

# Effects of *Bacillus amyloliquefaciens* on Volatile Components and Nutrient Element Contents of *Mentha piperita* L. Grown under Salt Stress

Songül Tuğba Üner, and Aslıhan Cesur Turgut \*

*Bacillus amyloliquefaciens* (*Ba*) was applied to *Mentha piperita* L. (peppermint) seedlings grown at various salt levels (0, 50, 75, and 100 mM) for 42 days (six weeks). The study was conducted in a total of eight groups, with 24 seedlings per group. At the end of the study, the seedlings were analyzed for plant nutrient elements and volatile compound contents. The negative effect of salt was observed in almost all parameters. When all groups were evaluated for plant nutrient elements, *Ba* had a positive effect on Zn, Mn, Cu, and Na values compared to the control, but it did not show any effect on B, Fe, K, P, Mg, and Ca. In volatile compounds, limonene was detected as the major component in all groups. As a result of the evaluation based on limonene, the highest rate was found in the control, and the lowest rate was found in 100 mM NaCl. The salt-dependent inhibition between the groups with the highest and lowest limonene was 73%. While the negative effects of salt were observed in almost all parameters, the promoter effects of *Ba* were not as pronounced.

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Contact information: Burdur Mehmet Akif Ersoy University, Burdur Food Agriculture and Livestock Vocational School, 15030, Burdur, Türkiye; \*Corresponding author: [acesur@mehmetakif.edu.tr](mailto:acesur@mehmetakif.edu.tr)

## INTRODUCTION

Climate change affects agriculture in many ways. Salinity, droughts, floods, and rainfall distribution changes greatly impact agriculture and land use (Nong *et al.* 2021). Crop yields, maturity periods, livestock feed shortages, animal health issues, and crop quality and quantity decrease due to climate change (Singh 2019). It may also lower crop yields and boost new crop growth (Setu *et al.* 2019). Climate change affects soil health, erosion, water availability, pests, diseases, and weeds, lowering agricultural productivity (Abdel-Maksoud 2018). It can also disrupt irrigation water, which is vital for food crop production (Joyo and Ram 2018). In some areas, climate change will intensify salt stress on agricultural soils (Etesami and Beattie 2018). Climate change causes crop and seed failure due to unpredictable weather (Syahputra and Sucahyono 2022). It dehydrates and salinates fertile soils, limiting food production (Teshaeva and Sadriddinova 2021). Climate change may aggravate drought, heat, salinity, and flooding (Andjelkovic 2018). Kumar and Gautam (2014) revealed that even a little increase in global warming-induced evapotranspiration demand can deplete fragile desert ecosystems' water resources.

Plant growth-promoting bacteria (PGPB) have been widely explored to reduce salt stress in numerous plant types. PGPB improves plant tolerance to abiotic stresses,

including salt (Santos *et al.* 2018). Endophytic PGPB preserves plant performance better than rhizospheric PGPB under salt stress, perhaps due to their closer relationship with the host (Heydarian *et al.* 2016).

Several studies have shown that PGPB induces the synthesis of osmolytes, which assists the resistance of plants to salt stress, promoting plant development, and salt tolerance (Hmaeid *et al.* 2019). For example, PGPB bio-inoculation also reduces salt stress by regulating plant antioxidant defense (Ma *et al.* 2022). PGPB may also improve plant performance in salt-affected soil (Al-Garni *et al.* 2019). To improve plant salt stress resistance, PGPB is a sustainable and cost-effective option (Ayuso-Calles *et al.* 2021).

Plant growth-promoting bacterium inoculation improves plant growth and stress tolerance. Salt stress has been studied in relation to *Bacillus amyloliquefaciens* (*Ba*), a PGPB species. Plant-associated *Ba* promotes plant growth and produces secondary compounds that reduce soil-borne plant diseases (Chen *et al.* 2007). *Ba* strains may colonize plant organs and endure intense plant response reactions, encouraging plant growth (Fan *et al.* 2017). This bacterium promotes plant development and removes organophosphorus herbicides (Ngalimat *et al.* 2021). *Ba* degrades plant polysaccharides and can withstand high temperatures, pressures, and acidic or alkaline conditions (Hong *et al.* 2019). *Ba* strains can help plants grow faster, fight off infections, and be healthier by changing the structure of the microbial community in the rhizosphere which makes the plants more resistant throughout their bodies (Liu *et al.* 2021). Inoculating corn seeds with *Ba* improves plant development and grain yield (Silva *et al.* 2023). The bacterium improves plant health by increasing disease and insect resistance (Li *et al.* 2015). *Ba* strains are frequently employed in agriculture to boost crop output, plant growth, and disease control (Zhao *et al.* 2015). They inhibit a wide range of plant pathogenic fungi, indicating their potential as agricultural biocontrol agents (Zhang *et al.* 2016). *Ba* also makes plants better able to handle *Fusarium oxysporum*, water, and nutrient stress (Prisa 2021). In salinized soils, *Ba* increases photosynthetic activity and oxidative stress tolerance (Rossi *et al.* 2021). *Bacillus amyloliquefaciens* affects salt-stressed plants through unknown methods. However, it is known that salt stress impacts plant ion homeostasis and Na<sup>+</sup> transport-related gene expression (Sun *et al.* 2021). PGPB, especially *Ba*, may affect ion homeostasis and gene expression in salt-stressed plants, improving salt tolerance. PGPB, including *Ba*, communicate and benefit when they form a consortium, especially under various stress circumstances (Santoyo *et al.* 2021).

In this study, the effects of *Bacillus amyloliquefaciens* (*Ba*) a halotolerant species, were examined on the volatile compounds and plant nutrients of *Mentha piperita* L. seedlings grown under salt stress.

## EXPERIMENTAL

### Materials

*Mentha piperita* L. (peppermint) seedlings, *Bacillus amyloliquefaciens* (Cilus Plus), NaCl (Merck KGA, Germany), torf (Klasmann), and perlite (Klasmann) were commercially purchased. Four-week-old seedlings were purchased from Kırcami Fide Agricultural Company in Antalya/Türkiye. Cilus Plus is a microbial preparation containing a high concentration ( $2 \times 10^{10}$  CFU/g) of *Bacillus amyloliquefaciens* strain IT45 isolated through Lallemand Research.

## Experimental Design

### *Planting and application details*

Seedlings were divided into eight equal groups, and the experiment was set up with 24 seedlings per group. There are three repetitions for each group and eight seedlings in each repetition.

**Table 1.** Experimental Groups

Groups	
1.	Control (non-bacterial and non-saline conditions)
2.	*Ba + non-saline conditions
3.	50 mM NaCl
4.	Ba + 50 mM NaCl
5.	75 mM NaCl
6.	Ba + 75 mM NaCl
7.	100 mM NaCl
8.	Ba + 100 mM NaCl
*Ba: <i>Bacillus amyloliquefaciens</i>	

In the first stage of the study, 4-week-old peppermint seedlings of similar sizes (10 to 12 cm) were planted in the seedling trays containing a mixture of 50% perlite and 50% sterile peat, and 24 samples were included for each group. All seedlings (24 seedlings) belonging to the same group were placed in the 24-cell tray (8\*3), but each seedling was watered separately. For statistical studies, each of these three rows was considered a repetition, and statistics were applied that way. Seedling trays were left to grow and develop for 6 weeks (42 days) at  $19 \pm 1$  °C in a climate chamber with a 16-h light (12000 lux) and 8-h dark photoperiod (Sabzalian *et al* 2014; Üner and Cesur Turgut 2023). The experiment started with the planting of four-week-old seedlings. On the day of planting, the seedlings were watered only with distilled water, and then NaCl solutions (in distilled water) were applied six times at six-day intervals. Measurements were made 42 days after planting (70-day-old seedlings).

### *Salt and bacteria applications*

Tort and Türkyılmaz (2003) report that NaCl is usually mentioned when soil salinity and salt stress are discussed. For this reason, only NaCl was used to create salinity stress. Salt levels for peppermint seedlings were determined at control (non-bacterial and non-saline), 50 mM, 75 mM, and 100 mM NaCl based on the literature and after preliminary studies (Tüzüner 1990; Tort and Türkyılmaz 2003; Baydar and Çoban 2017). Bacteria applications are grouped in four different ways (*Ba* + non-saline, *Ba* + 50 mM, *Ba* + 75 mM, and *Ba* + 100 mM NaCl). Salt applications were applied to the seedlings planted in the peat-perlite mixture at a rate of 30 mL/seedling.

Microbial fertilizer solution was prepared at the rate recommended on the package (1.6 g/10 L), taking into account the total peat-perlite volume. The prepared solution was applied to each eye (30 mL/seedling) twice, the day after planting and 28 days (4 weeks) later, in accordance with the instructions.

### Obtaining data

The study continued for six weeks (42 days after planting). At the end of 42 days, the following analysis of volatile components and plant nutrient elements was made in accordance with the literature.

Volatile component analyses were carried out in solid phase microextraction/gas chromatography (SPME/GC) at Burdur Mehmet Akif Ersoy University scientific and technology application and research center. Aerial parts of *Mentha piperita* L. were used for SPME (sample weight 1.0 g) analysis. The starting temperature in oven was kept at 60 °C. After waiting at 60 °C for 2 min, it was increased to 220 °C with an increase of 2 °C/min. It was held at this temperature for 20 min (Baydar *et al.* 2013; Taherpour *et al.* 2017). The detector and injector temperatures were set at 250 and 240 °C. Analyses were performed on the Agilent 5975 C Agilent 7890A device (Agilent Technologies, Santa Clara, CA, USA) with a CP WAX 52 CB column.

Plant nutrient elemental analysis was performed at Burdur Mehmet Akif Ersoy University scientific and technology application and research center. Dried aerial parts of *Mentha piperita* L. were used for ICP-OES analysis. By grouping the samples (1.0 g per sample), washed by tap water first and deionized water secondly, and dried at 70 °C for 48 hours. The dried samples were then pulverized using a blender. For P, K, Ca, Fe, Zn, Cu, Mn, B, Na, and Mg analysis, the samples were microwaved (Milestone Stard D, Sorisole Bergamo, Italy), and the readings were taken with the Perkin Elmer Optima-8000 (Waltham, MA, USA), which was taken using the ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) device (Saltan and Seçilmiş Canbay 2015).

### Statistical evaluation of results

The statistical significance of the differences between the averages in the data obtained was analyzed with the Duncan ( $P < 0.05$ ) multiple comparison test in SPSS (IBM-SPSS Inc., Armonk, NY, USA) version 25.0, and the values are given as mean  $\pm$  SD (standard deviation) (Efe *et al.* 2000).

## RESULTS AND DISCUSSION

### Nutrient Elements

When applying bacteria *Ba* to peppermint seedlings under salt stress, the effects on the intake of Zn (zinc), B (boron), Mn (manganese), Fe (iron), Cu (copper), Na (sodium), K (potassium), P (phosphorus), Mg (magnesium), and Ca (calcium) elements were examined. The results of this examination (Table 2) are discussed below.

When the results were evaluated, Zn values increased depending on salt levels. The highest value was found in *Ba* + 100 mM NaCl compared to the control (74.2% increase), and the lowest value was detected in the control. It was observed that at almost all salt levels (except 75 mM), bacteria-applied groups contained higher Zn content than non-bacterial groups. There are many studies in literature showing that salt stress increases Zn values, and this is compatible with the authors' results (Maas *et al.* 1972; Chavan and Karadge 1980; Martinez *et al.* 1987; Alpaslan *et al.* 1998). Additionally, there are studies showing that *Bacillus* species, as one of the PGPB species, increase zinc uptake and support the current study's results (Turan *et al.* 2007). Singh *et al.* found that *Bacillus amyloliquefaciens* increased zinc uptake in wheat despite salt stress. It is thought that

mechanisms such as bacteria's phosphate solubilization abilities, ion exchange capacities, and antioxidant production are effective in increasing Zn uptake (Singh *et al.* 2021).

When B results were examined, unlike Zn, the highest value was observed in the control, while the lowest value was detected in *Ba* + 100 mM NaCl (more than 50% decrease). With increasing salinity, a regular decrease in B amounts was observed in both bacteria-applied and non-applied groups. The decrease in stomatal conductance under salinity stress limits the movement of water and nutrients, including boron, within the plant, thereby reducing boron uptake (Carvajal *et al.* 2023). Additionally, high concentrations of sodium and chloride ions in the soil solution under salinity stress can have detrimental effects on plant growth and yield, potentially leading to nutrient imbalances, including boron deficiency (Tavakkoli *et al.* 2010). Studies in the literature that show salinity reduces B intake are in agreement with the results obtained (Holloway and Alston 1992). However, the presence of bacteria did not have a positive effect on B intake in the groups. There are studies on the positive effects of *Bacillus* species on boron intake (Masood *et al.* 2019), but they do not match the current results.

Manganese levels increased compared to the control in almost all groups (except *Ba* + non-saline). The Mn uptake increased in parallel with salt concentration. The highest values were detected at 75 and 100 mM NaCl, and the increase was ~110% compared to the control. The increase in Mn uptake with increasing salt levels in the current study is consistent with the literature (Maas *et al.* 1972; Chavan and Karadge 1980; Martinez *et al.* 1987; Alpaslan *et al.* 1998). There are reports indicating that PGPB can promote Mn uptake (Xue *et al.* 2011). In this study, it was observed that *Ba* had a salt stress-reducer effect only at 50 mM NaCl, but this level did not increase Mn uptake compared to the control or at other salt levels.

Iron uptake was highest in the control group with 68.7 mg, while the lowest uptake was in 50 mM NaCl with 42.6 mg (38% decrease). In non-bacterial groups, Fe intake decreased with increasing salt levels. Studies in the literature that show salinity limits Fe uptake in plants support these results (Rabhi *et al.* 2007). However, while Fe intake decreased in the presence of salinity compared to the control, it was observed that Fe intake increased with increasing salinity when salt levels were compared. Numerous factors are believed to be able to affect the increased absorption of Fe in higher salinity conditions. Some studies have demonstrated that plants may enhance the absorption of certain nutrients, such as iron, in response to salinity stress. Research has also shown that the application of iron nanoparticles can boost iron content in plants under salinity stress, suggesting a potential mechanism for increased iron absorption in saline conditions. Under saline-alkaline stress, plants may exhibit higher iron accumulation, potentially as a mechanism to cope with the stress condition (Askary *et al.* 2017; Zhou *et al.* 2017). *Bacillus amyloliquefaciens* alleviated salt stress only at 50 mM NaCl, just like Mn uptake, but could not increase it to the control level. There are studies showing that volatile organic compounds released by *Ba* increase Fe uptake (Wang *et al.* 2017). However, in this study, the positive effect of *Ba* on Fe uptake in the presence of salt was not clearly observed.

Copper levels increased in parallel to the increase in salt levels in almost all groups, except 100 mM. It was determined that *Ba* decreased Cu uptake only in *Ba* + 75 mM NaCl compared to 75 mM NaCl but increased it in 100 mM. As a result, an irregular increase or decrease was observed. Differences in other salt levels were statistically insignificant. Literature supports current results that Cu intake increases due to salt intake and concentration increases (Achakzai *et al.* 2010).

As expected, sodium intake increased significantly in parallel with the increase in salt (NaCl) levels. With this increase, the Na value, which was 0.7 g in the control, increased approximately 10 times to 6.8 g at the lowest concentration of 50 mM. These values continued to increase depending on the salt level. In *Ba* + 100 mM NaCl, it increased to the highest value of 17.4 g (~25 times). *B. amyloliquefaciens* application reduced Na uptake ~50% at 75 mM but increased ~12% at 100 mM. These results are intriguing. Multiple studies have demonstrated that the application of Ba can restrict the uptake of Na in saline conditions (Ashraf *et al.* 2004; Chen *et al.* 2016). Table 2 highlights the significant difference in Na uptake at higher rates compared to the control. It was determined that bacterial application caused a decrease in Na uptake at almost all salt concentrations (except 100 mM). In particular, there was a significant decrease of approximately 50% at 75 mM (between the bacteria-applied and non-bacterial groups). This reduction in Na uptake helps to alleviate Na toxicity and improve salt tolerance in the plant.

In terms of potassium intake, there was a decrease in the groups without bacterial application compared to the control, which was likely due to the effects of salt. However, there was no statistically significant difference observed in the bacterial groups. This is consistent with the results of Ruiz *et al.* (1997) and Tolay (2021), who also found a decrease in potassium intake under salt stress.

No significant differences were observed in phosphorus, magnesium, and calcium levels due to either salinity or bacterial application. This is in line with previous studies, such as Poss *et al.* (1985), who also found no effect of salinity on the uptake of these minerals.

In the literature, the inhibition of salt stress on growth parameters is generally mentioned. Salt (NaCl) stress affects plants in two ways: specific and osmotic effects (Al-Karaki 2001; Ghoulam *et al.* 2002). According to the first mechanism, it is stated that the greatest harms on physiological events are related to germination (Levit 1980). As a second mechanism, it is mentioned that salt inhibits post-germination morphogenetic developments due to its toxic effect on plant tissues in ion form (Chrominski *et al.* 1986; Üner and Cesur Turgut 2023). In addition, salt has many indirect effects. It can prevent germination by stopping mitosis (Cesur and Tabur 2011); it can prevent development and growth by preventing protein synthesis and nucleic acid synthesis; it can affect the permeability of the cell membrane (Yakit and Tuna 2006); and it can affect the metabolic rate of the cell due to the antagonistic effect of Cl<sup>-</sup> or Na<sup>+</sup> ions taken into the cells. It prevents the uptake of other ions, such as K<sup>+</sup> and Ca<sup>++</sup>, which are necessary for their functions (Özcan 2000).

**Table 2.** Effect of *B. amyloliquefaciens* on Some Nutrient Elements in *Mentha piperita* L. Seedlings Grown Under Salt Stress

Groups	Zn (mg)	B (mg)	Mn (mg)	Fe (mg)	Cu (mg)	Na (g)	K (g)	P (g)	Mg (g)	Ca (g)
Control (non-saline and non-bacterial)	24.9 ±2.9 <sup>aa</sup>	65.9 ± 5.5 <sup>f</sup>	20.7 ± 1.9 <sup>ab</sup>	68.7 ± 9.1 <sup>b</sup>	3.8 ± 0.9 <sup>a</sup>	0.7 ± 0.5 <sup>a</sup>	25.0 ± 1.5 <sup>b</sup>	1.4 ± 0.0 <sup>a</sup>	2.3 ± 0.1 <sup>a</sup>	14.9 ± 0.8 <sup>a</sup>
**Ba (non-saline)	27.6 ±5.1 <sup>a</sup>	52.5 ± 2.9 <sup>e</sup>	17.8 ± 4.9 <sup>a</sup>	58.3 ± 13.1 <sup>ab</sup>	3.1 ± 0.8 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	19.7 ± 3.9 <sup>ab</sup>	1.4 ± 0.3 <sup>a</sup>	2.1 ± 0.8 <sup>a</sup>	13.4 ± 0.3 <sup>a</sup>

50 mM NaCl	27.8 ± 7.3 <sup>a</sup>	46.9 ± 3.8 <sup>d</sup>	31.1 ± 6.2 <sup>abc</sup>	42.6 ± 7.2 <sup>a</sup>	4.0 ± 0.8 <sup>ab</sup>	6.8 ± 0.9 <sup>b</sup>	18.4 ± 0.8 <sup>a</sup>	1.4 ± 0.1 <sup>a</sup>	2.6 ± 0.5 <sup>a</sup>	11.3 ± 0.1 <sup>a</sup>
Ba + 50 mM NaCl	38.3 ± 4.9 <sup>b</sup>	44.0 ± 1.7 <sup>cd</sup>	37.0 ± 7.1 <sup>bc</sup>	67.9 ± 10.0 <sup>b</sup>	4.2 ± 0.2 <sup>ab</sup>	4.9 ± 0.9 <sup>b</sup>	20.7 ± 1.7 <sup>ab</sup>	1.5 ± 0.1 <sup>a</sup>	2.7 ± 0.4 <sup>a</sup>	14.3 ± 0.2 <sup>a</sup>
75 mM NaCl	39.5 ± 4.8 <sup>b</sup>	38.9 ± 1.4 <sup>bc</sup>	42.3 ± 19.4 <sup>c</sup>	46.7 ± 12.2 <sup>ab</sup>	5.5 ± 0.9 <sup>b</sup>	12.9 ± 0.1 <sup>c</sup>	22.9 ± 0.1 <sup>ab</sup>	1.8 ± 0.0 <sup>a</sup>	2.8 ± 0.1 <sup>a</sup>	14.4 ± 0.2 <sup>a</sup>
Ba + 75 mM NaCl	38.4 ± 3.0 <sup>b</sup>	36.9 ± 2.5 <sup>ab</sup>	33.9 ± 2.5 <sup>abc</sup>	44.1 ± 11.0 <sup>a</sup>	3.4 ± 0.7 <sup>a</sup>	6.3 ± 0.1 <sup>b</sup>	20.9 ± 1.0 <sup>ab</sup>	1.3 ± 0.1 <sup>a</sup>	2.2 ± 0.1 <sup>a</sup>	13.1 ± 0.1 <sup>a</sup>
100 mM NaCl	34.7 ± 1.6 <sup>ab</sup>	35.3 ± 1.67 <sup>ab</sup>	42.8 ± 8.4 <sup>c</sup>	54.2 ± 3.1 <sup>ab</sup>	4.3 ± 0.6 <sup>ab</sup>	15.5 ± 2.6 <sup>cd</sup>	22.4 ± 3.8 <sup>ab</sup>	1.5 ± 0.3 <sup>a</sup>	2.9 ± 0.5 <sup>a</sup>	13.9 ± 0.2 <sup>a</sup>
Ba + 100 mM NaCl	43.4 ± 8.3 <sup>b</sup>	31.8 ± 2.9 <sup>a</sup>	40.9 ± 15.0 <sup>c</sup>	58.1 ± 19.5 <sup>ab</sup>	5.4 ± 1.5 <sup>b</sup>	17.4 ± 0.4 <sup>d</sup>	20.1 ± 6.1 <sup>ab</sup>	1.7 ± 0.9 <sup>a</sup>	2.5 ± 0.5 <sup>a</sup>	11.3 ± 0.3 <sup>a</sup>
*Shows values with insignificant difference ( $p < 0.05$ ) for each column shown with same letters ( $\pm$ standard deviation)										
** Ba: <i>Bacillus amyloliquefaciens</i>										

Plant growth-promoting rhizobacteria (PGPR) are soil bacteria that can contribute to plant growth and increase tolerance to stresses. As a result of the application of PGPRs in agriculture, benefits have been achieved in seed germination, crop yield, and disease resistance, and direct or indirect effects have been observed (Kloepper 1993; Van Loon 2007; Kuan *et al.* 2016). Plant hormones are made, ethylene levels are lowered, and plant nutrients (*via* the release of phosphate and micronutrients from insoluble sources and the fixation of non-symbiotic nitrogen) (Van Loon 2007) and microbial volatile organic compounds (mVOCs) (Ryu *et al.* 2004; Wenke *et al.* 2012; Santoro *et al.* 2015) are raised. There are studies showing that salt tolerance can be increased by adding beneficial microorganisms (PGPB, such as *Bacillus amyloliquefaciens*) to plant environments under salt stress conditions (Kim *et al.* 2013). In addition, such applications, which increase the ability to scavenge reactive oxygen species (ROS) in plants exposed to salt stress, increase antioxidant activities (Frag *et al.* 2013; Kim *et al.* 2013).

In the study of *Bacillus amyloliquefaciens*' intake of plant nutrients, it was determined that it increased the intake of Na, Zn, Mn, and Cu compared to the control, that it could not exceed the control values in B and Fe, and that there was no statistical difference between them and the control in P, Mg, and Ca (Table 2). The  $\text{Ca}^{2+}$  ion is one of the basic elements required for growth and development.  $\text{K}^+$  and  $\text{Ca}^{2+}$  uptake are affected by salt stress.  $\text{Na}^+$  replaces  $\text{Ca}^{2+}$  in the cell membrane, increasing the  $\text{Na}^+/\text{Ca}^{2+}$  ion ratio in the apoplast part of the membrane. In this case, both the physiological and functional structure of the membrane are disrupted, and the  $\text{Ca}^{2+}$  balance in the cell is affected. High  $\text{Na}^+$  concentrations in the inner membrane structures of the cell cause internal  $\text{Ca}^{2+}$  stores to be emptied and free  $\text{Ca}^{2+}$  to increase in the cell. It is also thought that too much NaCl can depolymerize microtubules and stop the formation of spindle fibers during cell division (Rengel 1992; Yokoi *et al.* 2002; Çulha and Çakır 2011). However, similar to our findings, there are also studies showing that high Na levels do not cause an increase in Ca and Mg, or even decrease them (Poss *et al.* 1985; Cook and Veseth 1991). It was found that increased  $\text{K}^+$  uptake due to *Bacillus licheniformis* helped tomatoes grow better in soils that were stressed by salt. However, there seem to be no increases in potassium in this study (Muthuraja and Muthukumar 2022). Inhibition of salt-dependent growth and development in plants may be due to the excess of  $\text{Na}^+$  ions in the environment and the plant being

deprived of an essential nutrient by preferentially taking in  $\text{Na}^+$  instead of potassium ions ( $\text{K}^+$ ), as well as the inhibition of some enzyme systems (Okon 2019).

### Volatiles Components

The complete list of volatile components analyzed by SPME/GC for all groups is also provided. The proportions of volatile components in *Mentha piperita* L. seedlings of *B. amyloliquefaciens* under salt stress were evaluated. In the prepared Table 3, only volatile components present at 5% or more are stated.

When the volatile component numbers and major components were compared, limonene was detected as the major component in the control group (non-saline and non-bacterial), and there were 38 chemical components in total. Similarly, there are studies in the literature that identify limonene as the major component in *M. piperita* L. (Buleandra *et al.* 2016), which is compatible with current results. The effects of the bacteria on limonene, the major component in peppermint seedlings grown under salt stress, are also presented graphically.

There are studies in the literature showing that the components detected between the hydro-distillation and SPME methods may be different (Taherpour *et al.* 2017). For this reason, major components (menthol, menthone, *etc.*) that are generally detected as a result of hydro-distillation (Verma *et al.* 2010) were not found in this study. This difference is thought to be due to the method difference used.

In *Ba* (non-saline), 36 volatile components were detected, and the major component was limonene. Forty-nine volatile compounds were detected in 50 mM NaCl, 46 volatile compounds were detected in *Ba* + 50 mM NaCl, and the major component in both was carvotanacetone. Thirty-three volatile components were detected at 75 mM NaCl, and the major component was 2,4-decadienal. Thirty-nine volatile components were detected in *Ba* + 75 mM NaCl, 19 volatile components were detected in 100 mM NaCl, and the major component was carvotanacetone in both. Finally, 40 volatile components were detected in *Ba* + 100 mM NaCl and the major component was limonene.

Salt stress was found to reduce limonene and *cis*-dihydrocarvone values, but it was observed that application of bacteria did not increase the component percentages and only alleviated the effect of stress on limonene at 100 mM. It was determined that salt or bacterial application did not cause a regular increase or decrease in dihydrocarvone, carvotanacetone (2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)), and eucalyptol (1,8-cineole) values. The values were fluctuating. Cyclohexene, 1-methyl-4-(1-methylethenyl),  $\alpha$ -pinene,  $\beta$ -pinene, 2,4-decadienal, *trans*-caryophyllene, and carvol were the components detected in a single group. For this reason, no decrease or increase in these components could be observed. There are many studies that report salt stress changes the volatile component ratios in the plant and even causes the synthesis of components that are not present in the control group (Khorasaninejad *et al.* 2010; Roodbari *et al.* 2013; Baydar and Çoban 2017). This literature is consistent with the results obtained.



**Table 3.** Effect of *B. amyloliquefaciens* on Volatile Components in *Mentha piperita* L. Seedlings Grown Under Salt Stress

Groups (NaCl) \ Volatile Components *	Limonene (%)	Dihydrocarvone (%)	<i>cis</i> -Dihydrocarvone (%)	Carvotanacetone (%)	Eucalyptol (1,8-cineol) (%)	$\alpha$ -Pinene (%)	$\beta$ -pinene (%)	2,4-Decadienal (%)	<i>trans</i> -Caryophyllene (%)	Carvol (%)
Control (non-saline and non-bacterial)	49.3	13.3	-	-	7.8	-	5	-	-	-
**Ba (non-saline)	36	12.1	-	21.8	8.7	5	-	-	-	-
50 mM NaCl	26.2	10.9	-	34.1	11.1	-	-	-	-	-
Ba + 50 mM NaCl	20.9	17.3	-	24	7	-	-	-	-	-
75 mM NaCl	29.1	6	-	-	8.6	-	-	32.6	-	-
Ba + 75 mM NaCl	25.2	-	19.2	28.2	-	-	-	-	-	-
100 mM NaCl	13.3	18.3	-	57.6	5	-	-	-	-	-
Ba + 100 mM NaCl	22.5	-	7.8	15.1	6.1	-	-	-	5	13.8

\*Only components present at 5% or more are given in this table. Volatile component lists for all groups are shared at the end of the document

When the contents of the volatile components are summarized, in the control group, limonene was determined as the major component, and in the other groups, carvotanacetone and 2,4-decadienal were major components along with limonene. It was determined that salt stress reduced limonene and *cis*-dihydrocarvone values, but bacterial application did not increase the percentages of the components. The components given in Table 3, except for limonene, dihydrocarvone, eucalyptol (1,8 cineole), and  $\beta$ -pinene, which had the highest values in the control, were determined by stress/bacteria application. It was observed that *B. amyloliquefaciens* did not positively affect the accumulation of volatile compounds and secondary metabolites, contrary to the literature (Zhao *et al.* 2016) in the peppermint seedlings.

## CONCLUSIONS

In this study, the effects of salt stress on plant nutrients and volatile compounds in *Mentha piperita* L. seedlings grown at various salt levels and periodically applied with *Bacillus amyloliquefaciens* were examined.

1. The inhibition effect of salt stress manifested itself in many parameters, as expected.

2. It was found that *B. amyloliquefaciens* did not increase the rates of volatile components or help *Mentha piperita* L. take in all nutrient elements, which was different from that had been observed before in other studies.
3. It is thought that the reason for this lack of effectiveness of *B. amyloliquefaciens* may be due to the insufficient six-week growth period of the seedlings or the insufficient number (twice) or rate of bacterial applications. Subsequent studies with longer-term and different doses of bacterial application may reveal more striking results.
4. This study was conducted to guide farmers and producers commercially who want to obtain information about the volatile component contents of *M. piperita*, grown from more qualified seedlings.

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