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Effect of Microbial Fertilizer on The Development of Pepper (*Capsicum annuum* L.) Seedlings Exposed to Salt Stress

Halil İbrahim ÖZTÜRK[1*] **Atilla DURSUN**[2,3]

[1] Health Services Vocational School, Erzincan Binali Yıldırım University, Erzincan, TÜRKİYE
[2] Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum, TÜRKİYE
[3] Department Horticulture and Agronomy, Faculty of Agriculture, Kyrgyzstan -Turkey Manas University, Bishkek, KYRGYZSTAN

*Corresponding author: hiozturk@erzincan.edu.tr

A B S T R A C T

Salinity is an important abiotic stress factor that negatively affects plant growth and yield. The study, the effect of microbial fertilizer containing *Bacillus subtilis* bacteria, commercially named Subtima, on salt stress in pepper was observed. Two different saline solutions (100 mM and 200 mM) were prepared to create salt stress in the plant growing medium. The fertilizer solution was applied to the plants in 4 different concentrations (300, 400, 500 and 600 ppm) from the leaves in the form of a spray. Morphological parameters such as plant height, stem length, leaf length, leaf width, and petiole length were measured. It was found that salt stress resulted in a decrease in plant morphological characteristics compared to the control group (0 mM salt + 0 ppm fertilizer). It was found that the most effective fertilizer dose to increase plant height (25.23 cm) at 100 mM salt stress was 400 ppm. At 200 mM salt stress, it was found that the most effective fertilizer dose for increasing plant height (22.67 cm) was an application of 300 ppm application. In general, fertilizer applications were found to be effective in reducing the negative effects of salt on the morphological traits studied.

A R T I C L E I N F O

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INTRODUCTION

Stress factors that cause many regressions resulting in low yield in the plant are classified in two ways as abiotic and biotic (Yildiz et al. 2022). Salinity stress, which is the subject of this study, is one of the important abiotic stress factors that limits plant productivity all over the world (Tuna and Eroğlu 2017; Yaman et al. 2020). In general, salt damage shows its effect on the form of a smaller structure, a slowdown in growth due to a decrease in the number and area of leaves. It has been reported in different studies that salinity in irrigation water and soil creates negative effects on plant growth and productivity. Salt, with the high osmotic pressure it creates, reduces the water intake of the roots and negatively affects the uptake of nutrients such as K^+, Ca^{+2}, Mn^{+2} and NO_3^- (Altunlu 2020). The main goal increasing in the development of plants grown in a salty environment should be to restore the impaired osmoregulation. In saline conditions, the use of resistant cultivars as rootstock (Colla et al. 2010; Huang et al. 2010), applications that increase in the water uptake of the plant (Aroca et al. 2012), plant nutrients such as K and Ca, whose intake is decreased, from leaves or soil application (Amjad et al. 2016), the exogenous application of some substances such as proline, salicylic acid and melatonin to increase in resistance (Li et al. 2012) have been reported to increase in the plant's resistance to salt. It is a known fact that there is a symbiotic bond between the microorganisms in the soil and the plants (Çirka et al. 2022). In recent years, the use of rhizobacteria (PGPR), which promote plant growth as a biofertilizer, has been increasing in different plant species. The use of rhizobacteria directly and indirectly promotes the development of the plant by competing with harmful microorganisms, increase in the uptake of nutrients in the form that cannot be taken in the soil, increase in the use of water by the plant by promoting seed germination and root development with its metabolites acting as plant hormones (El-Katatny and Idres 2014). The most important genera of PGPR are *Pseudomonas* and *Bacillus*. PGPRs have significant potential for reducing abiotic stresses and for sustainable, agricultural production systems. *Bacillus* species can form endospores that are extremely resistant to harsh environmental conditions and secrete metabolites that promote plant growth and health. The successful use of beneficial microbes is thus a model for increasing stress tolerance and adapting to climate change (Hashem et al. 2019). Salt stress significantly reduces plant growth and yield in pepper cultivation, which is a moderately sensitive species to salinity.

In this study, the effects of microbial fertilizer containing *Bacillus subtilis* bacteria on some plant growth parameters in pepper plants under different salinity conditions were investigated.

MATERIALS AND METHODS

The study was carried out in greenhouse conditions between March-July 2022. Görkem F1 pepper variety was used in the experiment. The seedlings were grown in 128 compartment viols, 40 x 40 mm each. A mixture of 60% peat and 40% perlite was used in the seedling growing medium. The seedlings were planted in pots (50x15x20 cm) 35 days after sowing. Pots were filled with a 2:1 ratio of soil:sand and a bulk density of about 1.30 g cm^3. Irrigation was done with 3 levels of NaCl (0, 100 and 200 mM). Subtima (Content: $1x10^9$ Kob/ml *Bacillus subtilis* NGSR) commercial fertilizer supplied from NG-BioTechnology Company was used as microbial fertilizer. The fertilizer solution was prepared in 5 different concentrations as 0, 300, 400, 500 and 600 ppm doses. The solutions were sprayed on the upper and lower sides of the leaves the day before planting and then regularly (15 ml per plant) every week until harvest. Salinity treatments were started 2 days after the seedlings were planted and NaCl was added in 50 mM increments to prevent osmotic shock to the plants. Salt water was applied regularly until the end of the harvest. At 45 days after planting, morphological parameters were measured.

Grow characteristics

Plant height (cm), hypocotyl height (cm), leaf blade width (mm), leaf blade length (mm) and petiole height (mm) were measured to determine the seedling development. Plant height (cm) and hypocotyl height (cm) were measured with tape measure and leaf blade height (mm) leaf blade width (mm), petiole height (cm) was measured with a digital caliper.

Analysis of Data

The research was carried out in accordance with the "factorial trial" trial design with 2 factors (fertilizer application and salt stress) and 3 replications. The data obtained from the research were evaluated in the SPSS 22 statistical package program; Duncan test, one of the multiple comparison tests, was used to determine the differences between applications.

RESULTS

In the study, statistically significant differences were found at 1% significance level among all morphological parameters examined after salt and fertilizer applications (Table 1).

Table 1. *The effect of salt and microbial fertilizer applications on some plant morphological parameters*

Salinty	Fertilizer	PHE	HH	LBL	LBW)	PTH
(mM)	(mg L^{-1})	(cm)	(cm)	(mm)	(mm)	(mm)
0	0	39.33±5.50^{a}	9.00±1.00^{a}	110.57±5.37^{a}	69.73±0.89^{a}	53.33±2.97^{a}
100	0	20.67±0.57^{b-e}	5.17±0.76^{bc}	83.60±4.45^{cd}	45.57±2.80^{c-e}	37.03±3.96^{bc}
	300	24.83±4.85^{bc}	7.83±1.75^{a}	89.43±4.02^{bc}	53.60±4.15^{b}	39.07±1.40^{b}
	400	25.23±1.16^{b}	8.00±1.00^{a}	85.77±4.29^{b-d}	52.60±2.78^{b}	39.00±3.10^{b}
	500	19.33±1.32^{c-e}	5.67±0.76^{b}	92.67±4.66^{b}	48.27±1.85^{b-d}	37.93±4.65^{b}
	600	19.50±2.29^{c-e}	5.27±0.76^{bc}	85.53±6.01^{b-d}	50.03±2.34^{bc}	39.03±6.67^{b}
200	0	16.33±1.52^{e}	3.67±0.57^{c}	72.33±4.38^{f}	41.70±5.48^{e}	30.90±3.24^{cd}
	300	22.67±2.08^{b-d}	5.33±0.57^{bc}	80.37±6.07^{c-f}	48.40±3.55^{b-d}	36.60±3.08^{bc}
	400	20.17±0.28^{b-e}	5.67±0.76^{b}	81.47±6.13^{c-e}	46.80±2.07^{c-e}	33.03±1.61^{bc}
	500	19.33±4.72^{c-e}	5.17±0.76^{bc}	77.27±3.66^{d-f}	43.37±1.64^{de}	31.37±1.20^{cd}
	600	17.67±1.52^{de}	5.00±0.50^{bc}	73.70±2.13^{ef}	43.43±2.92^{de}	31.83±0.55^{cd}

PHE: Plant height, HH: Hypocotyl height, LBL: Leaf blade length, LBW: Leaf blade width, PTH: Petiole height; Means, denoted by different letters in each column, differ significantly at $p \leq 0.01$.

Significant reductions in plant height were detected in the study due to salt stress. The lowest plant height was observed in 200 Mm NaCl application. While it was determined that fertilizer application alleviated salt stress in general, it was determined that the most effective dose was 400 ppm fertilizer solution at 100 mM salt stress. However, the most effective dose was found to be 300 ppm at 200 mM salt stress (Figure 1).

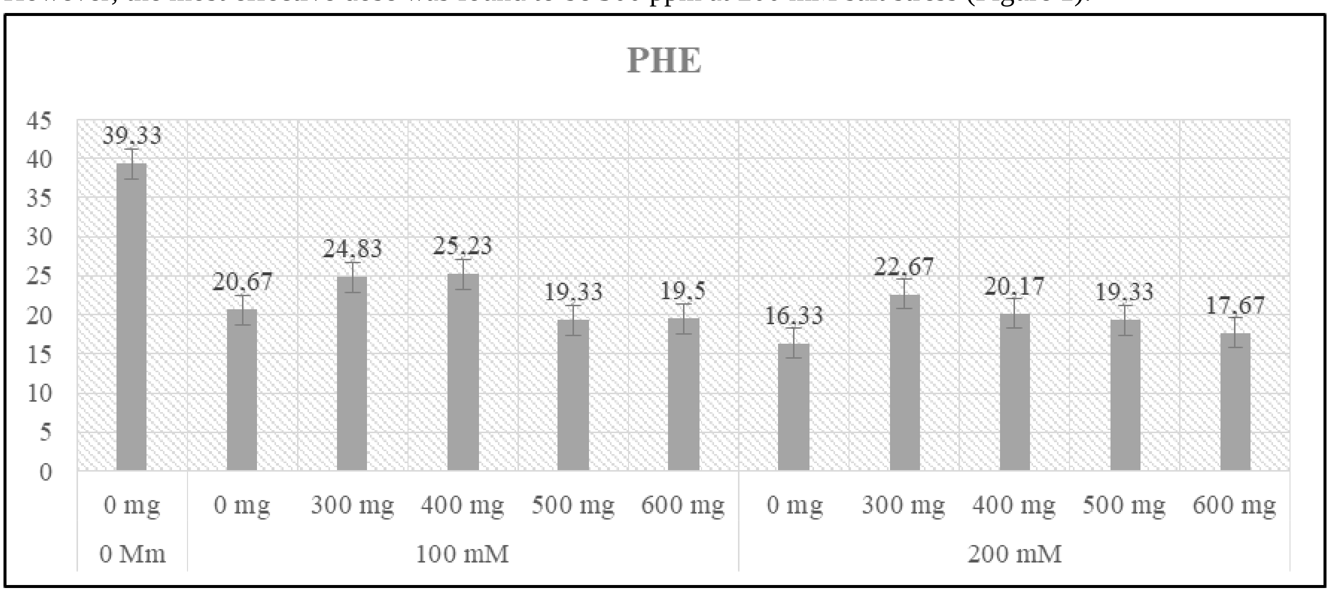

Figure 1. *Effect of salt stress and fertilizer applications on plant height*

The hypocotyl height measured in the control application, 0 mM salt and 0 mg L^{-1} fertilizer application, was measured as 9 cm. While 100 mM of salt application caused a decrease of approximately 42.5% in hypocotyl height, this rate was determined as 59.2% in 200 mM salt application. In the evaluation made considering the hypocotyl height, the most effective fertilizer concentration in reducing salt stress in 100 mM and 200 mM salt applications was 400 mg L^{-1} (Figure 2).

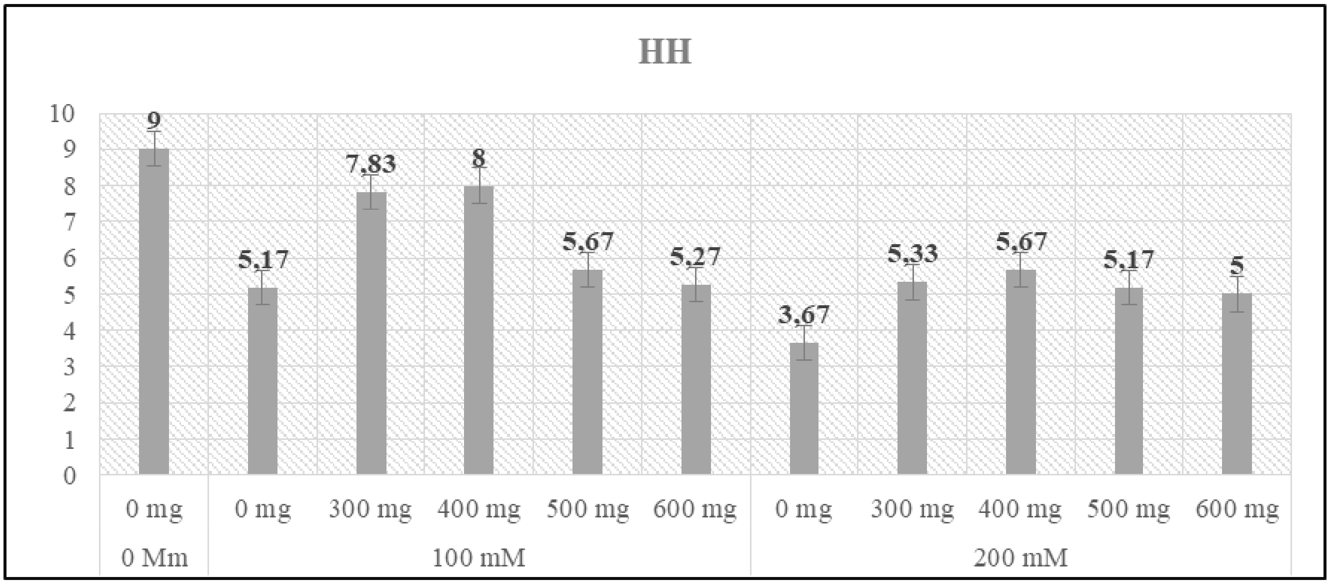

Figure 2. *Effect of salt stress and fertilizer applications on hypocotyl height*

Leaf blade length decreased due to salt stress. At 100 mM and 200 mM salt stress, leaf blade length was measured as between 83.60 mm and 72.33 mm. While the most effective fertilizer application was 500 mg L^{-1} at 100 mM salt stress, it was 400 mg L^{-1} at 200 mM salt stress (Figure 3).

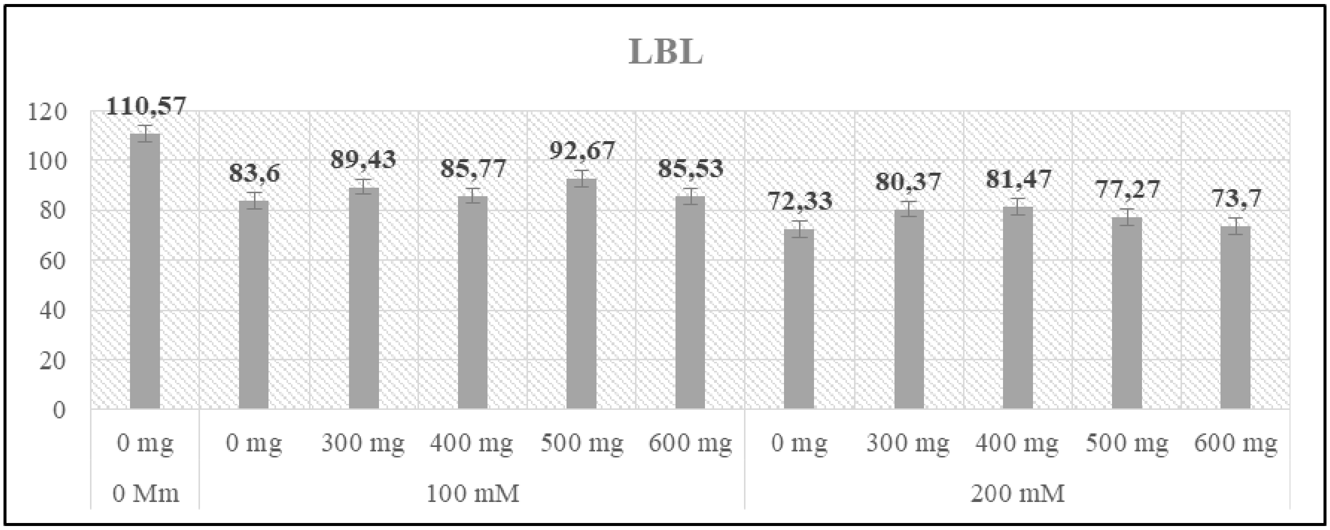

Figure 3. *Effect of salt stress and fertilizer applications on leaf blade length*

There was a 34.65% and 40.20% reduction in leaf blade length at 100 mM and 200 mM salt stress, respectively. Fertilizer application of 300 mg L^{-1} was the most effective dose to reduce the negative effects of both salt levels on leaf blade length (Figure 4).

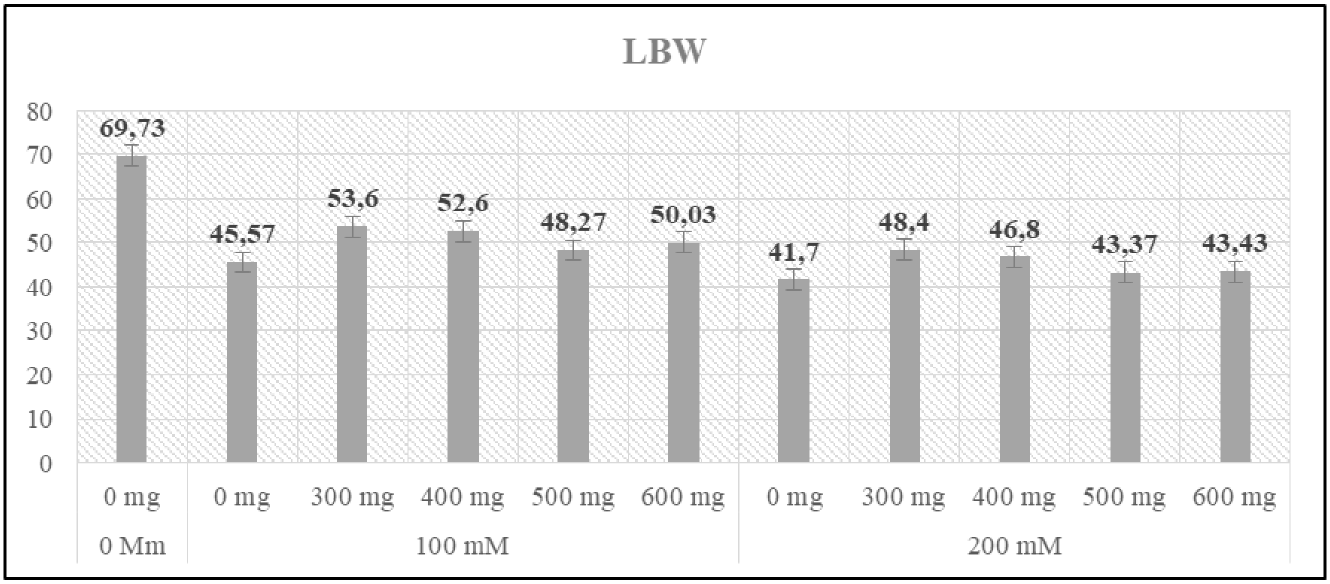

Figure 4. *Effect of salt stress and fertilizer applications on leaf blade width*

As with other morphological features, petiole height was adversely affected by salt stress. However, fertilizer applications were generally effective in reducing the effect of salt stress. Considering the petiole length, 300 ppm microbial fertilizer application under both 100 mM and 200 mM salt levels were the most effective application in alleviating the effect of stress (Figure 5).

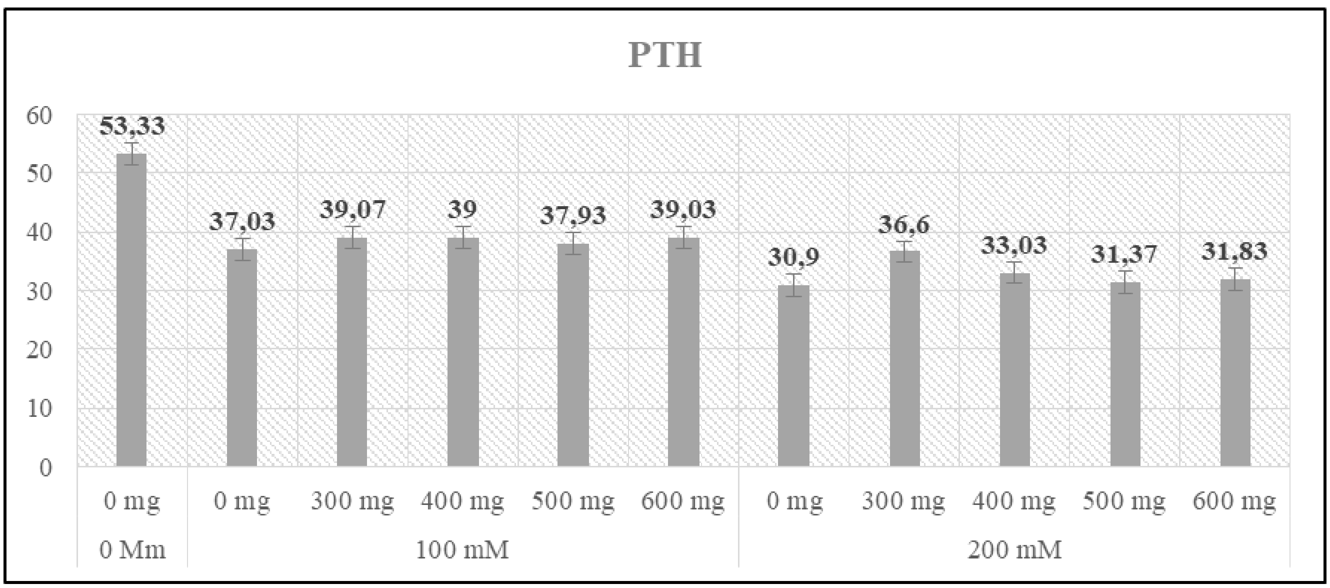

Figure 5. *Effect of salt stress and fertilizer applications on petiole height*

DISCUSSION

In many studies, it has been reported that the plant growth of cultivated plants is adversely affected in salty conditions (Del Amor et al. 2001; Abd El-Azeem et al. 2012; Wu et al. 2019; Zhang et al. 2020). Salt minerals in the water and soil increasing in the osmotic pressure around the root and cause a decrease in the water uptake of the roots (Nizam 2011). In addition, the intake of nutrients is adversely affected. After the salt is taken by the plant, when it rises above a certain density, it destabilizes the membranes and breaks down organelles such as chloroplast (Loudari et al. 2022). Therefore, the development of plants slows down or in case of prolonged exposure to salt, death may occur in plants (Altunlu 2020). In this study, it was determined that salt stress affected the development of pepper plants negatively. In a study, it was determined that there was a significant decrease in the height of pepper plants due to salt stress (Yakupoğlu 2020). The microbial fertilizer containing *Bacillus subtilus* used in the study alleviated the stress caused by salt application on plant height. Aydın et al. (2022) reported that the application of bacteria (*Trichoderma harzianum* T78) in tomato plants grown under salt stress reduced the negative effect of stress on plant height. In another study, mycorrhiza (*Glomus intraradices*) and rhizobacteria (*Bacillus subtilis*) applications had a positive effect on plant and stem growth in pepper against to salt stress (Altunlu 2020). In many similar studies, it has been reported that plant growth promoting bacteria (PGBR) have positive effects on the growth of many vegetable species such as tomatoes, peppers, beans and lettuce in salty conditions (Grover et al. 2011). It has been reported that some volatile organic compounds emitted from Bacillus regulate Na^+ recirculation in the whole plant under saline conditions (Zhang et al. 2008). *Bacillus* bacteria applied externally in lettuce plants exposed to salt stress were effective in stem development of the plant (Vivas et al. 2003). In another study, it was determined that different Plant Growth-Promoting Rhizobacteria promoted the development of plant height and stem height in pepper plants against salt stress (Hahm et al. 2017). In a study conducted; Three PGPR strains (*Microbacterium oleivorans* KNUC7074, *Brevibacterium iodinum* KNUC7183, and *Rhizobium massiliae* KNUC7586) have been reported to help pepper seedlings tolerate salinity stress, although 200 mM salt concentration creates salt stress in pepper seedlings and the adverse effects of salt stress on plant growth and development have not been completely eliminated. In study, the height of pepper seedlings under salt stress was 11.7%, 17.7% and 14.0% higher in M. oleivorans KNUC7074, B. iodinum KNUC7183 and R. massiliae KNUC7586 applications compared to the control (0 NaCl, 0 PGPR) (Hahm 2017). In the study in which Bacillus strains were used against the negative effects of salt stress on wheat plants; It was determined that 200 mM salt concentration had a negative effect on plant growth parameters, but the applied Bacillus strains positively encouraged plant growth under salt stress conditions (Ayaz et al. 2022). The results of the researchers and our findings support each other.

CONCLUSION

Salinity is an extremely important problem that restricts agricultural production in the world. In our study, increasing in salt concentration had a negative effect on pepper plant growth. It has been determined that *Bacillus subtilis* rhizobacteria, which have important functions in plant growth, alleviate the effect of stress under salt stress conditions. According to the research findings, the feasibility of *Bacillus subtilis* microbial fertilizer in pepper plants under salt stress conditions was revealed.

CONFLICT OF INTEREST

The authors declare no conflict of interest in the study.

AUTHORS CONTRIBUTION

All authors contributed equally.

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