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INVESTIGATION OF PGPR EFFECT ON *SALVIA* L. VARIETIES GROWN IN SALINE CONDITIONS AND DIFFERENT ENVIRONMENTS IN EARLY SEEDLING PERIOD

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Abstract

Soil salinity in arid and semi-arid regions, which is one of the abiotic stress factors, is a major problem worldwide. Different salt concentrations have a negative effect on plant growth by preventing or delaying the fundamental structure of the soil, seed germination, seedling growth, and thus adversely affecting yield. To investigate the effect of bacteria on the germination and early stages of *Salvia* L., seeds under saline conditions, extracts and water obtained from *Hypericum scabrum* and *Helichrysum plicatum* flowers were used as nutrient solutions in the study carried out in a growth chamber (Microtest, -20 to +70◦C) in a factorial experimental design with ten replicates using polyurethane foam. In the research, various indicators of sage varieties were examined in different environments (3 nutrient solution/medium) at different salt concentrations (0, 50 and 100 mM) of bacterial applications, and it was determined that germination was low at 50 mM and 100 mM salt concentrations, and shoot water holding capacity and relative water content in the examined parameters experienced negative effects due to increasing salt concentrations. Despite the visible effect of increasing salt, bacterial treatment minimized the negative effect on the parameters. It was determined that *S. nemorosa* L. obtained the highest values with a *B. subtilis* + 0 mM salt concentration in a *Hypericum scabrum* nutrient solution/medium.

Key words: Soil; NaCl; Media; Bacteria; Nutrient solution/medium

Introduction

Water scarcity, soil pollution, soil and water salinity are among the impacts incurred by climate change in recent years. Stress on plants increases and species extinction accelerates as population growth continues and arable agricultural land continues to decrease (Shokat *et al.,* 2019). Increased salt concentrations in soil or irrigation water are a major environmental problem for agriculture (Srivastava *et al.,* 2020). Abiotic stress, especially salt stress, has a detrimental effect on plant growth, germination development and early seedling stages. Salt stress affects plants on a morphological, anatomical and molecular level (Yıldız *et al.,* 2017; Fan *et al.,* 2020; Chavarria *et al.,* 2020).

Salinity, an abiotic stressor, is a prominent factor limiting plant growth and development (Vriezen *et al.,* 2007). Soil-related stress conditions have a significant impact in inhibiting plant growth and productivity globally (Liu *et al.,* 2023). Soil salinity and degradation are caused by erosion, inappropriate irrigation, climate and impermeable soil layers. The high costs and difficulty of reclamation of saline soils make it difficult. Therefore, it is necessary to determine and grow plant species suitable for halomorphic soils (Kadıoğlu, 2022).

Salinity has a negative effect on plant biology (growth, water uptake, germination, etc.) (Kadioglu, 2022). As a result of the toxic effects of sodium and chloride ions, salinity limits plant growth and affects yield (Roy *et al.,* 2017). Due to salinization, it becomes difficult for plant roots to absorb water. Salinity causes the germination period to extend and the shoot and root length to decrease (Demiroğlu Topçu *et al.,* 2020). The researches have reported that different salt rates have negative effects on fresh and dry weight, stem and shoot development (Hosseini *et al.,* 2006; Akbari *et al.,* 2007).

Climatic conditions play a major role in plant tolerance to salt. The effect of salinity is observed more in hot and dry conditions (Kotuby Amacher, 2000). Plants can tolerate heat, drought and salt (Yamazaki *et al.,* 2020). Considering the salinity levels, the selection of highly tolerant plant varieties is very important to reach the optimum level in plant yield (Altuner *et al.,* 2022). Increased soil salinity during germination inhibits growth (Nedjimi *et al.,* 2019). The decrease in germination leads to an increase in the osmotic pressure of the soil solution, preventing and limiting the necessary water absorption (Ouerghi *et al.,* 2016). Saline soils contain high concentrations of Na+, Cl- and SO4+, increasing osmotic stress and ionic toxicity, affecting plant growth. These conditions impair plant nutrient uptake and metabolism and lead to imbalances within the plant (Srivastava *et al.,* 2020). Agricultural activities are very difficult in soils with saline waters and saline and alkaline soils because plants cannot get the water they need as a result of the difference in osmotic pressure (Kacar & İnal, 2008). The cultivation of salt and drought tolerant plants in such soils can improve soil conditions as well as prevent erosion and desertification. This is why studies have been carried out recently on the tolerance of plants to salt stress by soil conditioners and PGPR bacteria. The use of soil amendments and PGPR bacteria has a positive impact on soil structure, plant metabolism, root and stem growth and nutrient uptake (Çakmakçı, 2005; Kadıoğlu, 2022).

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In soilless agriculture, different organic and inorganic media, nutrient solution are used as soil conditioners/mediums to help regulate soil structure, physical, chemical and biological properties. *Hypericum* and *Helichrysum* extracts are preferred as nutrient media due to their bioactive compounds and the microenvironment they create. Both plants possess a rich phytochemical profile that has proven its value in agriculture and microbiology. The origins of these extracts in agricultural applications lie in the traditional healing and preservation practices of these plants. In traditional agriculture, extracts of these plants are used as natural agents to suppress soil-borne pathogenic fungi and harmful bacteria. This antimicrobial mechanism provides a protective selectivity in the laboratory environment, allowing target microorganisms to feed and multiply while keeping out foreign contaminants (undesirable bacterial/mold growth). They are also used in plant nutrition formulations. These extracts are included in the nutrient medium not only as a nutrient source but also as organic biochemical complexes that stabilize the medium, exhibit bio-stimulating effects, and prevent environmental contamination (Kadıoğlu, 2021; Taşgın & Aytaç, 2025). The aim of our study was to determine the macro-mineral uptake, relative water content, water retention ability and tolerance index of sage cultivars at different salt concentrations in the early seedling stage of sage cultivars by using bacteria treatments in different media (Control, *Hypericum scabrum*, *Helichrysum plicatum*) seeds from the extensively growing Lamiaceae (Labiatae) family and *Salvia* and *Sideritis* genera (*S. nemorosa* L., *S. verticillata* L., *S. officinalis* L.) from this family. Stress factors in plants slow down their growth and development and impair their metabolic functions, leading to plant mortality. Different plants or even different species of a plant can respond differently to salt concentrations.

Materials and Methods

Experimental design: The experiment was carried out in a growth chamber (Mikrotest, -20 to +70◦C) using polyurethane foam in a factorial experimental design with ten replicates to investigate the effect of bacteria on *Salvia* L. seeds under saline conditions during germination and at the early stage. Nutrient solutions and water (control) obtained from *Hypericum scabrum* and *Helichrysum plicatum* flowers were used as growing media. The plant nutrient elements of *Hypericum scabrum* and *Helichrysum plicatum* are listed in Table 1 (Kadıoğlu, 2021).

Table 1. Nutrient elements of *Hypericum scabrum* and *Helichrysum plicatum* plants.

	N%	P%	K%	Ca%	Mg%	Fe ppm	Cu ppm	Zn ppm	Mn ppm
H. scabrum	0.24	0.30	2.01	0.22	0.19	185	17.6	24.0	19.1
H. plicatum	0.21	0.31	0,89	0.23	0.20	1531.1	8.7	69.5	52.8

Germination tests: The experiment was designed according to a factorial experimental design with ten replications. Plastic tanks with a capacity of 3 liters were used in the experiment and polyurethane foam was placed in each tank. *A. agilis* and *B. subtilis* were chosen as bacteria. Plant seeds inoculated with bacteria were planted, one plant per viola. The applied nutrient solutions were changed every 7 days. An aeration device was set for the experiment to supply oxygen to the nutrient solutions used as plant growth media. The nutrient solutions were aerated for 15 minutes every 2 hours every day. The research, in which *S. nemorosa* L., *S. verticillata* L., *S. officinalis* L. seeds were used, was carried out in a total of 810 tanks according to factorial experimental design as 3 genera *(S. nemorosa* L., *S. verticillata* L., *S. officinalis* L.*)* × 3 treatments *(A. agilis, B. subtilis,* and control (non-bacterial) × 3 media (nutrient solutions) × 3 salt doses (0, 50 and 100 mM) × 10 replicates. A 5% NaClO solution was used for the surface sterilization of the seeds. The seeds were sterilized for 10 minutes. The sterilized seeds were inoculated with bacteria. The inoculated seeds were planted in vials. Water (control), *Hypericum scabrum* (HS) and *Helichrysum plicatum* (HP) extracts were applied to the tanks by the tidal method (Karaşahin, 2015). The germination time of the seeds was determined according to the principles specified in ISTA (Anon., 2003). After the seeds were placed in the germination medium, the number of germinated seeds was determined with daily checks. After 50 days, the harvested plants were dried at room temperature and then dried in an oven at 70°C until they reached constant weight. The dried samples were ground to an average thickness of 2 mm in a grinder with Teflon blades and prepared for analysis (Kaçar & İnal, 2008).

Determination of tolerance indices: Measurements were taken from the root crown to the apex of the root to determine the root length and from the root crown to the apex of the shoot to determine the shoot length (Tenikecier *et al.,* 2013). Root and stem parts of plants weighed on precision scales, root and stem fresh weights were determined. The root and stem dry weights of plant samples dried in an oven at 70°C for 48 hours were determined (Tenikecier & Ateş, 2018). water retention (RCS), relative water content (RWC), tolerance index (TI) are calculated with the formulas given below (Clarke, 1986).

RCS (mg) = (SFM − SDM)/SDM (1)
SFM: shoot fresh matter, SDM: shoot dry matter.
RWC (%) = (SFM − SDM/SFM) × 100 (2)
TI = (SDM in salinity stress/SDM in control) × 100 (3)

Chemical analyses: Total nitrogen content of plant samples prepared for analysis was determined by "Mikrokjeldahl Method" after wet combustion with H_2SO_4 mixture (Anon., 2019). Macro and micro mineral substance contents (P, K, Ca, Mg, Fe, Cu, Zn, Mn) were determined by Perkin Elmer (Optima 2100) Model ICP-OES device after wet combustion with $HClO_4$ (Plank, 1992; Isaac *et al.,* 1998). K+/Na+ ratios of shoots were calculated. All samples were analyzed in duplicate.

Statistical analysis

The differences between analysis of variance and mean LSD multiple comparison tests were performed in the IBM SPSS STATISTICS 26 program.

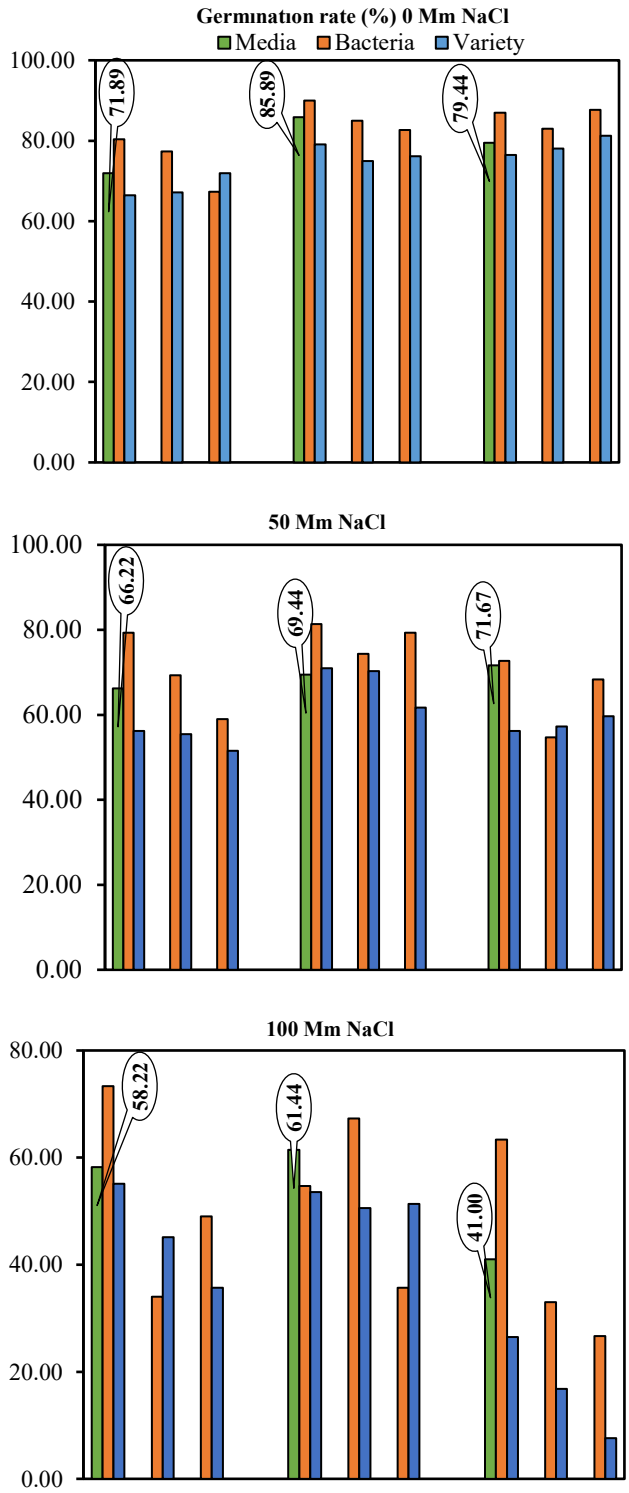


Fig. 1. GR % contents of medium, bacteria and species at different salt concentrations.

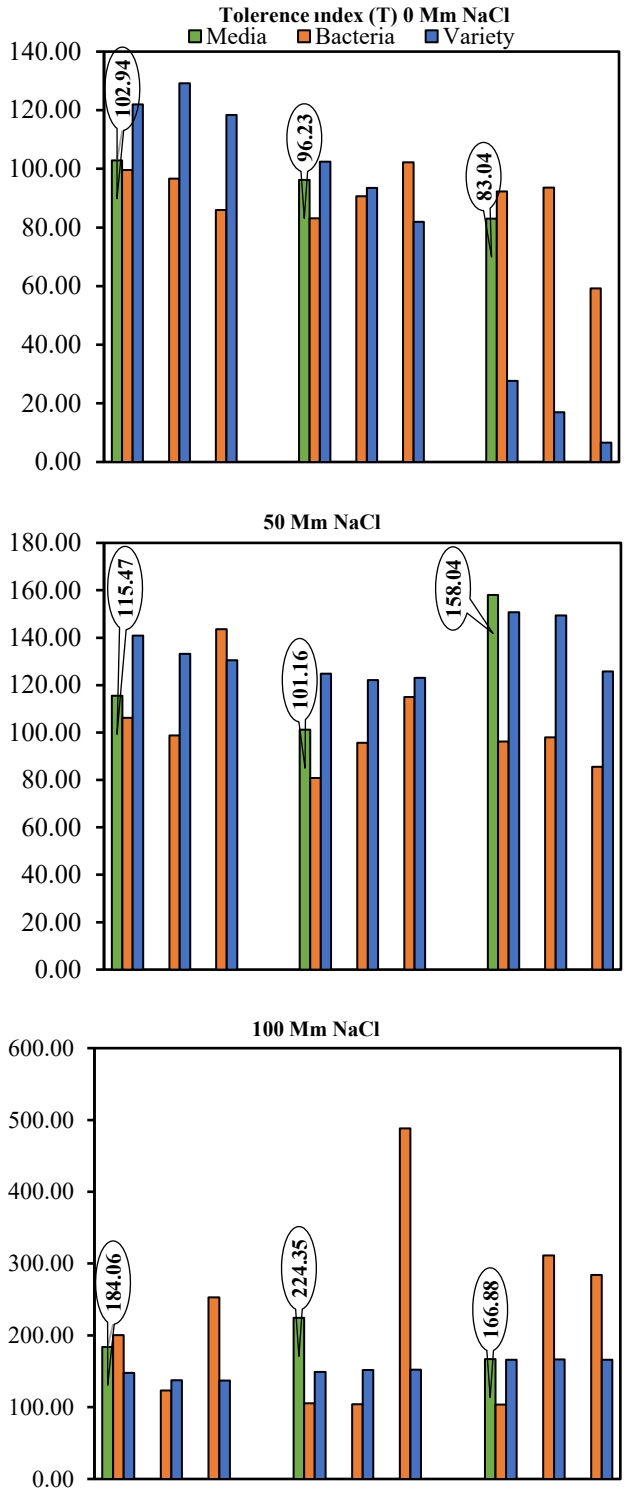


Fig. 2. Tolerance index contents of medium, bacteria and species at different salt concentrations.

Results

The effect of different salt concentrations of three bacterial treatments in three different nutrient solutions on germination, macro-mineral uptake and various physiological characteristics of sage cultivar seeds were investigated in this study. Analyses were performed at 0 mM, 50 mM and 100 mM salt concentrations. Germination rates ranged from 14% to 98% and the highest rate was determined in *S. nemorosa* cultivar with the *B. subtilis* + 0 mM concentration in an HS medium (Fig. 1). The average germination time of sage seeds was affected by salt concentrations and bacterial treatments minimized the negative effects of salt on the examined parameters. The fastest average germination time was observed in bacterial treatment in HS medium (3.46 days). The slowest germination was determined in an HP medium at 50 mM and 100 mM salt concentrations. The best result was given by *S. nemorosa* among the used sage cultivars.

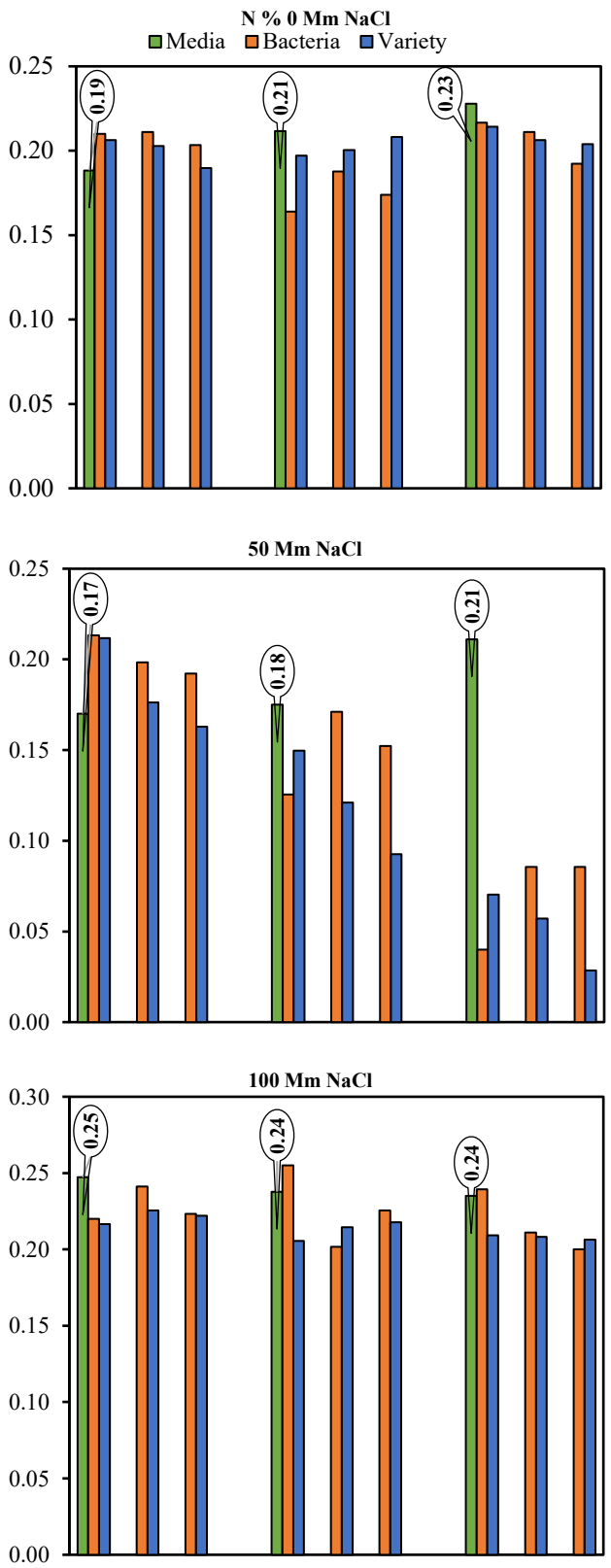


Fig. 3. N% contents of medium, bacteria and species at different salt concentrations.

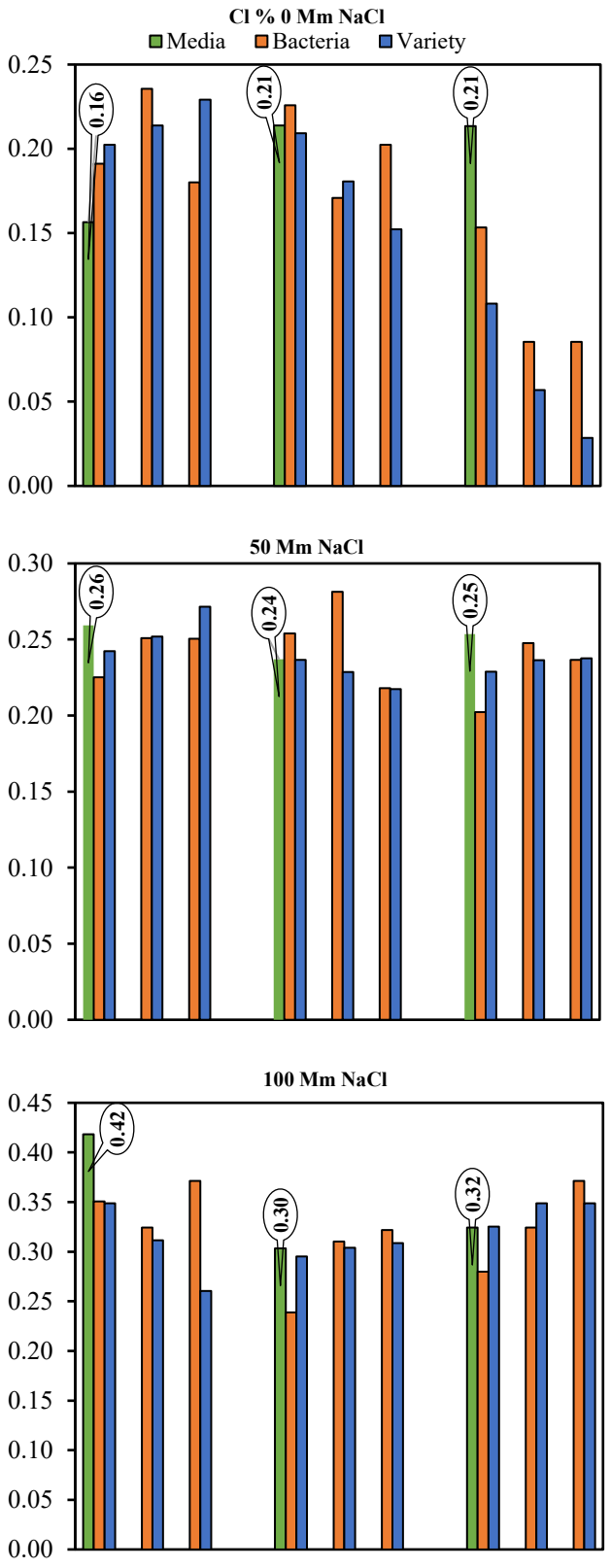


Fig. 4. Cl% contents of medium, bacteria and species at different salt concentrations.

Shoot length, root length and root fresh weight were negatively affected by increasing salt concentrations. Shoot length, root length and root fresh weight varied between 40.33-88.33 cm, 43.33-86.67 cm and 0.24-2.22 mg, respectively. The highest shoot length, root length and root fresh weight were determined in the *S. nemorosa* cultivar (54.45 cm, 45.03 cm and 0.79 mg) in the HS medium (64.76 cm, 57.27 cm and 0.91 mg) with a concentration of *B. subtilis* + 0 mM (65.93, 61.90, 1.00 mg). The highest dry weights were obtained in *S. nemorosa* (0.40 mg) in the HS medium (0.47 mg) and in *B. subtilis* + 0 mM (0.54 mg).

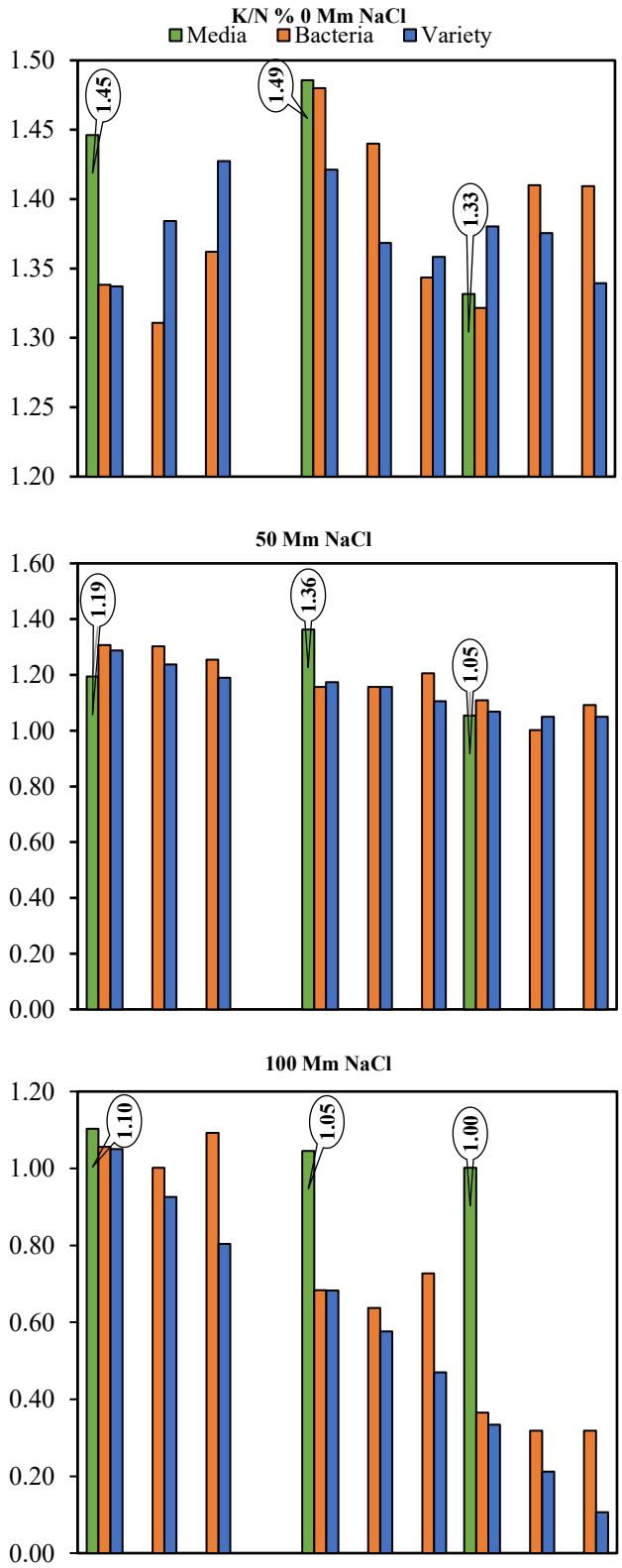


Fig. 5. K/Na% contents of medium, bacteria and species at different salt concentrations.

The fresh weights of shoots treated with bacteria in different media at different salt concentrations ranged from 1.69 to 13.73 mg. *S. nemorosa* (5.72 mg) had the highest values in HS medium (6.42 mg) and *B. subtilis* + 0 mM salt concentration (3.27 mg). The highest shoot fresh weight was determined in the medium with bacteria treatment without salt effect (0 mM), while the lowest shoot fresh weight was observed at the highest concentration of 100 mM. Shoot water retention (RCS) and relative water content (RWC) reacted negatively to increasing salt concentrations. Bacterial treatments minimized this effect. The highest water retention and relative water content among the shoots was obtained from *S. nemorosa* (4.47 mg, 49.51%) in the HS medium (2.70 mg, 54.48%) with a *B. subtilis* + 0 mM (0.65 mg, 26.08%) salt concentration. The tolerance index was highest at 0 mM (224.35) and lowest at 100 mM (83.04) (Fig. 2).

All examined mineral averages showed statistically significant changes in the cultivars, medium, bacteria and the interactions of these parameters at different salt concentrations ($p<0.01$). Nitrogen contents fluctuated with increasing salt concentrations. However, the highest nitrogen contents were determined with a concentration of 100 mM (0.23%) and the lowest with 50 mM (0.20%) (Fig. 3). Salt concentrations did not have a significant impact on the P and Ca contents of sage shoots.

K, Mg, Na and Cl concentrations ranged between 0.47-0.86%, 0.30-0.80%, 0.27-0.85% and 0.05-0.56%, respectively. The highest Mg contents were determined at 0 mM and 50 mM salt concentrations. As expected, Cl contents increased with salt concentrations. The lowest Cl concentration was determined with 0 mM (0.24%) and the highest with 100 mM (0.30%) (Fig. 4).

The highest K contents were determined with 0 mM, 50 mM and 100 mM (0.69%, 0.66% and 0.62%), respectively. The lowest K content was determined at the highest salt concentration (100 mM, 0.62%). The K+/Na+ ratio was highest with 0 mM (1.25) and lowest with 100 mM (1.21) (Fig. 5). The highest K, Mg, Cl and K/Na contents were obtained from *S. nemorosa* (0.54%, 0.43%, 0.26% and 1.03%) in the HS medium (0.65%, 0.48%, 0.27% and 1.24%), respectively.

Discussion

Benefitting from nature and natural vegetation in different areas and for different needs demonstrates the centuries-old human-plant interaction. The positive effect of grass juices and herbal nutrient solutions obtained from cereals on health is known. That is why research on different plant solutions has gained momentum. Extract obtained from the flowers of *Hypericum* and *Helichrysum* species were used as nutrient solution/medium in our study to examine the early seedling period of sage varieties under saline conditions. (Karaman *et al.,* 2021) examined the germination parameters of legume seeds such as oats, barley, beans, wheat and lentils in a study in which oat grass juice was applied at different doses such as 25%, 50%, 75% and 100%, and reported that the application of oat and grass juice doses had a negative impact on early seedling parameters development. Another study investigating the effect of wheatgrass juice on the seed physiology of cereals and forage crops, germination rate, germination index, root and stem lengths reported that wheatgrass juice application decreased germination index, germination rate, root and stem length (Akgün *et al.,* 2018). It was reported that extracts obtained from the leaves of sage and celery plants had a negative effect on the germination and seedling development of lentil seeds and inhibited root development (Stratu *et al.,* 2012).

Lamoureux & Koning (1998) investigated the effect of fennel, caraway, celery, dill, anise and coriander extracts on lettuce seed germination and reported that the plant extracts had a negative effect on lettuce germination. In our study, the highest values at which *Hypericum scabrum* was distributed were determined. This was because some bacteria are able to break down and neutralize toxic allelochemicals. Microbial communities and rhizobacteria in the soil utilize these toxins as a source of carbon and energy, thus reducing phytotoxicity and detoxifying the soil. Specific soil bacteria, such as *Bacillus subtilis* and *Pseudomonas putida*, are highly effective in degrading allelochemicals and promoting plant growth. In research on controlling tobacco bacterial wilt through microbial degradation of phenolic allelochemicals in tobacco, eight *Bacillus* strains, one *Brucella* strain, one *Enterobacter* strain, and one *Stenotrophomonas* strain, capable of degrading PAs in culture solutions and soil, were found to significantly reduce isolated cases of tobacco bacterial wilt by 61.44% (Xiaohan *et al.,* 2022). A study in which the effects of bacterial strains on the agronomic properties of fenugreek seeds were examined with NaCl salt doses and bacterial strains determined that bacterial treatments were statistically significant in the parameters under salt stress, gave better results with 30 mM salt + bacteria treatments and the bacterial strains had a positive effect on all parameters examined under salt stress (Kadıoğlu, 2024). The results of our study investigating the effect of bacteria application in different nutrient solutions and salt concentrations on sage varieties parallel the results of other studies. Studies report that characteristics such as the root, stem and shoot growth of plants exposed to salt stress are reduced, and in parallel, characteristics such as yield are also reduced (Köşkeroğlu, 2006). Some morphological changes caused by salinity stress include reduced root and shoot length along with restricted rooting (Evers *et al.,* 1997). Salt concentrations have a negative impact on the germination and early growth of different plant species. Biofertilizers are used to reduce soil salinity and drought and increase germination. PGPR applications affect plant metabolism and increase plant growth, nitrogen fixation, germination rate, plant yield, root development as well as the sensitivity of plants to stress conditions. The study in which bacteria application was made on flax at different salt doses determined that germination parameters decreased as salt doses increased while bacteria application minimized the negative effect of salt application (Kadıoğlu, 2022). PGPRs applied to seed or soil affect plant growth by increasing root development (root number, length and weight), nutrient uptake, nitrogen fixation, inorganic phosphate solubility and uptake (Çakmakcı, 2005a). It has been reported that the activities of microorganisms on plant growth are different, *Azotobacter* and *Azospirillum* species in particular are very effective in increasing production while PGPR application to seeds or soil increases root growth, plant nutrient uptake, nitrogen fixation, inorganic phosphate solubility and uptake (Çakmakcı, 2005; Çakmakcı, 2005a).

Plants need macro and micro nutrients to complete their life cycle. Nitrogen (N), phosphorus (P), sulfur (S), potassium (K), calcium (Ca) and magnesium (Mg) constitute macro nutrients (de Bang *et al.,* 2021). These nutrients have various functions as ions or components in plant physiology (Kathpalia *et al.,* 2016). Soil salinity generally suppresses plant growth and reproduction by accumulating Na+ and Cl- ions and causes nutrient deficiencies (Arzani *et al.,* 2016). Under salt stress, high Na+ in plant tissues usually prevents the absorption of other essential nutrients such as K+, Ca^{2}+ and Mg^{2}+, leading to nutrient deficiencies (Assaha *et al.,* 2017). Mineral deficiency inhibits plant growth by limiting biosynthesis Soil salinity commonly suppresses plant growth and reproduction through an initial phase of osmotic stress, followed by ionic toxicity resulting from the accumulation of Na+ and Cl− ions within the cell cytosol. This ultimately leads to oxidative stress and nutritional deprivation. Oxidative stress perturbs the equilibrium between the production of reactive oxygen species and their scavenging, resulting in damage to cell membranes and leakage of ions (Grusak, 2001). The intake of high concentrations of salt (NaCl) competes with the uptake of other nutrient ions, especially potassium, leading to potassium deficiency. Increased NaCl treatment leads to an increase in Na+ and Cl- and a decrease in Mg^{2}+, K+ and Ca^{2}+ levels in various plants (Khan, 2001). Salt stress and ionic stress directly harms plants and disrupts metabolic processes (Akhtar *et al.,* 2015). Salinity enhances the content of Na+, Ca^{2}+, and Cl− in *Vicia faba* L., and the ratio of K+/Na+ decreases. A positive correlation is observed between Na+ and Cl− concentrations, while a negative correlation is evident between Na+ and K+ concentrations in both roots and leaves. The concentration of Mg^{2}+ remains unaltered in both leaves and roots, irrespective of changes in the Na+ concentration. Similarly, the concentration of Ca^{2}+ exhibits no variation with the Na^{2}+ concentration in leaves; however, it demonstrates an inverse relationship in the roots (Ferreira *et al.,* 2001). The results of the study show that salt concentrations which are important in early seedling formation and development in different types of plants grown in different environments have a negative impact when the concentrations increase whereas bacterial applications tolerate this effect. Germination and early growth stages are the most sensitive stages affected by salinity (Mbarki *et al.,* 2020). Although germination alone is not sufficient to identify genotypes resistant to salt stress, cultivars that tolerate salinity during germination usually retain their resistance at later growth stages.

Conclusion

Drought-tolerant Salvia L. grows on rocky slopes, sandy ridges, meadows, oak and pine forests, fields and roadsides. It is moderately tolerant to halomorphic soils. Results obtained from a controlled environment show that increasing the salt concentration in nutrient extracts negatively affects the early seedling development of Salvia L. It was determined that germination and tolerance index decreased at 50 mM and 100 mM salt concentrations. Increasing salt concentrations negatively affected the examined seedling traits. In parallel with the increase in salt concentrations, there was an increase in Na and Cl contents. The results of the study, in which bacterial applications minimized this negative effect, have important implications for further studies to be carried out in field conditions. *Hypericum scabrum* nutrient extracts containing *B. subtilis* + 0 mM salt concentrations can be recommended as a growth medium for early plant development of *S. nemorosa* L.

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Author's Contribution: Banu Kadıoğlu: Methodology, Formal analysis, Investigation, Writing Original Draft, Conceptualization, Resources, Writing Review and Editing, Supervision, Softwares, Formal analysis, Writing-Review and Editing.

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