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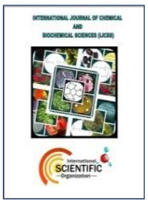

Evaluation of the salinity tolerance of *Lavandula dentata* grown in association with the halophyte plant *Atriplex prostrata* by measuring morphological and biochemical parameters

Issam El-Khadir[1], Saida Rezki[1], Safae Ktaoui[1], Meryem Doubi[1], Halima Ameziane[1], Ahmed Chriqui[1], Yassine Mouniane[1*], Driss Hmouni[1]
[1]Laboratory of Natural Resources and Sustainable Development, Faculty of Sciences, Ibn Tofail University – KENITRA - University Campus, Kenitra 14000, Morocco.

Abstract

This study examines the impact of salt stress on *Lavandula dentata* (a glycophyte plant) when grown alone and in association with *Atriplex prostrata* (a halophyte plant), by assessing various morphological, physiological and biochemical parameters. In experiments without *Atriplex prostrata*, the number of *Lavandula dentata* leaves decreased progressively with increasing NaCl concentration, reaching a drastic reduction at 9 g/l. On the other hand, with the association of the halophyte plant (*Atriplex prostrata*), even under high NaCl concentrations, the growth of *Lavandula dentata* seems to be better preserved. Morphological parameters also reveal significant differences: the height of the aerial part decreases more markedly without the association of *Atriplex prostrata*, whereas the presence of this halophyte plant attenuates these negative effects. Furthermore, the mortality rate was significantly higher in experiments without association (62.5% for the 9 g/l concentration), whereas in association with the halophyte plant the mortality rate was 37.5% for the 9 g/l concentration, underlining the importance of association with *Atriplex prostrata* for maintaining plant viability under salt stress. Biochemical analyses show variations in total soluble sugar content, with *Lavandula dentata* appearing to secrete an increased amount of total soluble sugars to cope with stress. However, when combined with *Atriplex prostrata*, this sugar secretion is reduced. In conclusion, these results highlight the beneficial role of association with a halophyte plant in mitigating the adverse effects of salt stress on *Lavandula dentata*, underscoring the potential importance of this strategy for improving crop tolerance to salt stress.

Keywords: Salinity, halophyte plant, glycophyte plant, coculture, *Lavandula dentata*, *Atriplex prostrata*.

Full length article **Corresponding Author*, e-mail: yassine.mouniane@uit.ac.ma

1. Introduction

Soil salinity is a major abiotic stress, with negative impacts on plant growth and development. It also disrupts ecosystem services and causes substantial reductions in agricultural yields [1-2]. Worldwide, around one billion hectares of land are affected to varying degrees by soil salinity. The global area affected by salt could increase significantly over the coming decades, due to the continued use of poor-quality water for irrigation and the consequences of climate change [3-5]. According to recent statistics, more than 50% of arable land will be affected by saline-alkaline soils by 2050. According to recent statistics, more than 50% of arable land will be affected by saline-alkaline soils by 2050 [6]. It is therefore essential to create practical defenses against salt stress for plants. Some plants, known as halophytes, can survive and thrive even in highly saline environments. They develop different strategies to counter salt stress at anatomical, biochemical and molecular levels. As a rule, these adaptations are the result of changes in gene and protein expression compared with non-salt-adapted plants (glycophytes) [7]. Plants of the genus Atriplex (Amaranthaceae) are considered halophytes due to their remarkable ability to thrive in saline environments. These annual plants grow on weathered or nitrified soils, as well as on saline sites in Europe, North Africa, Southwest Asia and North America. Atriplex species are valued for their role in restoring eroded areas, providing fodder for livestock and improving the productivity of marginal lands. *Atriplex prostrata*, in particular, is an annual species found in salt marshes, capable of adapting to both saline and non-saline soils. These plants use a variety of mechanisms to cope with salt stress, including the accumulation of inorganic and organic solutes such as proline, glycine betaine and soluble sugars, which help maintain a low osmotic potential and enable water uptake [8-9]. *Lavandula dentata* L. is a plant in the Lamiaceae family, found in the Mediterranean region, southwest Asia and the Arabian Peninsula [10]. It belongs to the genus Lavandula, which comprises some thirty-two species [11]. This medium-sized shrub has narrow, toothed green to grey-green leaves [12]. In Morocco, wild populations can be found along the northern Mediterranean coast as far as the Atlantic coast north of Agadir [13]. The *L. dentata* plant is renowned for its wealth of phytochemical compounds and medicinal properties, which encompass a wide range of beneficial effects such as antibacterial, antifungal, antioxidant, antidiabetic, anticancer, anti-inflammatory activities, and many others. Even at low concentrations, essential oils (EO) and organic extracts of *L. dentata* demonstrate a specific capacity to inhibit enzymes, membrane or intracellular receptors, proteins or signalling pathways involved in the genesis of these various pathologies [14]. The aim of this study is to assess the salinity tolerance of *Lavandula dentata* when grown alone and in association with the halophyte plant *Atriplex prostrata*. This evaluation will be carried out by measuring, comparing morphological, and biochemical parameters of the L. dentata plant under salt stress, in order to gain a better understanding of lavender's response mechanisms to this environmental constraint and the potential effect of co-cultivation with a halophyte plant on its ability to tolerate salt.

2. Materials and Methods

2.1. *Plant Material*

The plant material used in this study consists of two species belonging to different botanical families, *Lavandula dentata* (Figure 1. a), identified as a glycophyte plant, and *Atriplex prostrata* (Figure 1. b), characterized as a halophyte plant. It is essential to emphasize that *Atriplex prostrata* was collected in the Ouezzane region, specifically from the province of Asjen, located near Oued Maleh in Morocco (Figure 2). This location is of crucial importance, as Oued Maleh is renowned for its saline soils and demanding environment, providing an ideal natural habitat for halophytic plants. The provenance of the halophytic plant thus provides a significant context for understanding its specific adaptations to the harsh environmental conditions of this Moroccan region.

2.2. Experimental site and growing conditions

The experiment was carried out in plastic pots with a volume of 2.5 L at an experimental site located in the greenhouse of the Biology Department, Faculty of Science, Ibn Tofail University, Kenitra. A standard substrate, made from 2/3 soil (red sandy clay), 1/3 compost (dead leaves of maritime pine), and 1/3 peat, was selected for both studies to assess the impact of salinity on cultivated plants. As part of the lavender domestication process, 5-8 cm cuttings were prepared from healthy mother plants located in the greenhouse. These cuttings, comprising woody, semi-woody and herbaceous types, were grown in honeycomb trays, using standard substrate. The plants were transplanted in spring, on April 25, 2022, during the dormant period. Plants were moved to individual pots, all filled with the standard substrate [15]. This methodology ensures consistency in substrate conditions, enabling meaningful comparison of plant responses to salinity throughout the study.

2.3. Salt stress studies

Salt stress studies comprise two distinct components. In the first study, lavender (*Lavandula dentata*) plants were grown in isolation in plastic pots, subjected to different treatments with varying concentrations of NaCl 0 mmol/L (0 g/L), 51 mmol/L (3 g/L), 103 mmol/L (6 g/L), 154 mmol/L (9 g/L). In parallel, the second study involved growing lavender plants in association with *Atriplex prostrata*, in plastic pots. The same NaCl treatments were applied in this combined study. After a period of three months, the plants were subjected to a salt treatment. This procedure involved irrigating the pots with a solution of sodium chloride (NaCl). Two different irrigation frequencies were used: one every 36 hours in June, and another every 48 hours in September. Each irrigation was carried out with a standard 100 ml of NaCl solution. These treatments were designed to assess plant response to salt stress conditions, providing a detailed insight into the impact of salinity on plant physiology and morphology, both in isolated culture and in association with halophytes.

2.4. Measured parameters

Several morpho-physiological and biochemical parameters were meticulously assessed during the study, including leaf number and stem length, providing a detailed perspective on plant growth and development. The survival time of the plants was also recorded, providing crucial information on their adaptation to the experimental conditions. In addition, measurements of aerial and root length, as well as root volume, provided insight into the distribution and vigour of the plants' root systems. At the same time, fresh and dry matter analyses of leaves, stems and roots were carried out, enabling weight variations to be assessed and possible metabolic changes to be identified. The water content of various plant parts, including leaves, stems and roots, was also measured, providing valuable insights into hydration and plant adaptation to salt stress conditions. The biochemical parameters examined in this study include total soluble sugar content, measured by carbohydrate assay. This assessment was carried out using the phenol method of Dubois [16]. Analysis of total soluble carbohydrates provides detailed information on sugar metabolism in the plants studied, enabling an understanding of biochemical variations in response to salt stress conditions.

2.5. Statistical analysis

The results obtained were subjected to a descriptive statistical analysis, an analysis of variance (ANOVA), using IBM SPSS Statistics version 23 software. This comprehensive statistical approach enabled a detailed examination of the dataset to identify significant differences between the two studies and NaCl concentrations. Post-hoc tests were performed to determine which pairs of groups showed significant variations.

3. Results and discussion

3.1. Effect of salinity on leaf number

In experiments without halophyte association (Figure 3. a), examining *Lavandula dentata* cultivation alone, a progressive decrease in leaf number is observed as salt concentration increases. At 0 g/l NaCl (control), the number of leaves increases steadily over the days, but at higher NaCl concentrations, the number of leaves decreases rapidly. Indeed, at 9 g/l NaCl, the number of leaves dropped from 101 on day one to 0 on day 16. On the other hand, in the experiment in association with the halophyte plant (Figure 3. b), where *Lavandula dentata* is associated with *Atriplex prostrata*, the negative effects of salt appear to be partially attenuated. Even under high NaCl concentrations, *Lavandula dentata* growth seems to be better preserved when associated with *Atriplex prostrata*. At the NaCl concentration of 9 g/l, the number of leaves fell from 214 on day one to 20 on day 16, which is less pronounced than in the study without the halophyte plant (*Atriplex prostrata*).

3.2. Effect of salt stress on morphological parameters

3.2.1. Variation in height of aerial part

The effect of NaCl concentration on the height of the aerial part of *Lavandula dentata* was significantly demonstrated (Table 1). The results indicate a decrease in aboveground plant height with increasing NaCl concentration, suggesting that higher NaCl concentrations generally lead to a reduction in plant height. In experiments without *Atriplex prostrata*, a significant decrease in length was observed at different NaCl concentrations. At 3 g/l NaCl, the decrease was 27%, while at 6 g/l and 9 g/l, the decreases were 22.16% and 12.46% respectively. On the other hand, in experiments with *Atriplex prostrata*, the percentage reduction in length was less pronounced. At a concentration of 3 g/l NaCl, the decrease was 24.46%, while at 6 g/l and 9 g/l, the decreases were 14% and 7% respectively.

3.2.2. Variation in root length

The length of the root section of *Lavandula dentata* is significantly affected by NaCl-induced stress, with variations observed depending on whether the plant is grown with or without the *Atriplex prostrata* association (Table 1). Overall, a trend towards a decrease in the length of the root part of *Lavandula dentata* in response to higher NaCl concentrations is observed, although this decrease appears to be less marked when plants are grown in association with *Atriplex prostrata*.

3.2.3. Root volume variation

The analysis indicates that although higher concentrations of NaCl tend to reduce *Lavandula dentata* root volume, this reduction appears to be less significant (Table 1) when *Lavandula dentata* is grown in association with *Atriplex prostrata*. This suggests that the presence of *Atriplex prostrata* may partially mitigate the negative effects of NaCl on *Lavandula dentata* root volume.

3.2.4. Variation in mortality rate

Mortality rates were significantly higher in experiments without *Atriplex prostrata* than in experiments with *Atriplex prostrata*, indicating that higher NaCl concentrations do indeed lead to higher mortality rates in *Lavandula dentata* (Table 1). Furthermore, the interaction between the associated crop and salt stress showed a significant effect on the attenuating mortality rate is when the holophytic plant (*Atriplex prostrata*) is associated with *Lavandula dentata*. In other words, the presence of the halophyte plant appears to reduce the negative impact of NaCl on mortality rate, confirming its potential role in improving the salt stress tolerance of *Lavandula dentata*.

3.3. Effect of salt stress on physiological and biochemical parameters

3.3.1. Variation in fresh matter

The amount of fresh matter in the leaves, stems and roots of *Lavandula dentata* is strongly influenced by NaCl, with variations observed depending on whether the plant is grown with or without the *Atriplex prostrata* association, as revealed by the results in Table 2. Furthermore, the interaction between the halophyte plant association (*Atriplex prostrata*) and NaCl concentration also shows a significant influence ($p < 0.001$) on stems and roots. These observations suggest that the presence of the halophyte plant could modulate the effect of salt stress on fresh matter in different parts of the plant.

3.3.2. Variation in dry matter

Similarly for dry matter, the results in Table 2 indicate a strong influence of NaCl on the quantity of dry matter in the leaves, stems and roots of *Lavandula dentata*, whether without or in association with *Atriplex prostrata*. Furthermore, the interaction between the association of the halophyte plant (*Atriplex prostrata*) and NaCl concentration also shows a significant influence ($p < 0.001$) on leaves, stems and roots.

3.3.3. Variation in soluble sugar content

The content of total soluble sugars varies significantly in the case without association and in association with the halophyte plant (*Atriplex prostrata*) under different NaCl treatments, as evidenced by the observed variations (Figure 4). In the case without halophyte association, the values of total soluble sugar content gradually increase with the increase in NaCl concentration. In saline conditions, *Lavandula dentata* appears to increase its production of total soluble sugars to respond to stress. However, when it is associated with *Atriplex prostrata*, this sugar production is decreased. For instance, for *Lavandula dentata* cultivated alone, the average content of total soluble sugars is 0.6233 µg/g MF at 0 g/L of NaCl, then increases to 1.2233 µg/g MF at 3 g/L of NaCl, 3.7500 µg/g MF at 6 g/L of NaCl, and finally to 5.1233 µg/g MF at 9 g/L of NaCl.

Figure 1. (a): *lavandula dentate* **(b):** *Atriplex prostrata*

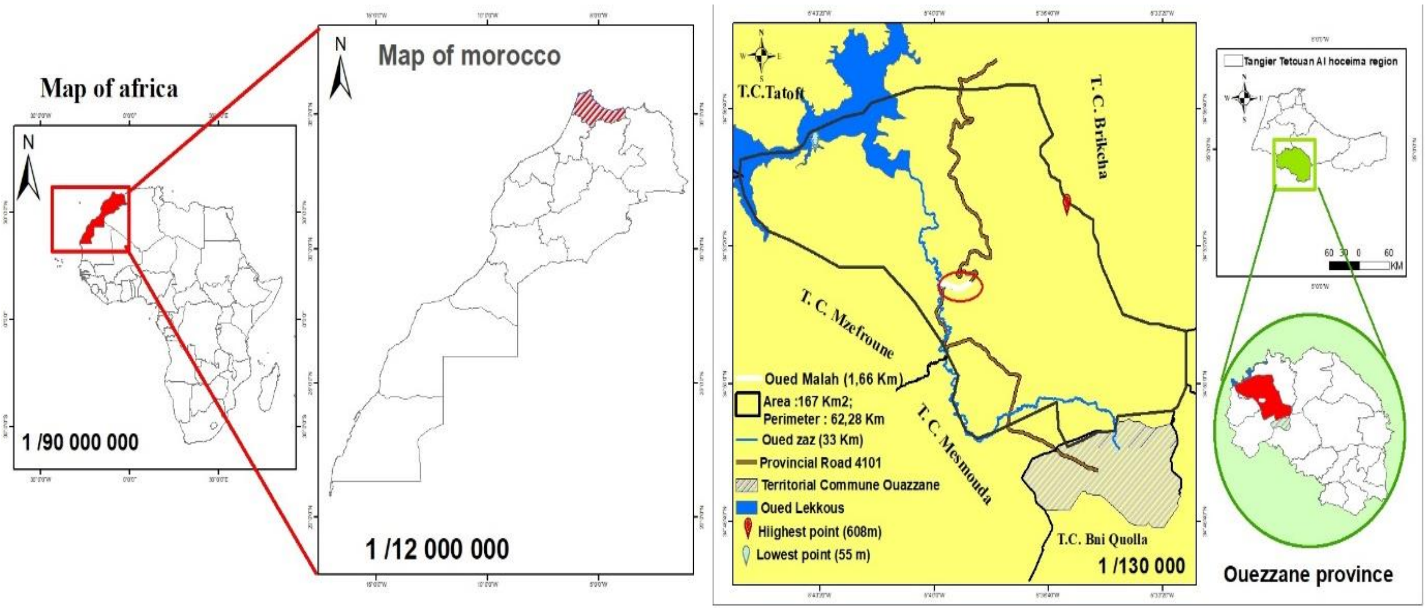

Figure 2. Localization of the study area for transplanted halophyte plants [20]

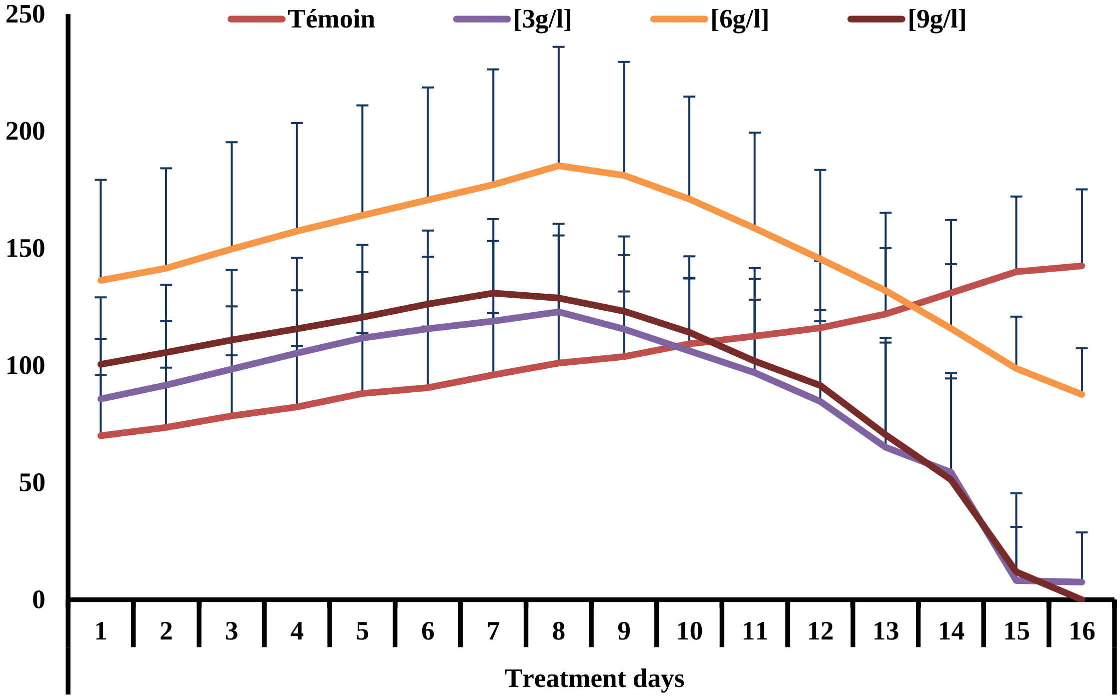

Figure 3.a: Variation in the number of leaves over time (days) in study 1

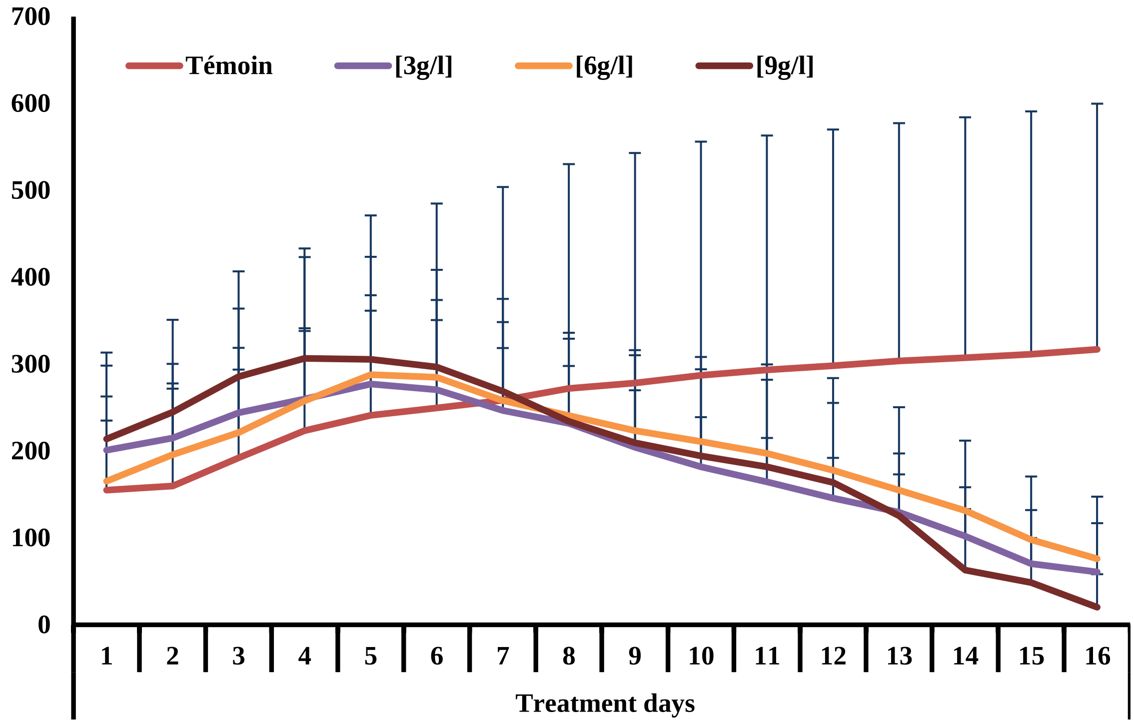

Figure 3.b: Variation in the number of leaves over time (days) in study 2

Table 1. Effect of salt stress on morphological parameters

Experiments	NaCl g/l	Length of the aerial part	Length of the root part	Root volume	Mortality rate
Experiments without association of *Atriplex prostrata*	0	26,93	24,5	4,23	0
	3	23,6	21,4	3,36	0
	6	20,96	19,8	2,95	37,5
	9	19,5	18,13	2,6	62,5
Experiments with combination of *Atriplex prostrata*	0	28,33	20,10	6,4	0
	3	26,16	21,83	5,26	0
	6	24,16	22,2	4,65	12,5
	9	21,4	21,28	3,9	37,5
Source of variation	Factor A (NaCl)	57,168***	7,282***	4,639***	3437,5***
	Factor B (Etude)	30,827***	0,940 ^{ns}	18,727 ***	937,5***
	A*B	0,923 ^{ns}	17,302***	0,2**	312,5***

Values with different letters in the column indicate statistical significance at a 5% level. ns: not significant; * significant at 5%; ** highly significant at 1%; *** very highly significant at 1 ‰.

Table 2. Effect of salt stress on physiological parameters

Experiments	**NaCl g/l**	**Fresh matter**			**Dry matter**		
		Leaves	Stems	Roots	Leaves	Stems	Roots
Experiments without association of *Atriplex prostrata*	0	7,5	4,5	1,44	1,67	1,58	1,37
	3	6,8	3,46	1,57	1,57	1,37	0,77
	6	4,3	1,9	1,41	1,42	0,88	0,75
	9	2,51	1,2	1,12	1,17	0,86	0,73
Experiments with combination of *Atriplex prostrata*	0	7,5	2,75	0,75	1,8	1,58	1,77
	3	6,6	2,41	0,7	1,58	1,37	1,27
	6	4,46	1,55	0,8	1,37	1,27	1,17
	9	2,5	1,58	0,67	1,27	1,07	0,97
Source of variation	Factor A (NaCl)	31,072***	6,536***	0,073***	0,292***	0,470***	0,617***
	Factor B (Etude)	0 ^{ns}	2,877***	2,581***	0,13***	0,140***	0,913***
	A*B	0,023 ^{ns}	1,257***	0,047***	0,01***	0,055***	0,017***

Values with different letters in the column indicate statistical significance at a 5% level. ns: not significant; * significant at 5%; ** highly significant at 1%; *** very highly significant at 1 ‰.

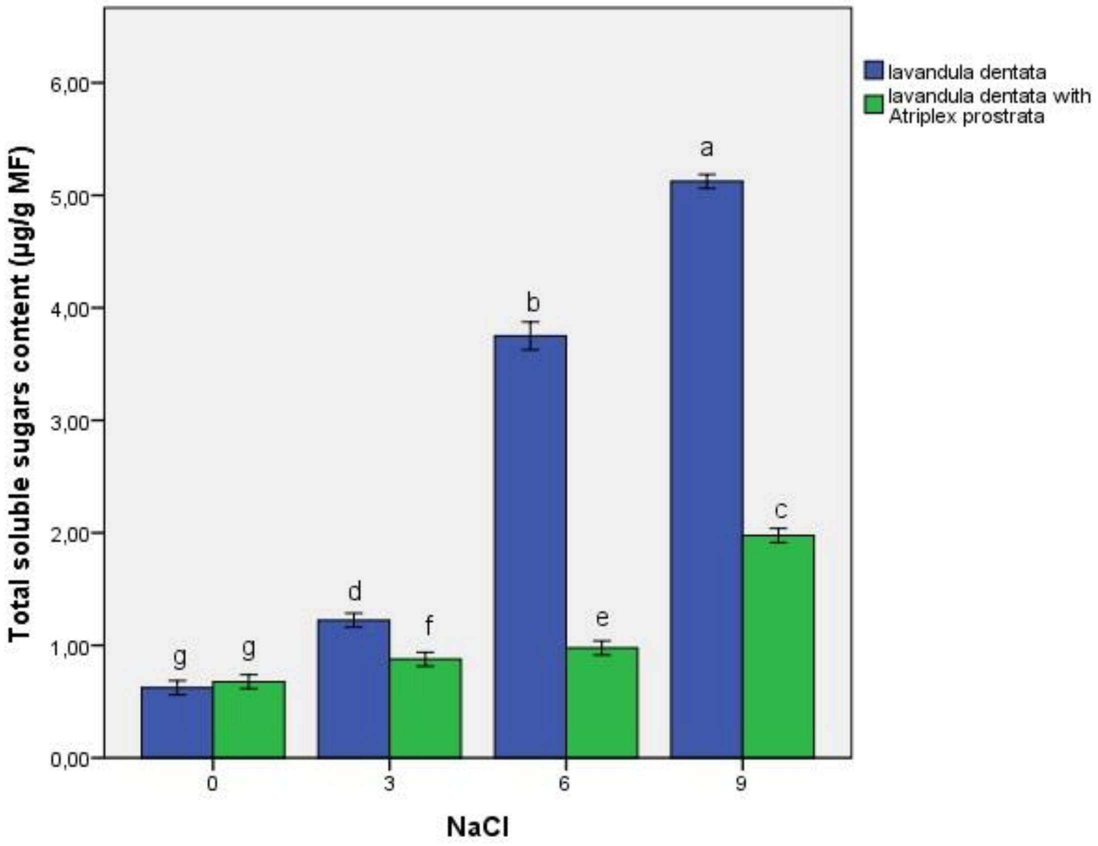

Figure 4. Total soluble sugar content for different NaCl treatments in two experiments

Table 3. Mean squares for total soluble sugars content of the two studies in the different NaCl treatments

Source	Type III Sum of squares	df	Mean square	F	Significance
Revised model	57,907^a	7	8,272	9545,055	,000
Constant	86,944	1	86,944	100320,308	,000
Experiments * NaCl	12,097	3	4,032	4652,615	,000
Experiments	14,477	1	14,477	16704,308	,000
NaCl	31,333	3	10,444	12051,077	,000

Conversely, in the case where *Lavandula dentata* is associated with *Atriplex prostrata*, the trend is less linear. For this association, the average content of total soluble sugars is 0.6767 µg/g MF at 0 g/L of NaCl, then decreases to 0.8767 µg/g MF at 3 g/L of NaCl, before increasing to 0.9767 µg/g MF at 6 g/L of NaCl, and finally decreasing to 1.9767 µg/g MF at 9 g/L of NaCl. In the analysis of variance, both factors of association with the halophyte plant (*Atriplex prostrata*) and saline stress (NaCl) show significant effects on the total soluble sugar content (Table 3). This explains the differences in the response of total soluble sugar content between the two studies (without and with *Atriplex prostrata*) and highlights the potential impact of the association with *Atriplex prostrata* on this response to saline stress. This observation suggests that the association with *Atriplex prostrata* creates a more favorable environment for *Lavandula dentata*, where it can withstand saline stress with less reliance on soluble sugar production. Soil salinization is a widespread issue globally, representing one of the most concerning abiotic stresses for plant growth and development. This salinization leads to a decrease in both crop yield and quality, gradually becoming a major threat to global food security [17]. In the present study, we examined several morphological, physiological, and biochemical parameters to evaluate the response of *Lavandula dentata* to salt stress induced by NaCl. These parameters include variations in leaf number, shoot and root length, root volume, mortality rate, as well as fresh and dry matter content and total soluble sugars. Regarding the effect of salt stress, our results highlight a significant response of *Lavandula dentata* to different concentrations of NaCl. We observed variations in plant growth, development, and physiology in response to increasing soil salinity. For example, an increase in NaCl concentration was associated with a decrease in leaf number, root length, and root volume, as well as an increase in plant mortality rate. Additionally, we noted changes in fresh and dry matter content, as well as total soluble sugars, in response to salt stress. Previous studies have highlighted that salt stress leads to a rapid decrease in the root system's ability to absorb water. Concurrently, the progressive accumulation of sodium and chloride ions in the plant's aerial parts results in a decrease in the photosynthesis rate, leading to an overall reduction in plant growth [18-19]. Other studies have shown that salinity affects shoot and root growth differently, with a significant reduction in shoot length and a decrease in root fresh weight [20]. This reduction in shoot growth could result from the progressive accumulation of Na+ in the leaves [21-23]. Salt stress also induces an accumulation of Na+ and Cl− ions in the plant, reducing cellular osmotic potential by binding free water molecules. This creates a state similar to physiological drought [24]. It is worth noting that our study also examined the effect of association with the halophyte plant *Atriplex prostrata* on the response of *Lavandula dentata* to salt stress. We found that this association could mitigate some of the negative effects of salt stress on *Lavandula dentata*. For example, we observed that the presence of *Atriplex prostrata* could reduce the decrease in root length and root volume of *Lavandula dentata* in response to high concentrations of NaCl. Furthermore, this association also had a modulating effect on the total soluble sugar content, suggesting a potential adaptation of *Lavandula dentata* to saline stress conditions through this association. Plants have developed sophisticated strategies to adapt to salt stress, producing a variety of osmolytes such as proline, glycine betaine, and soluble sugars [25-27]. In the first study, *Lavandula dentata* alone must cope with salt stress, thereby increasing its secretion of soluble sugars to protect its cells. However, in the second study with *Atriplex prostrata* (a halophyte plant), the latter absorbs some of the salt, thus reducing salt stress for the lavender. Therefore, *Lavandula dentata* does not need to produce as many soluble sugars because it benefits from a less stressful environment in the presence of *Atriplex prostrata* due to various strategies to combat salinity [28]. Halophytes have the ability to reduce soil salt concentration [29] and are potentially beneficial in areas affected by salinity. However, the introduction of halophytes as companion plants has a significant impact on improving plant dry matter and fruit yield in tomato plants [30], contradicting previous findings [31]. These findings underscore the complexity of interactions between glycophyte and halophyte plants in saline environments. Additionally, it is worth noting that competition with sodium

(Na) and chloride (Cl) can lead to nutritional imbalances in the leaves of both types of plants [32-33].

4. Conclusions

In this comprehensive study on the impact of salt stress on *Lavandula dentata*, whether in isolated cultivation or in association with *Atriplex prostrata*, we observed significant alterations in the plant's growth, morphological, physiological, and biochemical parameters. Salt stress induced a progressive decrease in leaf number, reduction in aboveground height, variation in root length, as well as a decrease in root volume and an increase in mortality rate of *Lavandula dentata*. Additionally, a decrease in fresh and dry matter content along with changes in total sugars were observed. However, in the study where *Lavandula dentata* was associated with *Atriplex prostrata*, we observed a significant attenuation of these adverse effects of salt stress. The presence of *Atriplex prostrata* preserved the growth of *Lavandula dentata*, reducing the decrease in leaf number, mitigating the reduction in aboveground height, and limiting the decrease in root length. Notably, the mortality rate was significantly reduced when the two plants were associated, confirming the beneficial role of the halophyte plant in enhancing the salt stress tolerance of *Lavandula dentata*. In summary, this study highlights the significant impact of salt stress on *Lavandula dentata* and suggests that association with halophyte plants such as *Atriplex prostrata* could mitigate these adverse effects. These findings are important for understanding plant responses to environmental stress and propose promising strategies for sustainable management of ecosystems facing increasing salinity. An emerging approach involves using halophyte plants to remove salts from the soil, often by integrating them into intercropping or sequential cropping systems, combining cash or forage crops to ensure the economic viability of farming operations. Future research should delve deeper into these interactions for practical application in the field of sustainable agriculture.

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