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ORIGINAL PAPER

BORON TOXICITY AND PGPR PHYTOREMEDIATION EFFECTS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF MEDICAL SAGE (*SALVIA OFFICINALIS* L.)

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Abstract

In this study, the effects of boron doses (0=control, 5, 10, 20 mM) and some beneficial rhizobacteria (*Azospirillum lipoferum, Bacillus megaterium, Frateuria aurentia*) on the physiological and biochemical parameters of medicinal sage (*Salvia officinalis* L.) were investigated. The study was carried out in factorial order in a completely randomized experimental design with four replications in a controlled climate chamber. In the study, chlorophyll a, chlorophyll b, total chlorophyll, total carotenoid, total anthocyanin, nitrogen balance index, MDA, relative water in leaf tissues, ion leakage, membrane durability, total phenolic substance, total flavonoid substance, and total antioxidant activity parameters were investigated. Depending on the applied boron doses, increases were observed in total phenolic, total flavonoid, total carotenoid, ion leakage, and MDA values. In contrast, decreases in the nitrogen balance index and membrane durability were observed. It has been determined that PGPR applications effectively reduce the damage of boron by increasing the total anthocyanin, total flavonoid, chlorophyll b, total chlorophyll amounts, and membrane durability, and contributing to the decrease of MDA values compared to the control.

Keywords: anthocyanin, boron toxicity, MDA, PGPR, *Salvia officinalis*, total antioxidant

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INTRODUCTION

The Lamiaceae family contains approximately 240 genera and 7000 species worldwide (Botanica 2009). Salvia genus is the richest genus of the Lamiaceae family with 1000 species (Walker, Sytsma 2007). *Salvia officinalis* is a perennial herbaceous plant with woody stems, grayish leaves, and blue to purple flowers (Barrett et al. 2000). It grows naturally in the Mediterranean, Southeast Asia, and South America and is cultivated in many countries of the world with continental climates such as Ukraine, Moldova, Germany, Italy, England, Türkiye, India, Japan, and South Africa (Grdiša et al. 2015). *S. officinalis*, which does not have a natural distribution area in Türkiye, is cultivated especially in the Aegean, Mediterranean, and Marmara regions (Bağdat 2006). Due to the phenolic compounds, terpenoids, and essential oils it contains, medicinal sage has a wide range of uses such as antidiabetic, antimicrobial, anti-inflammatory, antispasmodic, cardiovascular support, and anticancer (Ghorbanpour et al. 2016). It is known that the flowers and leaves of medicinal sage are frequently used for mouth sores, pharyngitis, antiseptic and analgesic purposes, nervous system stimulant and sedative, and digestive facilitators in Turkey (Miraj, Kiani 2016).

The boron element in plants is mainly involved in the transport of metabolites, hormones, and various ions produced in the cell membrane and wall, many enzyme activities, and biochemical processes (Dordas et al. 2000). In case of insufficient amount of boron mineral from the soil in natural or cultural environments, weakening of the root organ, disruptions in carbohydrate metabolism and transport, problems in the production of nucleic acids, difficulties in transferring mineral substances from the soil to the plant, rots above and below the ground, plant height and dryness. It manifests itself as reductions in substance accumulation and prolonging fruit ripening time (Zhao Oosterhuis 2003, Wimmer, Eichert 2013, Behboudian et al. 2016). Boron has a toxic effect on the plant when found in high concentrations in the soil. The symptoms of boron, which is at toxic levels in the plant, show themselves with carbon dioxide assimilation, photosystem chemistry, carbohydrate metabolism, antioxidant systems, and typical chlorosis and necrosis observed in the leaf (Han et al. 2009, Reid 2013). Boron, which is an essential nutrient for plants, exists in nature in different concentrations like every element. While the presence of boron in different concentrations is excessive for some plants, it may be below the desired amount for some plants. In this respect, keeping boron at certain intervals plays a critical role in the growth and development of plants (García-Sánchez et al. 2020). They reported that as a result of the use of various agrochemicals, contamination of the soil increased and limited plant production (Martínez et al. 2019). Some methods are applied to prevent boron toxicity in plants. The main ones of these methods are; to reduce the boron level in the soil and thus to limit the plant's boron uptake; to reduce the transport of boron between cells with the help of various substances; to increase the physiological endurance of the plants with the use of nutrient supplements, plant growth regulators, and microorganisms involved in plant growth and development (Hua et al. 2021).

Rhizobacteria promoting plant growth and development (PGPR=Plant Growth Promoting Rhizobacteria) has been started to be used with many beneficial aspects such as; increasing the plant's mineral substance uptake from the soil; increasing the quality and yield of the crop; protecting the plant from the harms of synthetic fertilizers; increasing its resistance to adverse environmental conditions (Qiu et al. 2019, Khan et al. 2021).

Samreen et al. (2019), in their study on canola, determined that the strain belonging to the genus *Bacillus*, which is tolerant to boron, was effective in dissolving phosphate in alkaline-lime soil and contributed positively to the growth of the plant. It has been reported that the *Bacillus* strain contributes positively to phosphate uptake and growth of maize grown in chromium-contaminated soil (Afzal et al. 2020). Iftikhar, Toru (2010) determined that the *Bacillus* strain isolated from the soil can live at a high boron level (450 mmol L^{-1}) and that the plants living in the environment keep the intracellular boron level at a lower level than the extracellular one. Additionally, it was reported that species belonging to the genus *Azospirillum* support root growth and development, increase yield, and reduce drought stress damage (Czarnes et al. 2020). Therefore, it has become imperative to understand the molecular and physiological mechanisms that govern microorganism-plant interactions in order to successfully formulate beneficial microorganisms living in the soil and design efficient application programs (Lephatsi et al. 2021).

In the light of the information stated above, this study was carried out to determine the effects of different boron doses and some PGPR applications on the physiological and biochemical parameters of medicinal sage.

MATERIAL AND METHODS

Material

The trial was carried out in 2021 in a fully controlled climate cabinet belonging to the Van YYU Faculty of Agriculture, Field Crops Department. In the research, medicinal sage (*Salvia officinalis* L.) seeds, which is an important medicinal and aromatic plant used as a raw material in various perfumery, pharmaceutical, food, and cosmetic industries, were used as seed material. The seeds were obtained from the Van YYU medicinal and aromatic plants garden.

Method

The experiment was carried out in factorial order with four replications according to the completely randomized experimental design. While boron contributes to growth and development when found in trace amounts in the plant, it is an element that causes toxicity at high concentrations. In this study, some beneficial bacteria (PGPR=Plant) that survive in the soil rhizosphere contribute to plant growth and development with different doses of boric acid (H_2BO_3) form (B0 – control, B1 – 5, B2 – 10, and B3 – 20 mM) as a boron source. Growth Promoting Rhizobacteria) ((P0 – =Control, P1 – *Azospirillum lipoferum* (1x106 kob ml^{-1}), P2 – *Bacillus megaterium* (1x105 kob ml^{-1}) and P3=*Frateuria aurentia* (1x105 kob ml^{-1})). First of all, seed surfaces after sterilizing the seeds and sterilized with 3% sodium hypochlorite, they were planted in viols filled with peat (3/4) and perlite (1/4). Germination started on average three days after planting, and after one week, misfire was done so that only one plant remained in the viols. 28 days after planting, when the plants had 4-5 leaves, they were transferred to one-liter pots with 1/3 of peat (Klassman) and 2/3 of garden soil by volume. After planting, the pots were placed in a climate chamber with a temperature of 25°C and a humidity of 65% in a light/dark photoperiod of 16/8 hours. The pots, whose water holding capacity was measured as 230 ml on average, were given distilled water after planting, and they reached the pot capacity. Four days after all the seedlings were transferred to the pots, an average of 120 ml of distilled water was given every other day for 18 days. In the experiment, 20 days after germination, the solution consisted of ammonium sulphate (21%), triple super phosphate (46%), and potassium sulphate (50% K_2O and 16-20% S) fertilizer mixture was given once as basic fertilizer. Two days after the fertilizer application, bacterial solutions prepared at a dose of 10 ml L^{-1} were applied to the pots where bacteria should be applied, three times with an interval of four days, instead of irrigation water. Control applications were given pure water only. Boron applications were made three times in 3-4 day periods in the pots in which bacteria applications were made. Attempt; It was terminated when the signs of boron stress became evident (36 days after transfer to pots).

Nitrogen balance index (dualex value) and total anthocyanin (dualex value)

Before the experiment was terminated, nitrogen balance index and anthocyanin ratio were measured from leaves with dualex scientific+ (FORCE-A, France) device.

MDA (nmol g^{-1})

In the study, the amount of malondialdehyde (MDA), the end product of lipid peroxidation, was determined according to the methods of Heath, Packer (1968) and Sairam, Saxena (2000). 0.5 g of leaf tissue sample taken from the plant was homogenized with 10 ml of 0.1% trichloroacetic acid (TCA), and then the homogenate was centrifuged at 15000 rpm for 5 minutes. The 1ml of the centrifuged sample was taken from the supernatant, and 0.5% thiobarbituric acid (TBA) dissolved in 4 ml of 20% TCA was added. After the mixture was kept in a 95°C water bath for 30 min, cooled rapidly in an ice bath, and centrifuged at 10000 rpm for 10 minutes, the absorbance of the supernatant was determined at 532, and 600 nm wavelengths and the malondialdehyde (MDA) content was calculated.

Total phenolic substance amount (mg GA 100 g^{-1})

In the determination of the total amount of phenolic substance, the Folin-Cicaltea spectrophotometric method, which was modified by Obanda et al. (1997), was used. The Folin-Cicaltea solution was diluted at 1:3 in volume. For the saturated sodium carbonate (35%) solution, 87.5 g of sodium carbonate was dissolved in distilled water, made up to 250 ml and left overnight, then filtered. The stock solution (500 µg ml^{-1}) was prepared by dissolving 50 mg of gallic acid in 100 ml of distilled water. For the gallic acid working solution, every 500 µg ml^{-1} gallic stock solution was prepared in 5 ml measuring balloons as 9 separate solutions with concentrations ranging from 0-55 µg ml^{-1}. 1 ml of these solutions was taken and mixed with 1 ml of Folin-Cicaltea solution. After waiting for 5 min, 2 ml of sodium carbonate was added and shaken, and diluted with 2 ml of water. After this mixture was kept in the dark for 30 min, the absorbance value was read in the spectrometer at a wavelength of 700 nm. A calibration curve was obtained by graphing the absorbance values read against these different concentrations of gallic acid (r^2=97.47).

Total flavonoid substance amount (mg QE 100 g^{-1})

The determination of the total flavonoid substance was performed according to the method developed by Quettier-Deleu et al. (2000). 2 ml of 2% $AlCl_3$ was added to 2 ml of extract and left in the dark for 1 h at room temperature. The total flavonoid contents of the extracts were measured with a spectrophotometer at a wavelength of 415 nm by performing 2 parallel studies in each sample and calculated in mg QE (Quercetin Equivalent) 100 g^{-1} by using the calibration curve prepared using standard quercetin.

Total antioxidant activity amount (mg TE g^{-1})

In order to determine the total antioxidant activity, 2 g of sage leaves were weighed, 4 ml of methanol was added, and the material passed through the homogenizer was centrifuged at 10000 rpm for 10 minutes, and the supernatant remained on top was taken. Then, 10 mmol L^{-1} 2,4,6-tripyridyl-s-triazine (TPTZ) was prepared by dissolving 300 mM acetate buffer (pH 3.6) in 40 mM HCl. Then, the FRAP reagent was prepared by mixing the prepared TPTZ solution with 20 mmol L^{-1} $FeCl3.6H_2O$ solutions at 10:1:1 ratios. The mixture prepared for ABTS (2.2-Azinobis 3-ethyl-benzothiazoline-6-sulfonic acid) analysis with 2850 µL of FRAP reagent was diluted 50 times with ethanol, then 150 µL of sage leaf sample was mixed and kept at room temperature for 30 minutes. The resulting ferrustripyridyltriazine complex was measured at 593 nm in the spectrophotometer, and the results were reported as mg Trolox g^{-1} (Lutz et al. 2011). Trolox concentration range has been studied as 0-500 ppm.

Membrane strength index (%), Ion leakage (%), and Relative water content (%)

Membrane resistance index, ion leakage, and relative water content ratio in leaf tissues were determined according to the method described by Premachandra et al. (1990) and Sairam, Saxena (2000).

Amounts of chlorophyll a (μg g^{-1} FW), chlorophyll b (μg g^{-1} FW), total chlorophyll (μg g^{-1} FW), and total carotenoid (μg g^{-1} FW)

In the analysis of photosynthetic pigments determined according to Lichtenthaler (1987), 0.2 g (200 mg) fresh plant sample was extracted with 10 mL 80% acetone and centrifuged at 4600 rpm (rpm) for 15 minutes. The absorbance values of the aliquots taken after centrifugation at 663, 645, and 470 nm wavelengths were determined in a spectrophotometer (PG T60 UV-VIS) and recorded. Calculations were made with the help of the formulas given below:

1. chlorophyll a – $11.75 \times A662 - 2.350 \times A645$;
2. chlorophyll b – $18.61 \times A645 - 3.960 \times A662$;
3. total chlorophyll – chlorophyll a + chlorophyll b;
4. total carotenoid = $(1000 \times A470 - 2.270 \times \text{chlorophyll a}) - (81.4 \times \text{chlorophyll b}/227)$;

A – absorbance value;
FW – fresh weight.

Statistical analysis

Statistical analyzes of the data obtained as a result of the study were performed with the COSTAT (version 6.303) package program according to a two-way full random experimental design and grouped according to the LSD multiple comparison test at the 5% level.

RESULTS AND DISCUSSION

Total anthocyanin content

While the effect of boron applications and PGPR × boron interaction on the total anthocyanin amount was found to be statistically insignificant, the effect of PGPR applications was significant at the 5% level. According to PGPR applications, the highest value was obtained from P1 applications with 0.09, the lowest value (0.06) was obtained from P0 applications which show non statistically significant differences with P2 and P3 applications (Table 1). Boron application's mean values were in the range of 0.081 - 0.071 (Table 1). Although there was no statistically significant difference in total anthocyanin values in our study, it was observed that it gradually decreased

Table 1

The effects of boron doses and some PGPR applications on the biochemical parameters of medicinal sage

Applications**		Total anthocyanin (dualex value)	Total phenolic substance (mg GA 100 g^{-1})	Total flavonoid substance (mg QE 100 g^{-1})	Total antioxidant activity (mg TE g^{-1})
PGPR*	Boron				
Control (P0)	**B0**	0.07	222.04 *ab*	6.28 *de*	48.11 *a-c*
	B1	0.06	216.23 *b*	7.39 *de*	48.90 *a-c*
	B2	0.07	225.74 *ab*	10.86 *bc*	65.47 *ab*
	B3	0.07	231.86 *ab*	7.19 *de*	67.97 *a*
Mean		**0.07 B**	**225.27**	**7.93 B**	**57.61 A**
P1	**B0**	0.09	217.21 *b*	8.07 *c-e*	45.75 *bc*
	B1	0.09	224.00 *ab*	5.46 *e*	47.97 *bc*
	B2	0.08	221.05 *ab*	8.07 *c-e*	57.14 *a-c*
	B3	0.12	250.27 *a*	9.75 *b-d*	45.67 *c*
Mean		**0.10 A**	**228.13**	**7.89 B**	**49.13 B**
P2	**B0**	0.10	244.89 *ab*	8.39 *b-e*	58.53 *a-c*
	B1	0.08	222.66 *ab*	8.95 *b-d*	50.75 *a-c*
	B2	0.06	227.46 *ab*	14.64 *a*	40.84 *c*
	B3	0.07	240.73 *ab*	9.21 *b-d*	55.47 *a-c*
Mean		**0.08 B**	**233.93**	**10.29 A**	**51.39 AB**
P3	**B0**	0.06	220.49 *ab*	11.88 *a-c*	41.03 *c*
	B1	0.08	233.18 *ab*	12.53 *ab*	58.01 *a-c*
	B2	0.08	214.25 *b*	7.48 *de*	38.67 *c*
	B3	0.07	231.29 *ab*	8.93 *b-d*	40.89 *c*
Mean		**0.07 B**	**224.80**	**10.20 A**	**44.64 B**
Boron (B) ***	**B0**	0.078	226.15 *B*	8.65 *B*	48.35
	B1	0.077	224.01 *B*	8.58 *B*	51.40
	B2	0.071	222.12 *B*	10.26 *A*	50.53
	B3	0.081	239.54 *A*	8.76 *B*	52.50
PGPR LSD ($P \leq 0.05$)		0.01	ns	1.39	7.46
Boron LSD ($P \leq 0.05$)		ns	11.20	1.39	ns
PGPR×Boron LSD ($P \leq 0.05$)		ns	38.79	4.84	25.85
CV		19.04	5.90	18.53	17.70

* There is no difference between the means indicated by **capital bold** letters in the same column.

** There is no statistical difference between the means shown in small letters in the same column.

*** There is no difference between the means shown in *capital italic* letters in the same column.

GA – gallic acid, **QE** – quercetin equivalence, **TE** – trolox equivalence, ns – non-significant (at 5% level).

until the B3 dose but increased at the B3 dose. In studies with different plants, it can be seen that anthocyanin values can increase or decrease in various stress situations. It was reported that increases in total anthocyanin values were also observed due to increasing chromium doses in beans

(Mahdavian 2021). It has been reported that decreases in total anthocyanin value are observed in pigweed plants due to arsenic presence in the soil (Gajić et al. 2020). Additionally (Balci et al. 2020), in their study on raspberry, reported that as a result of PGPR applications, increases in the amount of anthocyanin were observed compared to the control.

Total amount of phenolic substance

As a result of the statistical analysis, the effect of boron doses and PGPR × boron interaction on the total phenolic substance was statistically significant at the level of 5%. In contrast, the effect of PGPR applications was found to be insignificant. As seen in Table 1, according to boron applications, the highest amount of total phenolic substance was obtained from B3 with 239.54 GA 100 g^{-1}, while the lowest amount (222.12 GA 100 g^{-1}) was obtained from B2, which did not differ statistically from B0 and B1. It was determined that the PGPR treatments' mean values were in the range of 233.93 - 224.80 mg GA 100 g^{-1}. The highest value in the PGPR × boron interaction was obtained from the interaction of 250.27 GA 100 g^{-1} with P1 × B3 application (Table 1). It was stated that non-enzymatic tocopherol, phenolics, flavonoids, and carotenoids, which are antioxidant compounds, play a role in reducing the harmful effects of free radicals due to toxic boron and increase in various stress situations (Hua et al. 2021). The increase in total phenolic, total flavonoid, total antioxidant, and total carotenoid amounts depending on boron doses in this study results confirms previous studies.

Total amount of flavonoid substance

The effect of PGPR, boron doses, and PGPR × boron interaction on the total flavonoid substance amount was found to be statistically significant at the rate of 5%. According to PGPR applications, the highest value was obtained with 10.29 mg QE 100 g^{-1} from P2 applications and was in the same statistical group as P3. The lowest value was obtained from P1 applications with 7.89 mg QE 100 g^{-1} and did not show any statistical difference with P0. According to boron applications, the highest value was obtained from B2 with 10.26 mg QE 100 g^{-1}, and the lowest value was obtained from B1 with 8.58 mg QE 100 g^{-1} (statistically indifferent with B0 and B3). The highest value in PGPR × boron interaction was obtained from 14.64 mg QE 100 g^{-1} and P2 × B2 interaction (Table 1). It is reported that flavonoids, which are secondary metabolites, protect the plant in biotic and abiotic stress situations and interact with various beneficial rhizobacteria living in the soil and alleviate stress symptoms (Shah, Smith 2020).

Total antioxidant activity

It was determined that the effect of PGPR applications and PGPR × boron interaction on total antioxidant activity was statistically significant at the level of 5%, while boron doses had no significant effect. According to the PGPR applications, the highest value was obtained from P0 with 57.61 mg TE g^{-1}, and the lowest value (44.64 mg TE g^{-1}) was obtained from the applications of P3, which had no statistical difference with the P1 application. As seen in Table 1, mean values obtained as a result of boron applications were found in the range of 52.5-48.35 mg TE g^{-1}. The highest value in PGPR × boron interaction was obtained from 67.97 mg TE g^{-1} and P0 × B3 applications. It has been reported that various rhizobacteria are effective in protecting the plant against stress by increasing enzymatic and non-enzymatic antioxidant compounds such as flavonoids and carotenoids in different plants grown under salt stress (Sapre et al. 2019). It was determined that the study results are in agreement with the literature.

Relative water content, membrane strength index, and rate of ion leakage

The effects of PGPR, boron doses, and PGPR × boron interaction on the relative water content in leaf tissues were not statistically significant. While the range of PGPR applications was 88.06-85.32%, the range of boron applications was 90.56-84.88% (Table 2). In some studies, which are in harmony with the study results, it is reported that different stress conditions with different plants do not change the relative water content in leaf tissues (Duman el al. 2014, Hamurcu el al. 2015), while it is reported to decrease it in some studies (Balal el al. 2017). In this study, it is thought that the reason why there is no significant difference in the relative water content of the leaf tissues as a result of boron applications is due to the fact that the boron levels have not reached the toxic dose in the plant or the defense mechanism may be resistant to boron.

The effect of PGPR, boron doses, and PGPR × boron interaction on the ion leakage in leaf tissues was statistically significant at a rate of 5%. According to PGPR applications, the highest value was obtained from P1 applications with 35.67%, and it was in the same statistical group as P3. The lowest value was obtained from P2 with 22.17%, and it was in the same statistical group as P0. According to boron applications, the highest value was obtained from B3 with 60.05%, while the lowest value was determined from B0 with 9.13% and did not differ statistically from B1. In the PGPR × boron interaction, the highest value was obtained from P1 × B3 with 82.38% (Table 2).

The effect of boron doses, PGPR, and PGPR × boron interaction on the membrane durability index in leaf tissues was found to be statistically significant at the rate of 5%. According to PGPR applications, the highest value was obtained from the P2 application with 80.89%, and it was in the same statistical group with P0 applications. The lowest value was obtained from P1 with 68.28%. According to boron applications, the highest value was obtained from B0 with 92.70%, while the lowest value was found in B3 with

Table 2

The effects of boron doses and some PGPR applications on the physiological and biochemical parameters of medicinal sage

Application **		Relative water content (%)	Ion leakage in leaf tissues (%)	Membrane durability index in leaf tissues (%)	Nitrogen balance index (dualex value)	MDA (nmol g^{-1})
PGPR *	Boron					
Control (P0)	**B0**	86.74	5.15 *f*	96.88 *a*	45.20 *ab*	2.05 *c-g*
	B1	89.19	10.05 *f*	91.35 *a*	44.08 *ab*	1.87 *d-g*
	B2	89.89	26.04 *de*	82.59 *a*	46.18 *a*	2.26 *b-e*
	B3	83.69	61.18 *b*	38.83 *d*	41.53 *ab*	2.77 *ab*
Mean		**87.38**	**25.60 B**	**77.41 A**	**44.24 A**	**2.23 A**
P1	**B0**	86.20	15.94 *ef*	90.42 *a*	51.55 *a*	1.68 *e-g*
	B1	88.06	28.11 *de*	77.52 *ab*	33.87 *ab*	1.92 *d-g*
	B2	90.24	16.28 *ef*	81.38 *a*	35.20 *ab*	2.32 *b-d*
	B3	87.77	82.38 *a*	23.68 *e*	34.43 *ab*	2.47 *bc*
Mean		**88.07**	**35.67 A**	**68.25 B**	**38.76 AB**	**2.09 AB**
P2	**B0**	93.69	7.00 *f*	89.48 *a*	35.17 *ab*	2.22 *b-f*
	B1	91.10	5.99 *f*	90.87 *a*	41.37 *ab*	1.42 *g*
	B2	90.57	32.94 *d*	86.02 *a*	38.53 *ab*	2.06 *c-f*
	B3	76.07	42.78 *c*	57.22 *c*	28.49 *b*	2.97 *a*
	Mean	**87.86**	**22.17 B**	**80.89 A**	**35.88 B**	**2.16 A**
P3	**B0**	72.92	8.42 *f*	94.05 *a*	41.80 *ab*	1.87 *d-g*
	B1	90.12	9.27 *f*	82.04 *a*	37.10 *ab*	1.68 *fg*
	B2	91.56	54.22 *b*	62.88 *bc*	36.40 *ab*	1.55 *g*
	B3	86.70	53.88 *b*	62.81 *bc*	37.97 *ab*	2.60 *a-c*
	Mean	**85.32**	**31.44 A**	**75.44 AB**	**38.76 AB**	**1.92 B**
Boron (B) ***	**B0**	84.88	9.13 *C*	92.70 *A*	43.42 *A*	1.95 *B*
	B1	89.61	13.35 *C*	85.44 *AB*	39.10 *AB*	1.72 *C*
	B2	90.56	32.36 *B*	78.21 *B*	39.07 *AB*	2.04 *B*
	B3	85.55	60.05 *A*	45.63 *C*	35.60 *B*	2.70 *A*
PGPR LSD ($P\leq0.05$)		ns	4.64	8.37	6.33	0.21
Boron LSD ($P\leq0.05$)		ns	4.64	8.37	6.33	0.21
PGPR×Boron LSD ($P\leq0.05$)		ns	16.09	29.01	21.94	0.75
CV		10.43	19.45	13.33	19.38	12.43

ns non-significant (at 5% level);

* There is no difference between the means indicated by **capital bold** letters in the same column.

** There is no statistical difference between the means shown in small letters in the same column.

*** There is no difference between the means shown in *capital italic* letters in the same column.

45.63% (Table 2). It has been reported that there is a decrease in the durability of the cell membrane and an increase in ion leakage due to environmental stress in plants (Blokhina et al. 2003). It has been reported that the use of rhizobacteria in drought stress situations is an effective method in increasing membrane durability and reducing ion leakage (Li et al. 2019).

Nitrogen balance index

The effect of PGPR, boron doses, and PGPR × boron interaction on the nitrogen balance index was found to be statistically significant at the rate of 5%. According to PGPR applications, the highest value was obtained from P0 with 44.24, and the lowest value was obtained from P2 with 35.88. According to boron applications, the highest value was obtained from B0 with 43.42, and the lowest value was obtained from B3 with 35.60. The highest value of 51.55 in PGPR × boron interaction was obtained from the P1 × B0 interaction, and it was in the same statistical group with P0 x B2 applications (Table 2). It was reported that the nitrogen balance index parameters were used to determine the change in the amount of nitrogen that plants take from their environment (Cerovic et al. 2012). Guittonny-Philippe et al. (2015) reported that in heavy metal concentration, the plant had difficulty taking nitrogen from the soil, and there were decreases in the nitrogen balance index.

MDA

It was determined that the effect of PGPR, boron doses and PGPR × boron interaction on MDA was statistically significant at the rate of 5%. According to PGPR applications, the highest value was obtained from P0 (no statistical difference with P2) with 2.23 nmol g^{-1}, and the lowest value was obtained from 1.92 P3. According to boron applications, the highest value was determined from B3 applications with 2.70 nmol g^{-1}, and the lowest value was determined from B1 applications with 1.72 nmol g^{-1}. According to the PGPR × boron interaction, the highest value was obtained from the P2 × B3 interaction with 2.97 nmol g^{-1} (Table 2). It is known that in abiotic stress situations, lipids in the cell membrane are oxidized, and accordingly, malondialdehyde (MDA) increases in the environment (Yolcİ et al. 2021). The level of stress can be determined by looking at the amount of MDA produced in plants in stress situations. In studies conducted with different plants, it has been reported that MDA values increase in parallel with increasing boron doses (Kaya et al. 2020). Yilmaz, Kulaz (2019), in their study of chickpeas exposed to salinity stress, reported that PGPR applications reduced MDA values compared to the control.

Chlorophyll a, chlorophyll b, and total chlorophyll amount

While the effect of PGPR and boron doses on the amount of chlorophyll a was statistically insignificant, the impact of PGPR × boron interaction was found to be significant at the rate of 5%. While average values of the PGPR applications were in the range of 21.45-19.13 μg g^{-1} FW, the average values of the boron applications were in the range of 21.09-19.11 μg g^{-1} FW. According to the PGPR × boron interaction, the highest value (24.87 μg g^{-1} FW) was obtained from P2 x B2, which had no statistically difference with P1 × B2 and P2 × B1 interactions (Table 3).

As seen in Table 3, while the effect of PGPR applications and PGPR × boron interaction on the amount of chlorophyll b was statistically significant at the level of 5%, it was determined that boron doses had no significant effect. According to PGPR applications, the highest value was obtained from P1 with 9.98 μg g^{-1} FW, and the lowest value was obtained from P3 (statistically indifferent with P0) with 8.32 μg g^{-1} FW (Table 3). Boron applications ranged from 8.59-9.67 μg g^{-1}. The highest value of 13.50 in PGPR × boron interaction was obtained from P2 × B2 (Table 3).

While the effect of PGPR and PGPR × boron interaction on the total amount of chlorophyll was statistically significant at the rate of 5%, the effect of boron doses was found to be insignificant. According to PGPR applications, the highest value was obtained from P1 with 31.43 μg g^{-1} FW, and the lowest value was obtained from P0 with 27.50 μg g^{-1} FW. According to the PGPR × boron interaction, the highest value was obtained from 38.37 μg g^{-1} FW and P2 × B2 interaction (Table 3). Riaz et al. (2021) reported that chlorophyll a, b, and total chlorophyll amounts decreased due to the increase in boron doses in rice (Kayıhan et al. 2017), in their study investigating the effects of boron doses on chlorophyll a, b, and total chlorophyll amounts in different rice cultivars, reported that while no significant changes were observed in Bolal cultivar depending on the increase in boron doses, decreases occurred in chlorophyll amounts in Atay cultivar. It is known that the element boron participates in the production of chlorophyll and causes increases up to a certain level, while it causes decreases in the amount of chlorophyll at the toxic level. The results of this study show that boron doses have a positive effect on chlorophyll amounts, and the applied boron doses do not reach toxic levels. (Khan et al. 2016) reported that the chlorophyll amounts of *Bacillus pumilis* rhizobacteria increased in rice, where they applied boron and salt, compared to the control.

Total amount of carotenoids

It was determined that the effect of boron doses, PGPR and PGPR × boron interaction on the total carotenoid amount was statistically significant at the rate of 5%. According to PGPR applications, the highest value was obtained from P2 (no statistical difference with P1) with 5.78 μg g^{-1} FW, and the lowest value was obtained from P0 with 4.99 μg g^{-1} FW. According to boron applications, the highest value was obtained from B3 with 5.86 μg g^{-1} FW (no statistical difference with B2), and the lowest value was obtained from B0 with 4.81 μg g^{-1} FW. According to the PGPR × boron interaction,

Table 3

The effects of boron doses and some PGPR applications on the biochemical parameters of medicinal sage

Applications**		Chlorophyll a (µg g^{-1} FW)	Chlorophyll b (µg g^{-1} FW)	Total Chlorophyll (µg g^{-1} FW)	Total Carotenoid (µg g^{-1} FW)
PGPR*	Boron				
Control (P0)	**B0**	18.55 *ab*	9.80 *a-e*	28.34 *b-d*	4.67 *bc*
	B1	18.97 *ab*	6.94 *de*	25.91 *cd*	4.69 *bc*
	B2	17.16 *b*	5.51 *e*	22.67 *d*	4.83 *bc*
	B3	21.88 *ab*	11.20 *ab*	33.08 *a-c*	5.80 *ab*
Mean		**19.13**	**8.36 B**	**27.50 B**	**4.99 B**
P1	**B0**	20.63 *ab*	9.30 *b-e*	29.93 *a-d*	5.30 *a-c*
	B1	20.05 *ab*	10.53 *a-c*	30.58 *a-c*	5.37 *a-c*
	B2	22.93 *a*	10.75 *a-c*	34.65 *ab*	5.99 *ab*
	B3	21.25 *ab*	9.34 *b-e*	30.59 *a-c*	6.31 *ab*
Mean		**21.45**	**9.98 A**	**31.43 A**	**5.74 A**
P2	**B0**	14.96 *b*	5.04 *e*	20.00 *d*	3.55 *c*
	B1	22.93 *a*	10.32 *a-d*	33.25 *a-c*	6.19 *ab*
	B2	24.87 *a*	13.50 *a*	38.37 *a*	7.27 *a*
	B3	21.14 *ab*	9.58 *b-e*	30.72 *a-c*	6.12 *ab*
Mean		**20.97**	**9.61 AB**	**30.58 AB**	**5.78 A**
P3	**B0**	22.30 *ab*	10.24 *a-d*	32.54 *a-c*	5.75 *a-c*
	B1	21.93 *ab*	8.80 *b-e*	30.73 *a-c*	5.55 *a-c*
	B2	15.71 *b*	5.69 *e*	21.40 *d*	4.04 *c*
	B3	20.09 *ab*	8.58 *c-e*	28.67 *b-d*	5.22 *bc*
Mean		**20.00**	**8.32 B**	**28.33 AB**	**5.14 AB**
Boron (B) ***	**B0**	19.11	8.59	27.70	4.81 *B*
	B1	20.97	9.14	30.11	5.45 *AB*
	B2	20.40	8.86	29.27	5.53 *A*
	B3	21.09	9.67	30.76	5.86 *A*
PGPR LSD ($P \leq 0.05$)		ns	1.46	3.62	0.71
Boron LSD ($P \leq 0.05$)		ns	ns	ns	0.71
PGPR×Boron LSD ($P \leq 0.05$)		8.52	5.06	12.55	2.41
CV		14.50	19.38	14.79	15.65

ns Non-significant (at 5% level)
* There is no difference between the means indicated by capital bold letters in the same column.
** There is no statistical difference between the means shown in small letters in the same column.
*** There is no difference between the means shown in capital italic letters in the same column.

the highest value was obtained from 7.27 µg g^{-1} FW and P2 × B2 interaction (Table 3). It has been reported that beneficial bacterial applications increase the amount of carotenoids in plants under various heavy metal stress (Singh et al. 2019). The study results are in agreement with the literature.

CONCLUSIONS

Boron, which is one of the essential nutrients in the growth and development of plants, can be noticed with various symptoms in its deficiency and excess. The needs and toxic levels of plants for boron vary. For this reason, it is important to determine the toxic levels of boron elements in plants. It is crucial to determine the interaction of various beneficial soil bacteria used in environmental stress situations with the boron and their responses at the toxic boron level. With the use of rhizobacteria in agriculture, many purposes such as reducing agricultural inputs, reducing the damage caused by abiotic stresses to the plant, and contributing to organic agriculture have become possible. As a result of the study, it has been determined that increasing boron doses are at toxic levels for many parameters of medicinal sage, and negative damages are seen, and the applied PGPRs are effective in compensating for the damages of toxic boron.

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