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The effects of *Glomus intraradices* on seedling growth parameters of Sage (*Salvia officinalis* L.) under salinity stress conditions

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Abstract Salinity is a significant abiotic stress that adversely affects plant growth and development, threatening global agricultural productivity, particularly in arid and semi-arid regions. Results indicated that increasing salt concentrations significantly inhibited seedling growth in all studied parameters, with control plants (0 mM) exhibiting the highest growth values. The tallest plants measured 23.16 cm in the control treatment, while those exposed to 200 mM salt reached only 13.12 cm. Although AMF applications did not show significant effects on plant height, they positively influenced growth parameters in salt-free conditions. Statistical analysis revealed significant interactions between salt levels and AMF on root fresh and dry weights, with the highest values recorded in the control group. Furthermore, AMF applications generally promoted growth, particularly in non-saline conditions, corroborating findings from previous research highlighting mycorrhizae's role in enhancing plant stress tolerance. In conclusion, the research findings emphasize the detrimental impact of salinity on plant development and the potential of mycorrhizal inoculation as a strategy to mitigate these effects. These findings contribute to understanding the beneficial role of AMF in improving salt tolerance and promoting sustainable agricultural practices in saline-prone environments.

Keywords: Salinity, Arbuscular mycorrhizal fungi, Plant growth, Seedling development

Introduction

Plants have been utilized by humans for nutrition, shelter, heating, and medicinal purposes throughout history. Approximately 250 plant species were used for therapeutic purposes around 5000 BC (Göktaş and Gıdık, 2019). Turkey boasts a rich flora, hosting approximately 11,000 plant taxa, with around 500 employed in alternative medicine (Türkan *et al.,* 2006). As living

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standards rise globally, the consumption of medicinal and aromatic plants has increased, leading to their extensive use in medicine, perfumery, cosmetics, toothpaste, soap, and the sugar industry (Baytop, 1999). This trend has been further accelerated by growing concerns regarding the adverse effects of synthetic drugs on human health (Bayraktar *et al.,* 2017). Turkey is a leading country in the cultivation of medicinal and aromatic plants, including sage, fenugreek, basil, coriander, fennel, poppy, anise, cumin, and laurel (Beyzi, 2011). Sage (*Salvia officinalis* L.) described by Carl Linnaeus in 1753, is native to the Western Balkans, particularly Dalmatia and Macedonia, as well as southern and central Europe (Güner and Aslan, 2012; O'Leary and Moroni, 2016). Research has demonstrated various therapeutic properties of this species, including antioxidant, antimicrobial, anti-stress, antidepressant, anti-cancer, anti-inflammatory, and antidiabetic effects (Miraj and Kiani, 2016). Understanding the effects of environmental factors on the growth and quality of medicinal sage is essential for optimizing cultivation practices. Plants often face unfavorable conditions that negatively impact their growth and limit productivity, categorized as stress factors, which can be biotic (e.g., pests, pathogens) or abiotic (e.g., drought, salinity, nutrient deficiency, temperature extremes) (Büyük *et al.,* 2012). Salinity, a significant abiotic stress, adversely affects plant development and agricultural productivity. It threatens 20% of agricultural land globally, a figure expected to rise to 50% in the next 20 years if no preventive measures are implemented (Hasanuzzaman *et al.,* 2013). Salinity limits nutrient uptake and disrupts ion balance (Ashraf and Bhatti, 2000), leading to physiological drought and reduced photosynthesis due to stomatal closure (Parida and Das, 2005). This stress also increases the formation of reactive oxygen species, resulting in lipid peroxidation and subsequent cellular damage (Tambussi *et al.,* 2000; Yılmaz *et al.,* 2011). To cope with such stresses, plants activate various adaptation mechanisms involving anatomical, morphological, physiological, and biochemical changes (Öztürk, 2015). Strategies to enhance salt resistance include increasing water uptake (Sheng *et al.,* 2008; Aroca *et al.,* 2007), applying calcium and potassium to correct nutrient imbalances (Yılmaz *et al.,* 2011; Amjad *et al.,* 2016), and enhancing antioxidant enzyme activity (Zhu *et al.,* 2004). Arbuscular mycorrhizal fungi (AMF) represent a promising microbial application to combat salinity stress. These fungi establish symbiotic relationships with plant roots, enhancing nutrient and water uptake while improving plant tolerance to abiotic stressors (Ruiz-Lozano, 1996; Carvalho *et al.,* 2004). AMF can mitigate the negative impacts of salinity by increasing leaf relative water content, chlorophyll, and carotenoid levels, as well as reducing malondialdehyde (MDA) formation, a marker of lipid peroxidation (Yıldız *et al.,* 2010; Çekiç *et* *al.,* 2012). This study aimed s to assess the effects of AMF (*Glomus intraradices*) applications on the growth and development of sage seedling under salt stress conditions.

Materials and methods

The research project was conducted in a fully controlled growth chamber at the Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University, in 2021. Seeds of *Salvia officinalis* were sourced from the Medicinal and Aromatic Plants Garden of the Van Yuzuncu Yil University. The AMF isolate, *Glomus intraradices*, was obtained from the culture collection of the Plant Protection Department at the same institution. The experiment was designed as a factorial experiment with two factors including salt stress concentration and mycorrhizal fungus application, using a completely randomized design with four replicates. The AMF inoculum contained 455 spores/g of soil. Inoculum was added to pots at a rate of 20% during seed sowing. Seeds were sterilized by soaking in 95% ethanol for 5 minutes, followed by treatment with 3% hydrogen peroxide for 5 minutes and thorough washing with distilled water (Öğütcü *et al.,* 2010). Salt stress was applied using NaCl solutions at varying concentrations: S_0 (control), S_1 (50 mM), S_2 (100 mM), S_3 (150 mM), and S_4 (200 mM). Also, in this study, the treatments with mycorrhizae and without mycorrhizae were labeled as M_1 and M_0, respectively. Five sage seeds were planted in 500 cc plastic cups filled with a mixture of 40% sand, 40% soil, and 20% mycorrhizal inoculum. Following seed emergence, pots were maintained in a growth chamber with a 16/8 hours light/dark photoperiod, a light intensity of 250±10 μmol/m²/s, at 25°C, and 65% RH. Irrigation was conducted with Hoagland nutrient solution when necessary, with pH adjusted to 5.5-6.5 (Taiz and Zeiger, 2002). Upon reaching maturity (30±5 days), salt stress treatments were initiated. Control plants received pure water. When physiological stress indicators became pronounced (45±5 days), plants were harvested using sterile scissors. Measurements of root and stem lengths were recorded, and fresh weights were noted. Dry weights were determined after drying root and stem samples at 105°C for 24 hours (Kacar and Inal, 2008). The recorded data were subjected to variance analysis and the means were compared using the least significant difference (LSD) method at $P<0.05$. All statistical analyses were carried out using the COSTAT software (Version 6.3).

Results

In this study, the effects of salt doses and the interaction between salt and AMF on plant height were statistically significant differed at the 1% level, while the effects of AMF applications alone were found to be insignificant. Plant height decreased with increasing salt doses; the tallest plants (23.16 cm) were recorded in the control treatment, while the shortest plants (13.12 cm) were observed at the 200 mM salt dose (S_4). No statistically significant difference was detected between the 150 mM (S_3) and 200 mM (S_4) salt doses. For AMF treatments, plant height values ranged from 17.68 cm to 17.93 cm, with the highest plant height of 24.91 cm recorded in the $S_0 \times M_1$ interaction. The effect of salt doses and their interaction with AMF on stem fresh weight were found to be significant at 1% and 5% levels, respectively. However, the effect of AMF application was insignificant. The highest stem fresh weight (5.13 g) was obtained from the control group, whereas the lowest (3.27 g) was observed at the 200 mM salt dose (S_4). Stem fresh weights for AMF treatments ranged from 3.94 g to 4.08 g, with the highest weight (5.41 g) recorded for the $S_0 \times M_0$ application. The effects of salt doses and AMF on stem dry weight were significant at the 1% level, while, the effects of their interaction on stem dry weight were significant at 5 % level. The highest stem dry weight (2.34 g) was noted for the 50mM salt dose (S_1), which was statistically similar to the control, while the lowest value (1.63 g) was recorded at the 200 mM salt dose (S_4). Among AMF treatments, the highest value (2.24 g) was observed for M_1 applications, while the lowest (1.76 g) was from the control group. The highest value in the Salt ×AMF interaction was also recorded in $S_3 \times M_0$ treatment (2.56 g). Root fresh weight was significantly affected by salt doses and AMF treatments at the 1% level, while, the interaction between salt doses and AMF was also significant at the 5% level. The control group exhibited the highest root fresh weight (1.71 g), whereas the lowest was (1.19 g) observed at the 200 mM salt dose (S_4). Among AMF applications, M_1 yielded the highest fresh weight (1.53 g), while M_0 had the lowest (1.24 g). The highest root fresh weight from the interaction was recorded in the $S_2 \times M_1$ treatment (1.91 g). Root dry weight was significantly affected by salt doses and AMF applications at the 1% level. Also, the Salt doses and AMF interaction was found to be significant at 5% level. The highest root dry weight (0.50 g) was recorded in the control group, while the lowest (0.30 g) was from the 200 mM salt dose (S_4). In terms of AMF applications, the highest dry root weight (0.40 g) was obtained from M_1 applications, with the lowest value (0.37 g) from the control group. The effects of salt doses and AMF applications on the root length of *Salvia officinalis* were significant at the 1% level, while the interaction of these two factors was also significant at the 5% level. The control treatment yielded the longest roots (22.95 cm), which were statistically similar to those from the 50mM (S_1) and 100 mM (S_2) salt doses. The shortest roots were recorded at the 200 mM (S_4) salt dose (17.75 cm). For AMF applications, the longest roots were achieved with M_1 treatments (22.15 cm), while the control had the shortest roots (19.97 cm). In the interaction treatment, the longest roots were recorded for the $S_2 \times M_1$ and $S_3 \times M_1$ treatments (23.16 cm).

Table 1. Effect of mycorrhiza applications on seedling growth parameters of sage plant grown under salt stress conditions

Treatments		Plant Height (cm)	Stem Fresh Weigth (g)	Stem Dry Weigth (g)	Root Fresh Weigth (g)	Root Dry Weigth (g)	Root Length (cm)
Salt doses	**AMF**						
Control (S_0)	Control (M_0)	21.41bc	5.41a	2.22bc	1.52bc	0.49ab	22.75ab
	AMF (M_1)	24.91a	4.85ab	2.13c	1.46bc	0.51a	21.5ab
S_0 Mean		**23.16a**	**5.13a**	**2.32a**	**1.71a**	**0.50a**	**22.95a**
S_1 (50 mM)	Control (M_0)	22.08b	4.39bc	1.73d	1.12ef	0.45c	20.95bc
	AMF (M_1)	18.75d	4.54b	1.43e	1.09f	0.48b	18.33d
S_1 Mean		**20.41b**	**4.47b**	**2.34a**	**1.53b**	**0.47b**	**22.16a**
S_2 (100 mM)	Control (M_0)	19.25cd	3.75cde	1.29e	1.01f	0.33fg	16.33e
	AMF (M_1)	17.0e	3.75de	2.41ab	1.91a	0.35de	23.16a
S_2 Mean		**18.12c**	**3.75c**	**1.95b**	**1.35c**	**0.34c**	**22.05a**
S_3 (150 mM)	Control (M_0)	13.83fg	3.09f	2.56a	1.60b	0.32g	22.83ab
	AMF (M_1)	14.58f	3.80cd	2.17c	1.59b	0.36d	23.16a
S_3 (Mean)		**14.20d**	**3.44cd**	**1.76c**	**1.19d**	**0.32d**	**20.37b**
S_4 (200 mM)	Control (M_0)	13.08g	3.08f	2.09c	1.29cd	0.27h	22.41ab
	AMF (M_1)	13.16g	3.46ef	1.96c	1.27de	0.34ef	19.16cd
S_4 Mean		**13.12d**	**3.27d**	**1.63c**	**1.14d**	**0.30e**	**17.75c**
AMF	Control (M_0)	17.93	3.94	1.76 b	1.24 b	0.37 b	19.97 b
Application	AMF (M_1)	17.68	4.08	2.24 a	1.53 a	0.40 a	22.15 a
Salt Dose (S)		**	**	**	**	**	**
AMF		ns	ns	**	**	**	**
S ×AMF		**	*	*	*	*	*
CV (%)		5.55	8.78	7.37	8.02	3.37	6.18
LSD (0.05)		2.95	1.02	0.43	0.32	0.039	3.86

*: Significant at P<0.05, **: Significant at P<0.01 and ns: Non significant

Discussion

Salinity is one of the most significant abiotic stress factors affecting plant growth and development, severely limiting yield and quality. This global issue, particularly pronounced in arid and semi-arid regions, currently threatens approximately 20% of agricultural land. Without intervention, this figure is projected to reach 50% within the next two decades (Hasanuzzaman *et al.,* 2013). In Türkşye, it is estimated that around 2 million hectares are affected by salinity (Anonymous, 2018). Due to their practicality and long-term effectiveness in enhancing salt tolerance, microbial applications have become widely adopted worldwide in the management of salinity. Among these, mycorrhizal fungi present a particularly effective alternative. Mycorrhizal fungi form symbiotic relationships with plant roots, allowing plants to access carbohydrates that they cannot synthesize. In return, the fungi extend the root system's area of influence, facilitating improved water and nutrient uptake (Smith and Read, 1997). The benefits of mycorrhizae extend beyond enhanced nutrient absorption; they also bolster plant tolerance to both abiotic and biotic stress conditions (Ruiz-Lozano, 1996; Carvalho *et al.,* 2004). In this study, we observed that growth was inhibited with increasing salt doses in all seedling growth parameters, with the best growth metrics recorded in control groups without salt application (S0). Salt stress negatively impacts plant growth and development by reducing nutrient uptake and disrupting ion balance (Ashraf and Bhatti, 2000). It increases soil osmotic pressure, leading to physiological drought (Parida and Das, 2005), and reduces photosynthesis through decreased CO_2 uptake as a result of stomatal closure. Additionally, salt stress promotes the formation of reactive oxygen species, causing membrane damage and lipid peroxidation (Tambussi *et al.,* 2000; Kalefetoğlu and Ekmekçi, 2005). Consistent with our findings, Altunlu (2020) reported that salt applications in pepper significantly decreased seedling length, stem diameter, and both fresh and dry weights of stems and roots. Specifically, seedling length decreased by 30.7%, stem diameter by 38.5%, fresh weight by 36.5%, dry weight by 40.3%, root fresh weight by 43.16%, and root dry weight by 59.7% compared to the control group. In the absence of salt (S_0), mycorrhiza applications positively influenced plant development parameters. Our observations suggest that mycorrhiza applications generally promote growth and development. Previous studies, such as Sannazzaro *et al.* (2007), found that *Glomus intraradices* mitigated the negative effects of saline conditions (200 mM NaCl) on *Lotus* flower cultivation. Similarly, Hajiboland *et al.* (2010) investigated the effects of *Glomus intraradices* inoculation on tomato plants, assessing two varieties (one tolerant and one sensitive) under three different salt levels (0.63, 5, and 10 dS m^{-1}). They concluded that mycorrhiza application positively influenced plant development in both varieties, with greater effectiveness observed in the salt-resistant variety. Recent studies have increasingly documented that mycorrhiza enhances plant resistance to salinity, an important abiotic stress factor. Its application as a bioregulator in saline soils alleviates the negative effects of salt on plant development and improves stress resilience (Al-Karaki, 2000; Ruiz-Lozano, 1996; Tain *et al.,* 2004; Kaya *et al.,* 2009). For instance, Shokri and Maadi (2009) demonstrated that mycorrhiza application significantly improved mineral nutrition and yield in *Trifolium alexandrinum* exposed to varying salt concentrations (2.2, 5.0, and 10 dS m^{-1}), resulting in a total dry weight that was 5.29 times higher than that of control plants. Furthermore, Asghari (2008) investigated the effects of arbuscular mycorrhiza (*Glomus intraradices*) on *Trifolium subterraneum* and *Festuca arundinaceae* in saline areas, noting enhanced plant growth and increased root volume in the salt-sensitive *Trifolium subterraneum*. These findings align closely with those of our study, underscoring the potential of mycorrhiza as a beneficial intervention for mitigating the impacts of salinity on plant growth.

This study demonstrated that increasing salinity levels negatively impact the growth and development of sage seedlings, as evidenced by significant reductions in plant height, fresh and dry biomass, and root length. Higher salt concentrations, particularly at 200 mM NaCl, led to substantial inhibition of seedling growth. However, the application of arbuscular mycorrhizal fungus mitigated the adverse effects of salt stress. AMF treatments improved plant growth parameters under saline conditions, with notable improvements in root and shoot biomass, as well as root length. The symbiotic relationship between AMF and plants enhanced the uptake of water and nutrients, improving the plant's tolerance to salinity and supporting its growth. These findings are consistent with previous studies highlighting the role of AMF in enhancing plant resistance to abiotic stress factors such as salinity. The interaction between salt and AMF treatments was particularly effective in alleviating the detrimental effects of moderate salt stress (up to 150 mM NaCl), suggesting that AMF inoculation can be a valuable biological tool for improving crop productivity in saline soils. The results underscore the potential of AMF as a sustainable and eco-friendly strategy to counteract the negative impacts of salinity in agriculture. Future research could focus on the long-term effects of AMF under varying environmental conditions and explore its use in different crop species to further validate its role in enhancing salt tolerance.

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