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# THE EFFECTS OF BACILLUS AMYLOLIQUEFACIENS ON MENTHA PIPERITA GROWN UNDER SALT STRESS

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**ABSTRACT**

Climate change threatens agricultural areas and food supply security not only in our country but also worldwide. As a result, plants are exposed to many abiotic stresses such as salt stress, drought stress, etc. Various methods are being tried to cope with abiotic stress, but sustainable alternative methods are needed in agriculture and one of them is biological fertilizers. Microbial fertilizers such as plant growth promoting bacteria (PGPB) reduce the use of chemical fertilizers and support environmentally friendly, soil-friendly, and more economical production in agriculture. In this study, the effects of Bacillus amyloliquefaciens, defined as a salt tolerant species, on the morphological parameters of Mentha piperita, which has significant commercial value and was grown under salty conditions, were investigated. Surprisingly, Bacillus amyloliquefaciens did not significantly alleviate the effects of salt stress on M. piperita grown under saline conditions (0, 50, 75, and 100 mM NaCl) in almost all parameters except for water content.

**Keywords:** Bacillus amyloliquefaciens, Mentha piperita L., PGPR, salt stress.

## 1. INTRODUCTION

The world's population is increasing rapidly and this increase is expected to reach 11.2 billion in 2100 (1). In today's world, there is an imbalance between the amount of food needed and meeting the need (2). This imbalance is not just caused by rapid population growth. In addition, the accumulation of human population in traditional agricultural areas, the decrease in arable land, the lack of water, the destruction of agricultural lands by urbanization, poor soil management, inappropriate agriculture and economic policies, the salinity of agricultural areas, acid rain and heavy metals, etc. exposure also causes imbalance (3, 4). All abiotic stresses (5, 6), especially salinity, affect plants at different stages of their growth and can even cause plant death in extreme conditions (7, 8). About 830 Mha of global arable land is affected by salt stress and 2 Mha of land becomes uncultivable every year due to salinity (9). Plants can be exposed to two or more of these stresses at the same time, and this exposure affects the plant more. Different abiotic stresses together are predicted to be responsible for more than 50% of crop loss worldwide (10). All of these lead to insufficient food needs. The main purpose of agricultural production is to produce efficient, quality and healthy nutrients for the increasing population. For these reasons, there is a need to develop sustainable methods that include environment- and human-friendly production systems, and that reduce the use of industrial pesticides and chemical fertilizers.

In sustainable agriculture, beneficial microorganisms obtained from the rhizosphere are used in order to minimize the use of fertilizers and to make maximum use of plant production (11, 12). Bacillus amyloliquefaciens strain is an important plant growth promoter. It can produce secondary metabolites against various soil-borne pathogens. In some of these studies, it has been stated that it can be a biological control agent against Alternaria solani (13), Botrytis cinerea (14) and Agrobacterium tumefaciens (15). Plant growth promoting rhizobacteria (PGPR) are considered the most promising agents in increasing crop yields. Bacillus spp. from PGPR are excellent antagonists with many advantages such as wide distribution, easy isolation and culturing, strong anti-adversity capacity, and especially producing various antibiotics and enzymes with broad spectrum antimicrobial activity. Biocontrol effectiveness may be weak with the direct application of PGPR to soil, but can be improved with the addition of organic matter. Some research on improving soil properties by the application of solid waste has also been reported. The use of bacteria, which are called bio fertilizers or control agents in agriculture, has become widespread after the 1990s. In recent years, the scope of biological fertilization has expanded and free-living rhizobacteria have been started to be used, which promote plant growth, which are used as biological warfare agents or as biofertilizers (16). Bacteria increase the production of growth hormones in plants and the uptake of some minerals such as phosphorus, inhibit ethylene synthesis, produce siderophores, vitamins, antibiotics, and increase resistance to diseases in plants (17, 18).

Mentha piperita L., a medicinally important plant belonging to the Family Lamiaceae commonly known as peppermint is a hybrid of M. spicata L. (spearmint) and Mentha aquatic. Peppermint is widely used as a spice all over the world. It is among the mint species that are mainly cultivated in the world. The fact that its essential oil is the most utilized species also plays a major role in this (19, 20). Peppermint essential oil obtained as a result of distillation ranks first in the world essential oil trade (21). In our country, although it is grown in small scales in almost all regions, it is grown for commercial purposes in our Mediterranean, Aegean and Marmara regions (22, 23).

In this study; The effects of Bacillus amyloliquefaciens, which is defined as a salt tolerant species, on some morphological parameters of peppermint seedlings grown in a climate cabinet under salt stress for six weeks were investigated. The measured parameters are root length (cm), stem length (cm), root width (cm), fresh weight (gr/seedling), dry weight (gr/seedling) and water content (%).

## 2. MATERIALS AND METHODS

In the study, M. piperita seedlings (with several leaves) were propagated vegetatively from cuttings. Bacillus amyloliquefaciens strains, a pure-tolerant species used as PGPB (Plant Growth Promoting Bacteria), were commercially obtained. All viols were placed in the plant growth cabinet 16/8 h light/dark cycle, 19°C and 65% moisture content. It was irrigated with salt solutions (0, 50, 75, 100 mM NaCl) suitable for each seedling group twice a week for six weeks (24, 31). The experiment was continued for six weeks in the plant growth cabinet, with 20 seedlings from each treatment group. There were four repetitive per treatment and five plant samples per repetitive. The steps of the method are given below.

### 2.1. Salt Concentrations

Tort and Türkyılmaz report that NaCl is usually mentioned when it comes to soil salinity and salinity stress (25). Therefore, only NaCl was used to create salt stress. According to similar studies for M. piperita, under field conditions, unsaline, low saline, saline and very saline levels (0-150 mM NaCl) solutions were treated to the potted soil (26). Khorasaninejad et al., in their study, stated that the plants could not tolerate the highest concentration of salt applied to Mentha piperita as 150 mM and they lost their vitality (27). For this reason, the salt concentration was not increased up to 150 mM in this study. Irrigation was done with 30 mL per plant in each irrigation. In the study, salt concentrations were determined as 0, 50, 75, and 100 mM NaCl.

### 2.2. Microbial Fertilizer Application

In the study, CILUS® PLUS brand microbial fertilizer was used, which contains only Bacillus amyloliquefaciens. The solution was prepared according to the usage instructions at a rate of 1.6g microbial fertilizer/10L distilled water. The solution was applied twice (on the day of planting and four weeks later) per plant, at a rate of 30 mL per plant in each irrigation (in addition to 0, 50, 75, and 100 mM NaCl). In the control group, no microbial fertilizer was applied, and the plants were only irrigated with 0, 50, 75, and 100 mM NaCl solutions.

### 2.3. The Morphological Measurements of M. piperita

The experiment was continued for six weeks in the plant growth cabinet, with 20 seedlings from each treatment group. There were four repetitive per treatment and five plant samples per repetitive. Based on the methods applied by other researchers

(28). Six weeks after transplanting into a pot; The root of each plant was removed from the soil before the measurement, washed when necessary and the water was removed with blotting paper. Then, the root and stem lengths and root width of the seedlings were measured with the help of a digital caliper. In order to determine the fresh weight of the plants, the roots of the plants were cleaned from the soil and then dried and weighed on sensitive scales. In the determination of dry weight, the plants were dried in an oven at 65°C for four days until they reached a constant weight and weighed on a sensitive balance. The water content (WC) can be expressed on a dry weight (DW) or fresh weight (32). For this study, calculation was made on fresh weight.

$WC_{(FW\ basis)} = [(FW-DW)/FW] \times 100$ (32)

### 2.4. Statistical Evaluation of Results

Each experiment was repeated at four times. Values are expressed as the means ± SD. For all experiments, the overall data were statistically analyzed in SPSS version 25.0 (IBM-SPSS Inc. USA). Duncan's multiple range tests were used ($p < 0.05$).

## 3. RESULT AND DISCUSSION

Salt stress inhibits plant growth and reduces agricultural production, but it also affects soil physicochemical properties and ecological balance (35, 36). Salinity is therefore an important issue for agriculture worldwide and has been extensively studied over the past decade (37, 38). Various strategies have been employed to mitigate this problem. In particular, developing salt-tolerant cultivars is an efficient strategy, but it is time-consuming, costly, seed-specific, and can pose potential environmental risks (39, 40). Therefore, there is a need to develop simple and inexpensive biological methods. Application of plant growth-promoting rhizobacteria (PGPR) is an effective approach to improve salinity tolerance to reduce agricultural losses caused by salt stress, according to previous studies.

In this study, the effect of Bacillus amyloliquefaciens, one of the plant growth-promoting bacteria, on some morphological parameters of Mentha piperita seedlings grown at various salt stress levels (0, 50, 75, and 100 mM) is presented (Table 1). No statistically significant difference was observed in parameters other than water content (%), when the same concentrations of control and PGPB groups (0, 50, 75 and 100 mM

**Table 1.** The Effect of B. amyloliquefaciens on some growth parameters in M. piperita seedlings grown under salt stress

| Group | NaCl (mM) | Root Lenght (cm) | Stem Lenght (cm) | Root Width (cm) | Fresh Weight (g) | Dry Weight (g) | Water Content (%) |
|---|---|---|---|---|---|---|---|
| Control | 0 | 17.3 ± 4.2$^{c*}$ | 29.8 ± 4.0$^{e}$ | 3.4 ± 0.6$^{f}$ | 16.1 ± 3.3$^{d}$ | 3.2 ± 1.2$^{c}$ | 80.6 ± 3.2$^{a}$ |
| | 50 | 15.7 ± 2.9$^{abc}$ | 19.3 ± 2.3$^{bc}$ | 2.8 ± 0.4$^{cd}$ | 9.7 ± 2.8$^{c}$ | 1.6 ± 0.4$^{b}$ | 83.8 ± 0.9$^{b}$ |
| | 75 | 15.0 ± 3.0$^{ab}$ | 19.8 ± 1.9$^{bc}$ | 2.6 ± 0.4$^{bc}$ | 6.0 ± 0.4$^{ab}$ | 1.0 ± 0.1$^{ab}$ | 84.0 ± 1.5$^{b}$ |
| | 100 | 14.0 ± 2.6$^{a}$ | 16.2 ± 2.0$^{a}$ | 2.3 ± 0.4$^{ab}$ | 5.5 ± 0.8$^{ab}$ | 0.7 ± 0.1$^{ab}$ | 87.1 ± 1.5$^{c}$ |
| PGPB** | 0 | 16.1 ± 2.1$^{bc}$ | 27.3 ± 3.7$^{d}$ | 3.1 ± 0.4$^{e}$ | 15.7 ± 0.9$^{d}$ | 2.5 ± 0.2$^{c}$ | 84.1 ± 0.8$^{b}$ |
| | 50 | 15.0 ± 1.6$^{ab}$ | 21.2 ± 3.0$^{c}$ | 2.9 ± 0.7$^{de}$ | 10.4 ± 1.4$^{c}$ | 1.6 ± 0.4$^{b}$ | 84.7 ± 2.1$^{bc}$ |
| | 75 | 16.6 ± 2.4$^{bc}$ | 18.9 ± 2.4$^{b}$ | 2.7 ± 0.5$^{bcd}$ | 8.4 ± 0.7$^{bc}$ | 1.0 ± 0.1$^{ab}$ | 87.7 ± 0.6$^{c}$ |
| | 100 | 15.2 ± 3.8$^{ab}$ | 15.3 ± 2.2$^{a}$ | 2.1 ± 0.7$^{a}$ | 3.8 ± 0.2$^{a}$ | 0.5 ± 0.1$^{a}$ | 87.8 ± 1.0$^{c}$ |

* Shows values with insignificant difference (P<0.05) for each column shown with same letters.

** Microbial fertilizer was applied in PGPB groups along with salinity

NaCl) were compared (Table 1).

Studies on the effects of B. amyloliquefaciens on M. piperita are very limited. When the effects of bacteria on growth-development parameters in different plants are investigated, Shahzad (2017) showed in a study with rice under salt stress that Bacillus amyloliquefaciens can increase the resistance of ABA synthesis in the plant against stress and as a result, increase agricultural productivity (30). It has been shown that Bacillus amyloliquefaciens can help increase salt tolerance in maize and Lotus japonicus cv Gifu. After being exposed to salt stress for 20 days, it significantly promoted the growth of maize seedlings and enhanced their chlorophyll content compared to the control group (33, 34). It is surprising that Bacillus amyloliquefaciens, which is generally described as a plant growth-promoting bacterium in the literature, did not show a significant increase compared to the control groups in PGPB groups. In fact, when the "0 (unsaline) groups" of the control and PGPB were compared, a significant decrease in stem length and root width was observed. Microbial fertilizer application only resulted in a significant increase in water content at certain salt levels (0 and 75 mM). However, this is not a generalized improvement state. This finding is not consistent with the literature (27, 29). This may be due to the inadequacy of bacterial application frequency. This suggests that it may be due to either insufficient frequency of bacterial application or the inadequate duration of the plant growth period.

## 4. CONCLUSION

Studies on the morphological, physiological, biochemical effects of B. amyloliquefaciens on Mentha spp. (which is grown under salt stress and has commercial importance), are still insufficient. It would be beneficial to focus on similar studies to develop alternative perspectives on sustainable agriculture. It is expected that this study will guide further research.

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**Ethical Statement**: -

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