions ( $25 \pm 1$  ° C); on the sage tea species obtained from Atatürk Horticultural Central Research Institute, woodland sage (*Salvia nemorosa* L.), lilac sage (*Salvia verticillata* L.) and common sage (*Salvia officinalis* L.) seven different salt concentrations (0, 25, 50, 75, 100, 125, 150 mM) were applied 10 mm NaCl solutions were added to petri dishes. The seeds sterilized with sodium hypochlorite (5%) were left to germinate. The germinated seeds (reaching 2 mm radicle size) were counted and removed from the environment (Prado et al., 2000). In the study, germination rate (%), germination time (days), average daily germination, peak value and germination value were examined (Czabator, 1962); (Ellis and Roberts, 1981); (Gairola et al., 2011).

$$\text{Germination percentage (GP)} = \frac{n}{\Sigma n} \times 100 \quad (\text{Eq.1})$$

n = Number of germinated seeds

$\Sigma n$  = Total number of seeds

$$\text{Germination speed (GS)} = \frac{n_1}{t_1} + \frac{n_2}{t_2} + \dots \quad (\text{Eq.2})$$

$n_1, n_2, \dots$  are the number of germinated seeds at times  $t_1, t_2, \dots$  (in days)

$$\text{Mean daily germination (MDG)} = \frac{\text{Total number of germinated seeds}}{\text{total number of days}} \quad (\text{Eq.3})$$

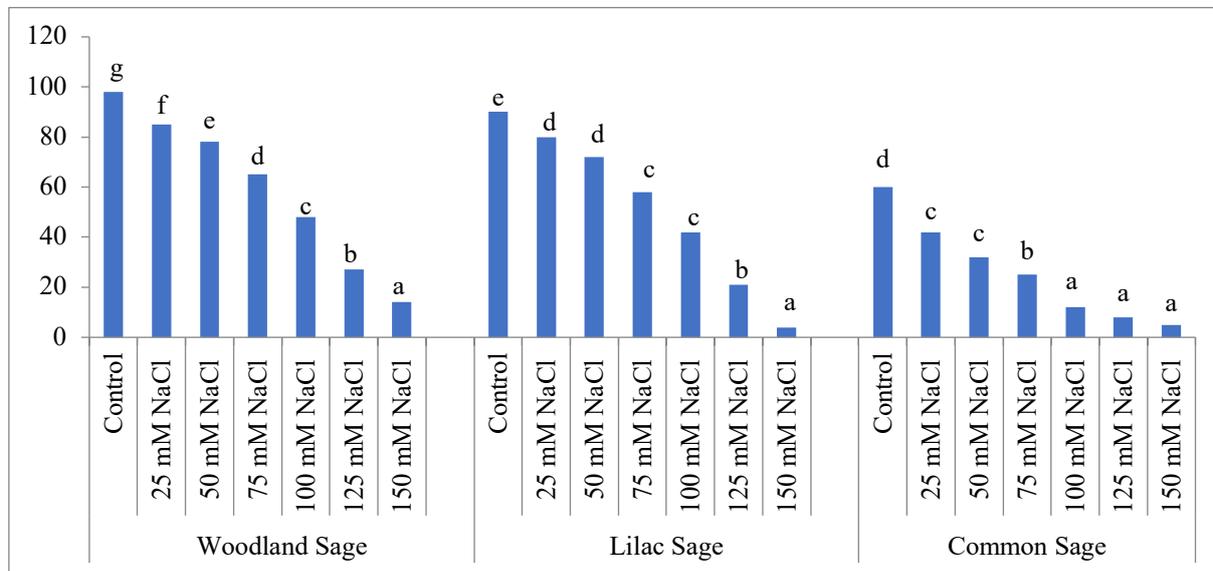
$$\text{Peak Value (PV)} = \frac{\text{highest seed germinated}}{\text{number of days}} \quad (\text{Eq.4})$$

$$\text{Germination Value (GV)} = \text{MDG} \times \text{PV} \quad (\text{Eq.5})$$

The study results were statistically tested using variance analysis and duncan multiple comparison tests (5%) (Dowdy and Wearden, 1983).

### 3. Results and Discussion

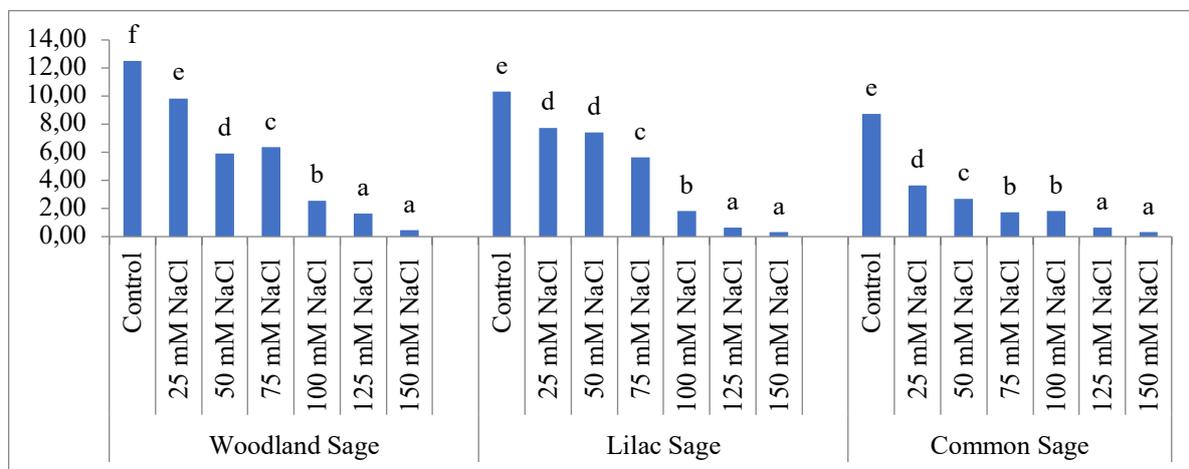
In the study carried out, all applications on sage species at 1% ( $P \leq 0.01$ ) all applications were important for the sage species. Salt stress has been determined to be effective on GP (Eq.1), there is no difference between the species and as the salt concentration rises, the GP value decreases with the highest value at woodland sage (85%), followed by lilac sage (80%) and common sage (42%), respectively (Figure 1).



There is no difference between the mean shown on bars in the same letter ( $P \leq 0.01$ ).

**Figure 1. Effect of salt stress on GP in sage species**

It was seen that GS (Eq.2) values in all three species decreased as the salt concentration increased. The woodland sage species gave the best result with 9.85 days in the application of 25 mM salt while the lilac sage and common sage species had the lowest values with 150 mM of salt application with 3 days (Figure 2).

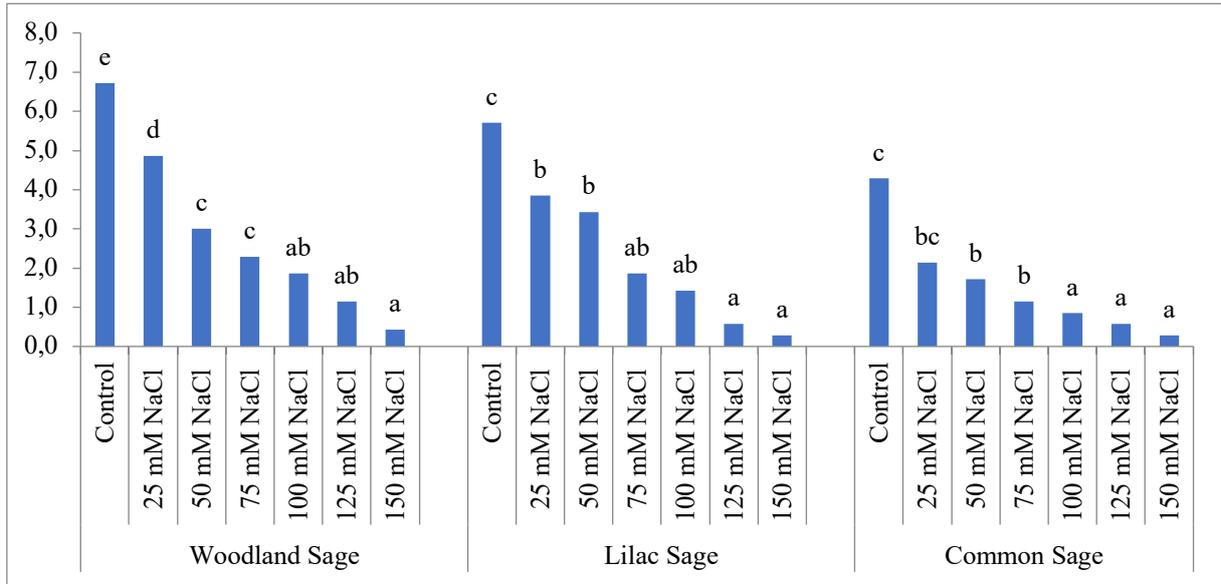


There is no difference between the mean shown on bars in the same letter ( $P \leq 0.01$ ).

**Figure 2. Effect of salt stress on GS in sage species**

When we examined the impact of salt stress on MDG (Eq.3), we determined that there was no difference between species. In woodland sage, lilac sage and common sage species, as the salt concentration increased, MDG

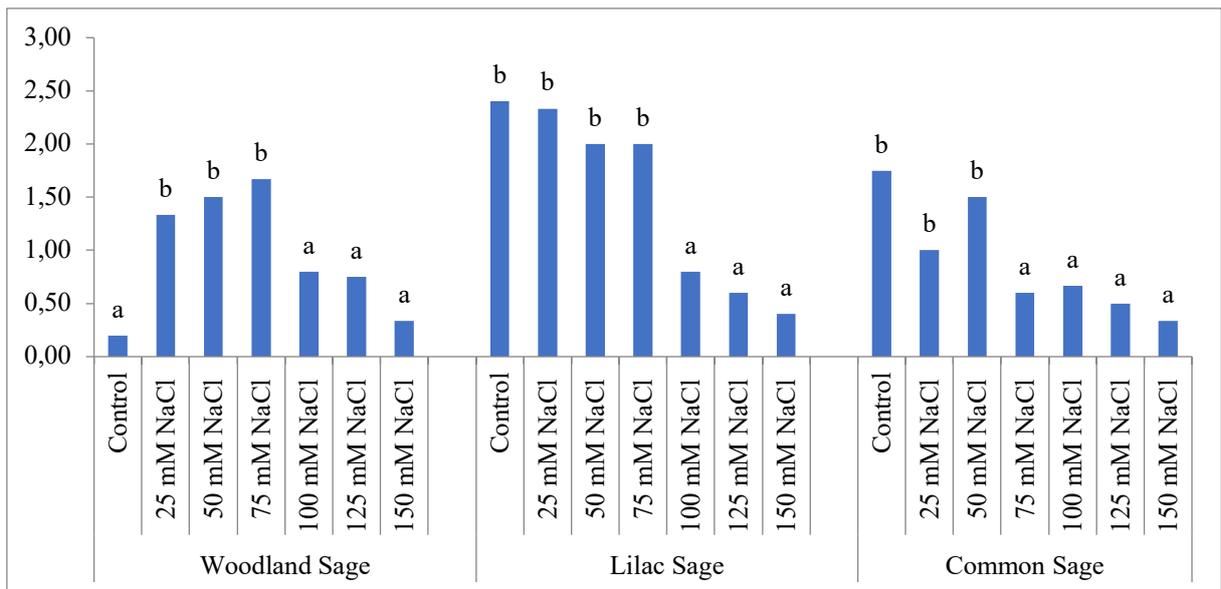
was negatively affected and 25 mM of salt application provided the best results compared to the control in all three varieties (Figure 3).



There is no difference between the mean shown on bars in the same letter ( $P \leq 0.01$ )

Figure 3. Effect of salt stress on MDG in sage species

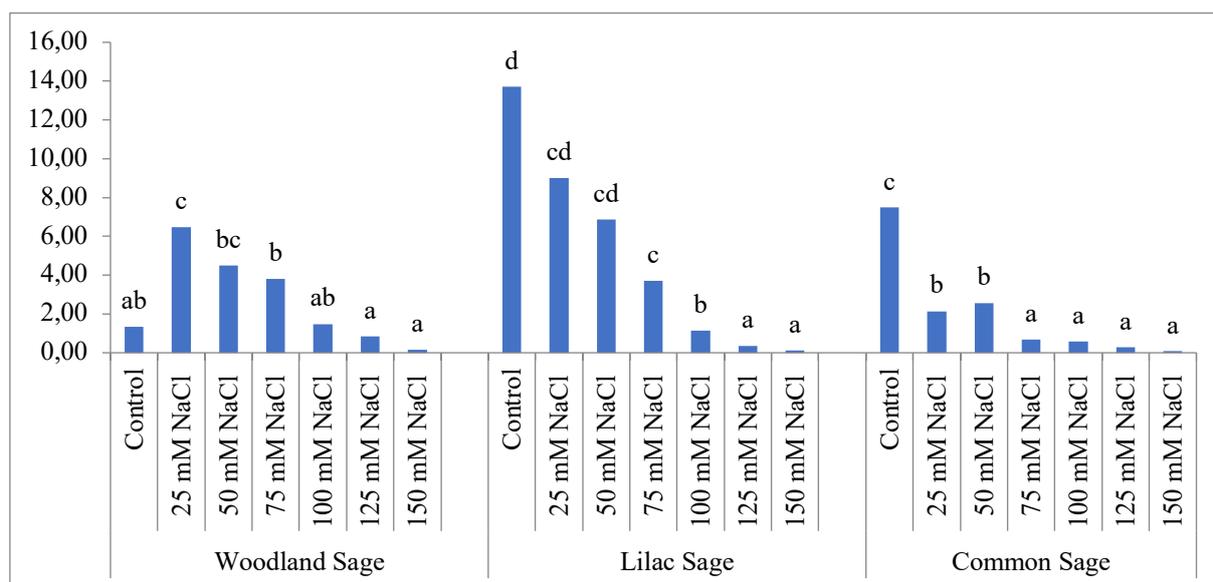
Concerning PV (Eq.4), it was observed that the lilac sage species gives the best results and the applications on the woodland sage species give better results than the control. In 75 mM, 25 mM and 50 mM salt applications, species (woodland sage, lilac sage and common sage) obtained the values of 1.67, 2.33 and 1.50, respectively (Figure 4).



There is no difference between the mean shown on bars in the same letter ( $P \leq 0.01$ )

Figure 4. Effect of salt stress on PV in sage species

In three species of sage, it was determined that 100 mM and 150 mM applications of salt stress had negative effects on GV (Eq.5) and lilac sage species gave the best results. 25 mM salt application in woodland sage and lilac sage type had the highest values with 6.45 and 9, whereas in common sage type, 50 mM salt application had the highest value with 2.57 (Figure 5).



There is no difference between the mean shown on bars in the same letter ( $P \leq 0.01$ ).

**Figure 5. Effect of salt stress on GV in sage species**

Salt stress and drought stress can also be confused with symptoms such as plant growth regression, slowing of growth in plant vegetative parts. The element "salt" may have a detrimental effect on plants and cause plant nutritional irregularities. Salinity in the soil can be addressed in various ways. These can be listed as providing sufficient drainage, washing the soil with water with a low salt concentration, or growing salt-tolerant plants. It is also very important to determine and grow medicinal and aromatic plants that can be cultivated in different topographies and problem areas, particularly in marginal areas, with various programs and that can tolerate environments such as saltwater or salty soil. The selection and development of plants whose reactions to salt are determined with such studies also gain importance. The data we obtained in this study conducted with three different species of sage show similarities with the results of other studies. In a study examining the impact of various salt applications (0, 50, 100, 150 and 200 mM) on the growth of sage (*Salvia officinalis* L.), which has an important position among medicinal and aromatic plants, it was determined that plant height, wet and dry root weight, wet and dry leaf weight, wet and dry stem weight were changed. Concerning the cultivation of sage in saline areas, adaptation studies on different species and varieties has been recommended (Kulak, 2011). In another study, it was reported that different salt concentrations, did not affect the sage growth much up to 100 mM salt level, but at amounts above this salt level, plant growth was negatively affected (Camlica et al., 2019). In another analysis, different salt concentrations (0, 5, 10, 20, 40, 80, 160  $\mu\text{M}$  NaCl) were added to seeds of sage (*Salvia officinalis* L.), black cumin (*Nigella sativa* L.) and flax (*Linum usitatissimum* L.) and it was reported that as the salt concentration increases, the amount of germination, germination rate, shoot and root length in these plants decreases (Yaldiz et al., 2016); (Kiremit et al., 2017). Seed germination and seedling development stage of plants is the most sensitive stage to salinity. Salt stress causes negative physiological and biochemical changes in germinated seeds. It has been reported that salt stress affects medicinal plants in three different physiological phases (germination, seedling and maturation and production periods) (Mondal and Kaur, 2017). In other studies, it was found that their tolerance to salt stress among plant species was different (Akçay and Tan, 2019); (Okcu, 2020); (Dadasoglu et al., 2020). A research study investigating the impact of concentration of NaCl on plant growth in *Salvia officinalis* determined that the application of 100 mM of NaCl reduced plant growth (Ben Taarit et al., 2010). Salinity delays or prevents germination of seeds by various factors such as reducing water content, making changes in the mobilization of stored reserves and affecting the structural organization of proteins (Ibrahim, 2016). Salinity significantly affects seed germination, seedling and plant growth (Kolsarici et al., 2005). In the study investigating the effects of 40 mM and 80 mM salt conditions on *Plectranthus scutellarioides*, it was determined that growth parameters and photosynthetic pigment concentration decreased, and anthocyanin and free proline

accumulation increased (Hawrylak-Nowak et al., 2019). In a study investigating the effect of increased salt stress on the oil (EO), phenolics and diterpene content of the leaves of sage tea, it was concluded that the effective substances in sage tea can be manipulated with salt stress applied for 4 weeks with 0, 50, 75, and 100 mM salt stresses (Tounekti and Khemira, 2015). In aquaculture, fatty acids were analyzed in sage teas (*S. officinalis*) grown under salt stress. It was concluded that the salt application (100 mM) reduced plant growth by 61% and the total fatty acid amount by 32%, and that sage was a moderately salt tolerant species (Ben Taarit et al., 2010). The effects of different doses of salt stress on plant growth were investigated, and it was reported that plant growth (42%), K uptake and plant water content decreased and Na uptake increased in the application of 75 mM NaCl (Ben Taarit et al., 2011). The effects of salt stress and kinetin application on plant growth, photosynthetic pigments, phenolic compounds and alpha tocopherols in sage (*S. officinalis*) were investigated and it was stated that the effects of salt stress can be improved by the application of kinetin in arid and semi-arid regions (Tounekti et al., 2011). In another study, examining the effects of the application of saline water during the germination period on the saline tolerance and morphological characteristics of linen, artichoke, safflower, and echinacea seeds, it was determined that linen and safflower seeds had more tolerance against salt stress when compared to echinacea and artichoke seeds and germination rate, offshoot and radicle lengths and plant dry weight characteristics of all plants decreased with the increasing salinity (Gholizadeh et al., 2016).

Based on the data obtained from the study, it was found that as salt concentrations increased, sage species had a negative effect on seed germination parameters. In all three species, it was determined that 100 and 150 mM salt applications had a decreasing effect on seed germination biology and the tolerance of woodland sage and lilac sage to salt stress was higher than common sage species.

#### 4. Conclusions

Soil salinity can adversely affect the biology of plant growth, water uptake and germination of seeds. Climate conditions, impermeable layer of soil, erosion, improper irrigation methods, etc. cause salinity and deterioration of soil. As it is difficult and expensive to upgrade salty soils, it is extremely important to identify and grow plants that are suitable for those types of soil. In this study, which aimed to determine the salt stress tolerance of sage species, which is a medicinal and aromatic plant with a high economic value, it was determined that there was no difference between species, 25 mM salt application gave better results compared to others and the common sage (*S. officinalis* L.) species was more affected by salt stress than other species. Considering all the factors examined, woodland sage (*S. nemorosa* L.) can be thought as the most tolerant sage species to salt stress. Also can be thought in stress conditions can be use especially in regions where salinity is difficult to eliminate. Therefore it is thought that *Salvia* species can be an alternative to be used in breeding programs, that species can be developed by taking them into field trials and will help the development of sage production in salty areas.

## References

- Akbari, G., Sanavy, S.A., Yousefzadeh, S. (2007). Effect of auxin and salt stress (NaCl) on seed germination of wheat cultivars (*Triticum aestivum* L.). *Pakistan Journal of Biological Sciences* 10 (15): 2557-2561.
- Akcay, E., Tan, M. (2019). The effects of different salinity levels on root and shoot development in some quinoa (*Chenopodium quinoa* Willd.) varieties. *Atatürk Univ., J. of the Agricultural Faculty* 50 (3): 292-298.
- Arslan, M., Aydinoglu, B. (2018). The effect of salinity (NaCl) stress on germination and early seedling growth in damson (*Lathyrus sativus* L.). *Academic Journal of Agriculture* 7(1):49-54.
- Bartels, D., Sunkar, R. (2005). Drought and Salt Tolerance in Plants. *Critical Reviews in Plant Sciences* 24: 23-58.
- Ben Taarit, M., Msaada, K., Hosni, K., Marzouk, B. (2010). Changes in fatty acid and essential oil composition of sage (*Salvia officinalis* L.) leaves under NaCl stress. *Food Chemistry* 119 (3): 951-956.
- Ben Taarit, M., Msaada, K., Hosni, K., Marzouk, B. (2011). Physiological changes and essential oil composition of clary sage (*Salvia sclarea* L.) rosette leaves as affected by salinity. *Acta Physiologiae Plantarum* 33 (1): 153-162.
- Czabator, F.J. (1962). Germination Value: An index combining speed and completeness of pine seed germination. *Forest Science* 8; 386-395.
- Camlica, M., Yaldiz, G. (2017). Effect of salt stress on seed germination, shoot and root length in basil (*Ocimum basilicum*). *International Journal of Secondary Metabolite* 4 (3): 69-76.
- Camlica, M., Yaldiz, G., Ozen, F., Basol, A., Askin, H. (2019). Effects of selenium applications on salt stress in sage and mountain tea. *Turkish Journal of Agriculture - Food Science and Technology* 7(2): 29-35. DOI: <https://doi.org/10.24925/turjaf.v7isp2.29-35.3098>.
- Dadasoglu, E., Ekinci, M., Yildirim, E. (2020). Effects of salt stress on seed germination of chick pea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.). *Atatürk Univ. J. of Agricultural Faculty* 51 (1): 53-62.
- Dowdy, S., Wearden, S. (1983). *Statistics for Research*. John Wiley and Sons Inc. New York, USA.
- Ellis, R.H., Roberts, E.T. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science Technology* 9 (2): 373-409.
- Gairola, K.C., Nautiyal, A.R., Dwivedi, A.K. (2011). Effect of temperatures and germination media on seed germination of *Jatropha curcas* Linn. *Advances in Bioresearch* 2 (2), 66-71.
- Gholizadeh, F., Manzari-Tavakkoli, A., Pazoki, A. (2016). Evaluation of salt tolerance on germination stage and morphological characteristics of some medicinal plants artichoke, flax, safflower and coneflower. *International Journal of Farming and Allied Sciences* 5(3), 229-237.
- Hamidi H., Safarnejad, A. (2010). Effect of drought stress on alfalfa cultivars (*Medicago sativa* L.) in germination stage. *American-Eurasian Journal of Agricultural and Environmental Sciences* 8 (6): 705-709.
- Hawrylak-Nowak B., Rubinowska, K., Molas, J., Woch, W., Matraszek-Gawron, R., Szczurowska, A. (2019). Selenium induced improvements in the ornamental value and salt stress resistance of *Plectranthus scutellarioides* (L.) R. Br. *Folia Horticulturae* 31(1): 213-221.
- Hosseini, H., Rezvani Moghadam, P. (2006). Effect of water and salinity stress in seed germination on isabgol (*Plantago ovata*). *Iranian Journal of Field Crops Research* 4 (1): 15-22.
- Ibrahim, E.A. (2016) Seed priming to alleviate salinity stress in germinating seeds. *Journal of Plant Physiology*, 192: 38-46. ISTA, 1996. *International Rules for Seed Testing*. Seed Science and Technology. 24, ISSN: 0251-0952.
- Kiremit, M. S., Hacikamiloglu, M., Arslan, H., Kurt, O. (2017) The effects of different irrigation water salinity levels on germination and early seedling development of flax (*Linum usitatissimum* L.). *Anadolu Journal of Agricultural Science* 32: 350-357.
- Kolsarici, O., Kaya, M.D., Ustuner, N.D., Sarihan, E.O. (2005) The Effects of Different NaCl Concentrations on the Germination of Some Oil Plants Seeds. Turkey VI. Field Crops Congress. I, 553-556. Some Confectionary Sunflower (*Helianthus annuus* L.) Genotypes. *Journal of Agricultural Sciences* 14, 230-236.
- Kulak, M. (2011). The effect of different salt applications on the development of sage (*Salvia officinalis* L.). (Master Thesis) Kilis 7 Aralık University. The Institute of Natural Sciences, Kilis, Turkey.
- Mahdavi, B., Sanavi, S., Balochi, H.R. (2007). The Effect of sodium chloride on the germination and seedling growth figures grass pea (*Lathyrus sativus* L.). *Iranian Journal of Biology* 20: 363-374.
- Mondal, H.K., Kaur, H. (2017). Effect of salt stress on medicinal plants and its amelioration by plant growth promoting microbes. *International Journal of Bio-Resource and Stress Management* 8 (2), 316-326.
- Munns, R. (2002). *Salinity, Growth and Phytohormones, Salinity: Environment-Plants-Molecules*, Published by Kluwer Academic Publishers, ISBN 1-4020-0492-3, Dordrecht, The Netherlands, 522p.
- Okcu, M. (2020). Impact of salinity stress on germination and seedling development in feeding cowpea (*Vigna unguiculata* L. Walp). *Journal of the Institute of Science and Technology* 10(1): 669-676.

- 
- Onal Asci, O., Uney, H. (2016). Effect of different salt concentrations on germination and plant growth in hungarian vetch (*Vicia pannonica* Crantz). Academic Journal of Agriculture 5(1), 29-34.
- Pastori, G.M., Foyer, C.H. (2002). Common components, networks, and pathways of cross-tolerance to stress. The central role of "redox" and abscisic acid-mediated controls. Plant Physiology 129: 460-468.
- Prodo, F.E., Boero, C., Gallardo, M., Gonzalez, J. A. (2000) Effect of NaCl on germination growth and soluble sugar content in *Chenopodium quinoa* (wild) seeds. Bot Bull Acad Sin 41; 27-34.
- Shavrukov, Y. (2013). Salt stress or salt shock: which genes are we studying? Journal of Experimental Botany 64(1): 119-27.
- Tiryaki, İ. (2018). Adaptation mechanisms of some field crops to salt stress. Kahramanmaraş Sutcu Imam University Journal of Agriculture and Nature 21(5):800-808.
- Tounekti, T., Hernandez, I., Muller, M., Khemira, H. & Munne-Bosch, S. (2011). Kinetin applications alleviate salt stress and improve the antioxidant composition of leaf extracts in *Salvia officinalis*. Plant Physiology and Biochemistry 49 (10): 1165-1176.
- Tounekti, T., Khemira, H. (2015). NaCl stress-induced changes in the essential oil quality and abietane diterpene yield and composition in common sage. Journal of Intercultural Ethnopharmacology 4 (3): 208-216.
- Turhan, A., Seniz, V. (2010). Salt tolerance of some tomato genotypes grown in Turkey. Journal of Food, Agriculture and Environment 8(3-4): 332-333.
- Vriezen, J.A.C., Bruijn, F.J., Nusslein, K. (2007). Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature. Applied and Environmental Microbiology 73: 3451-3459.
- Yaldiz, G., Camlica, M., Ozen, F. (2016). Effect on the Germination of Some Medicinal and Aromatic Plants of Different Salt Concentrations. 2nd International Conference on Science, Ecology and Technology. 14-16 October, p. 698-705. Barselona, İspanya.
- Yilmaz, E., Tuna, A.L., Burun, B. (2011). The tolerance strategies developed by plants against the effects of salt stress. Celal Bayar University Journal of Science 7(1): 47-66.