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ORIGINAL PAPER

EFFECT OF DIFFERENT BORON CONCENTRATIONS AND RHIZOBACTERIA APPLICATIONS ON PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF PURPLE BASIL (*OCIMUM BASILICUM*) PLANT

Muhammed Said Yolci[1], Rüveyde Tunçtürk[1],
Murat Tunçtürk[1], Tamer Eryiğit[2]

[1] Department of Field Crops
[2] Gevas Vocational School, Department of Plants and Animal Production
Van Yuzuncu Yil University, Van, Turkey

Abstract

It is known that applications of boron, which is known to play an essential role in nutrient transport by plant membranes, can affect the accumulation and use of other plant nutrients as a regulator or an inhibitor. Thus, in this study, the effects of boron doses (0-control, 5, 10, 20 mM) and some beneficial rhizobacteria (*Azospirillum lipoferum*, *Bacillus megaterium*, and *Frateuria aurentia*) on seedling growth, physiological, and biochemical parameters of purple basil (*Ocimum basilicum*) were investigated. The study was carried out in as a factorial experiment in a completely randomized design with four replications in a controlled climate chamber. In the study, plant root, seedling length, root and seedling fresh-dry weight, total chlorophyll, carotenoid, anthocyanin, flavonol, nitrogen balance index, MDA, relative water in leaf tissues, ion leakage, and membrane stability parameters were investigated. It was observed that all growth parameters were negatively affected by increasing boron doses. In contrast, while ion leakage, total flavonol, carotenoid, anthocyanin, and MDA contents increased, membrane stability, total chlorophyll content, and nitrogen balance index contents decreased. Rhizobacteria applications were found to be effective in reducing toxic boron damage in root length and seedling fresh weight, increasing total chlorophyll, and anthocyanin contents, and decreasing boron toxicity by reducing lipid peroxidation compared to control.

Keywords: boron toxicity, *rhizobacteria,* physiologic, biochemical, seedling growth.

Corresponding author e-mail: tamyigit@hotmail.com

INTRODUCTION

Basil (*Ocimum basilicum* L.) is an annual, herbaceous, medicinal and aromatic plant belonging to the *Ocimum* genus of the Lamiaceae (Labiateae) family (Balyan, Pushpangadan 1988). The natural distribution area of the genus *Ocimum*, which is represented by 65 species in the world, is known to be the temperate and warm regions of Asia, Africa, and South America (Paton et al. 1999). Although the basil plant does not have a natural habitat in Turkey, it is grown in gardens and on balconies, especially in the southern and western parts of Anatolia (Karaca et al. 2017). The basil plant has found many uses in many parts of the world due to its ornamental and therapeutic effects (Machado et al. 2019). It is used as antimicrobial, antifungal, insecticidal, antiparasitic, and antioxidant plants, as well as an immune system booster, anti-inflammatory, hepatoprotective, osteoporosis, cardiovascular protector, nervous system protector. The major secondary metabolites in the *Ocimum* basilicum species are linalool, geraniol, methyl eugenol, methyl chavicol, p-allylanisole, 1,8-cineol, trans-α-bergamoten, and neryl acetate (Dhama et al. 2021).

The outputs of many production systems, especially industrial waste, cause the soils to be polluted by metals and nonmetals and adversely affect many agricultural productions, especially plant production (Bhunia 2017). Boron is a crucial nutrient element that has functions in enzyme activities, hormone transport, cell membrane and wall structure, and the transmission of metabolites and ions in plants (Dordas et al. 2000).

Boron takes part in many physiological processes of plants and is found in the average range of $1\mu\ g^{-1} - 1mg\ g^{-1}$ in the plant. The element boron in plants has a narrow range between deficiency and excess levels, and the boron requirements of plants vary equally significantly (García-Sánchez et al. 2020). While it is possible to compensate the boron deficit in the soil with natural or synthetic fertilizers, building tolerance of its excess is difficult in terms of costs, time, and sustainability (Brdar-Jokanović 2020). The general symptoms in plants grown with insufficient levels of boron in the soil are the weakened growth and development in the root zone, problems in the production and transmission of sugars and derivatives, disruptions in nucleic acid production, difficulties in the uptake and transmission of micro- and macronutrients from the soil, and decay in many parts of the plant (Behboudian et al. 2016). Boron excess in plants generally manifests itself with lesion-necrosis in mature leaves, while it is reported that the root region does not differ morphologically under toxic levels of boron (Sarafi et al. 2017). When boron in the soil is found at a toxic level for plants, it accumulates in the plant and creates symptoms similar to ones induced by heavy metal toxicity. Boron toxicity in plants shows itself as many morphological and physiological abnormalities. Boron appearing in toxic levels in soils causes decreases in yield, economic losses and, indirectly, to different types of risk to humans. For these reasons, it is essential to prevent boron pollution in the soil and the damage it causes to the plant (Alengebawy et al. 2021). The application of rhizobacteria that promote plant growth to an agricultural production system is a profitable alternative owing to its role as a plant growth regulator and its effectiveness in abiotic stress management.

These bacteria have the potential to promote plant growth and assist in the management of plant diseases and abiotic stresses (Selem et al. 2022) in the soil through the production of bacterial phytohormones and related metabolites, as well as by leading to significant root morphological changes. These changes cause the improvement of plant-water relations and nutritional status in plants, and stimulate the defense mechanisms of plants to overcome adverse environmental conditions (Goswami, Suresh 2020).

In beans, *Azospirillum brasilence* species promoted root growth (Dardanelli et al. 2008), some *Bacillus* and *Pseudomonas* species increased growth parameters in basil (Golpayegani, Tilebeni 2011), and *Pseudomonas* and *Bacillus* species in legumes caused increases in plant height and seedling fresh weight (Metwali et al. 2015). It has been reported that some *Pseudomonas* and *Bacillus* species cause increases in root and seedling dry weights in cotton (Egamberdieva, Jabborova 2013).

This study was carried out to determine the effects of different boron concentrations on the seedling growth, physiological and biochemical properties of purple basil (*Ocimum basilicum*), and to determine the effects of some beneficial soil bacteria (rhizobacteria) against boron stress.

MATERIAL AND METHOD

Material

The experiment was carried out in 2021, in a controlled climate room of Van Yuzuncu Yil University Faculty of Agriculture, Field Crops Department. Purple basil (*Ocimum basilicum*) seeds were used as the plant material in the research.

Method

The study was carried out as a factorial experiment in a completely randomized design with four replications. In the study, different doses of boric acid (H_2BO_3) as a form of the element boron (B), which is known to contribute to the growth and development when found in trace amounts in plants, but can have a toxic effect when present in the soil at high levels (B0 – 0 (control), B1 – 5, B2 – 10 and B3 – 20 mM), and some beneficial rhizobium (R) bacteria (R0 – control, R1 – *Azospirillum lipoferum* ($1x10^6$ kob ml^{-1}), R2 – *Bacillus megaterium* ($1x10^5$ kob ml^{-1}) and R3 – *Frateuria aurentia* ($1x10^5$ kob ml^{-1})) that contribute the plant growth and development were used as factors of the study. Seed surfaces were sterilized with 3% sodium hypochlorite. The seeds, except the sterilized control group, were kept separately for two hours in solutions of *Azospirillum lipoferum*, *Bacillus megaterium*, and *Frateuria aurentia* rhizobacteria prepared at a concentration of 10 ml dm^{-3}. Treated and untreated seeds were planted as eight seeds per pot, each in one-liter pots filled with 1/3 peat (classman) and 2/3 garden soil by volume. The pots were placed in a fully controlled climate room with a light/dark photoperiod of 16/8 h, temp. of 25°C, and humidity of 65%. 220 ml of distilled water were added to the pots with an average water holding capacity of 220 ml. Germination started six days after sowing. The soil surface was wetted sufficiently (20 ml on average) from sowing to germination. Eight days after germination, one plant was left in each pot. From germination, 80 ml of distilled water were added to all pots every other day for 22 days. In the experiment, 23 days after germination, the solution consisting of 11.4 g dm^{-3} of ammonium sulphate (21%), 2.86 g dm^{-3} of triple super phosphate (46%), and 3.45 g dm^{-3} of potassium sulphate (50% K_2O and 16-20% S) fertilizer mixture was supplied to each pots once as basic fertilizer. Pots in the control group were given 80 ml of distilled water every other day until the end of the experiment. Two days after the basic fertilizer application, all bacterial doses prepared at an amount of 10 ml dm^{-3} solution were applied to the pots, except R0 pots, instead of irrigation water, twice at four-day intervals. The amount of 80 ml boric acid prepared from each boron dose was applied to the pots where bacteria were applied, once every two days, three times a day.When boron stress symptoms appeared before the flowering period (49 days after planting), the trial was terminated and plant samples were taken at this stage.

Growth parameters

Seedling lengths of plants; The distance between the soil and the extreme point as well as the root lengths were determined in cm by measuring the soil level from the pot with the help of a ruler after softening the soil with tap water and separating the roots. Wet weights of aerial and underground plant parts were determined by weighing the roots and seedlings separated from each other separately on sensitive scales. Roots and seedlings were placed in separate bags and kept in an oven at 70°C for 48 h, then their dry mass was weighed on a sensitive balance and determined as grams.

Membrane stability index, ion leakage, and relative water content in leaf tissues

Membrane stability index, ion leakage, and relative water content in leaf tissues were determined according to the method described by (Sairam 1994).

Nitrogen balance index, total flavonol, and total anthocyanin contents

Before the experiment was terminated, Nitrogen Balance Index, total flavonol, and total anthocyanin contents were measured from leaves with a Dualex 4 device (FORCE-A, Orsay, France) and recorded.

Lipid peroxidation (MDA) content

In the study, the content of malondialdehyde (MDA), the end product of lipid peroxidation, was determined according to the methods of Sairam, Saxena (2000). For this analysis, a 0.5 g leaf 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. 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 min, the absorbance of the supernatant at 532 and 600 nm wavelengths was determined, and then the malondialdehyde (MDA) content was calculated.

Total chlorophyll and total carotenoid contents

In the analyses performed to determine the photosynthetic pigments according to (Lichtenthaler 1987), a 0.2 g (200 mg) fresh plant sample was extracted with 10 mL 80% acetone and centrifuged at 4600 rpm for 15 minutes. Absorbance (A) values at 662, 645, and 470 nm wavelengths of aliquots taken after centrifugation were determined in a spectrophotometer (PG T60 UV-VIS) and recorded. Calculations were made with the help of the formulas given below:

chlorophyll a – $11.75 \times A662 - 2.350 \times A645$,
chlorophyll b – $18.61 \times A645 - 3.960 \times A662$,
A absorbance value of the sample
(1) total chlorophyll = a + b,
(2) total carotenoid = $(1000 \times A470 - 2.270 \times a) - (81.4 \times b/227)$.

Statistical analysis of data

Statistical analyses of the data obtained were performed using the COSTAT (version 6.03) package program, and multiple comparison tests were performed according to the Least Significant Difference ($LSD_{\alpha = 0.05}$) test.

RESULTS AND DISCUSSION

Root length

The effect of rhizobacteria, boron, and rhizobacteria × boron interaction on root length was found to be statistically significant ($P<0.01$). As seen in Table 1, in rhizobacteria applications, the highest root length values were obtained from R1 and R3 applications (13.34 and 12.86 cm), while the lowest value was obtained from R2 (9.20 cm). In boron applications, the highest value was obtained from B0 (15.93 cm), and the lowest value was obtained from B3 (6.61 cm). The highest value in the rhizobacteria × boron interaction was obtained from R3 × B0 (18.67 cm) – Table 1. In a previous study, in which the effectiveness of Azospirillum and Bacillus species in basil was investigated, azospirillum species increased plant height more than the control and Bacillus species (Tahami et al. 2017). Esringü et al. (2014) investigated the effects of inoculation with *Bacillus megaterium* bacteria in rapeseed grown in soil contaminated with B, Cd, and Pb, and reported that heavy metals negatively affected root length whereas bacterial application reduced heavy metal damage compared to the control. Donghua et al. (2000) reported that when boron is in a high concentration in the soil, root growth tips are inhibited, and root growth is negatively affected. Accordingly, reductions in root length, fresh and dry weights may occur. In another study, it was reported that decreases in seedling and root lengths and a reduction in root and seedling fresh and dry weights were due to increasing boron doses in olives (Rostami et al. 2017).

Seedling length

While the effect of rhizobacteria and boron doses on seedling length was statistically significant ($P<0.01$), the effect of rhizobacteria × boron interaction was not found to be significant. In rhizobacteria applications, the highest seedling length value (12.12 cm) was obtained from the control group (R0), and the lowest values were obtained from R2 and R3 (10.25 and 10.20 cm). According to boron applications, the highest value was obtained from B0 (14.62 cm), and the lowest value was obtained from B3 (7.91 cm) – Table 1. In a previous study, it was stated that decreases in seedling lengths occurred depending on the boron concentration in safflower (Sulus, Leblebici 2020). Rostami et al. (2017) reported that when boron is toxic to the plant in the soil, water availability decreases, the transfer of other nutrients to the aerial parts is inhibited and, consequently, reductions in seedling length, fresh and dry weight occur.

Root fresh weight

While the effect of rhizobacteria and boron applications on the root wet weight parameter was statistically significant at $P<0.01$, the impact of the rhizobacteria × boron interaction was significant at $P<0.05$. As seen in Table 1, depending on the rhizobacteria applications, the highest value in root wet weight was obtained from the control group (0.85 g), and the lowest value was obtained from the R1 application (0.48 g). As for the boron applications, the highest values of this trait (1.00 and 0.90 g) were obtained from the B0 and B1 applications, and the lowest value was obtained from the B3 application (0.37 g). The highest value in the rhizobacteria × boron interaction was obtained from the R3 × B0 interaction (1.32) – Table 1. In similar studies carried out on different plants, it has been reported, for instance, that the root fresh weight of *Arabidopsis thaliana* plants following the application of *Bacillus megaterium* bacteria (Dahmani et al. 2020); however, a reverse relationship was noted between boron doses and root fresh weight values in corn (Nawaz et al. 2020).

Seedling fresh weight

While the effect of rhizobacteria applications on seedling fresh weight was statistically significant at $P<0.01$, the impact of boron and rhizobacteria × boron interactions were found to be significant at $P<0.05$. As seen in Table 1, the highest value (2.88 g) was obtained from R3 according to rhizobacteria applications, and the lowest values were weighed from R0 and R2 applications (2.33 and 2.31 g). According to boron applications, the highest value was obtained from B0 (3.64 g), and the lowest value was obtained from B3 (1.19 g). In the rhizobacteria × boron interaction, the highest seedling fresh weight of 4.75 g was obtained from R3 × B0 (Table 1). In some previous studies, it has been reported that an increase of boron doses coincided with decreases in plant height, fresh and dry weights in safflower (Day et al. 2017), and in the seedling fresh weight in mint (Choudhary et al. 2021); additionally, *Bacillus megaterium* applications in beans led to increases in fresh and dry weights of the plant (López-Bucio et al. 2007).

Root dry weight

The effect of rhizobacteria, boron, and rhizobacteria × boron interactions on root dry weight was found to be statistically significant ($P<0.01$). Considering the rhizobacteria applications in Table 1, the highest root dry weight was obtained from the control (0.54 g), and the lowest value was obtained from R1 (0.21 g). According to boron doses, the highest value was obtained from the control group (0.79 g), and the lowest values (0.17 and 0.23 g) were obtained from B3 and B2, which did not differ between each other in a statistically significant way (Table 1). As shown in Table 1, the highest values in Rhizobacteria × boron interaction (1.01 and 1.12 g) were obtained from R0 × BO and R3 × B0 interactions (Table 1). It has been reported that there is a decrease in root dry weight in parallel with an increase in boron dose applied in soybean (Hamurcu et al. 2013), while *Azospirillum brasilense* applied to the soil in sorghum fields contributes to a higher dry weight of the plant (Malhotra, Srivastava 2006).

Seedling dry weight

As seen in Table 1, while boron applications had a statistically significant 1% effect on seedling dry weight, the impact of rhizobacteria and rhizobacteria × boron interaction was not found to be significant. Rhizobacteria

Table 1

Effects of boron doses and some rhizobacteria on some growth parameters of purple basil

Applications ** Rhizobacteria (R) *		Root length (cm)	Seedling length (cm)	Root fresh weight (g)	Seedling fresh weight (g)	Root dry weight (g)	Seedling dry weight (g)
	boron (B)						
R0 (control)	B0	16.78 ab	16.00	1.12 ab	3.19 bc	1.01 a	0.45
	B1	13.20 de	13.00	0.94 bc	2.66 bc	0.54 cd	0.37
	B2	10.36 f	10.50	0.77 cd	2.32 c	0.27 e	0.22
	B3	6.34 h	9.00	0.60 de	1.17 d	0.35 efg	0.11
	mean	**11.67 B**	**12.12 A**	**0.85 A**	**2.33 B**	**0.54 A**	**0.28**
R1	B0	14.76 bcd	14.50	0.69 cde	3.32 bc	0.39 de	0.44
	B1	15.17 bc	12.00	0.74 cd	3.00 bc	0.19 fghi	0.28
	B2	13.92 cd	10.33	0.30 f	2.34 c	0.21 fgh	0.22
	B3	9.53 f	8.67	0.21 f	1.39 d	0.07 i	0.12
	mean	**13.34 A**	**11.37 B**	**0.48 C**	**2.51 AB**	**0.21 C**	**0.26**
R2	B0	13.53 cde	14.00	0.89 bcd	3.33 bc	0.66 bc	0.34
	B1	10.41 f	10.50	1.07 ab	2.29 c	0.69 b	0.32
	B2	7.93 g	9.00	0.66 de	2.44 c	0.23 efg	0.22
	B3	4.96 i	7.33	0.40 ef	1.19 d	0.10 hi	0.1
	mean	**9.20 C**	**10.25 C**	**0.75 AB**	**2.31 B**	**0.42 B**	**0.24**
R3	B0	18.67 a	14.00	1.32 a	4.75 a	1.12 a	0.47
	B1	15.30 bc	11.00	0.88 bcd	3.34 b	0.33 ef	0.27
	B2	11.89 e	9.33	0.32 f	2.39 c	0.21 fgh	0.18
	B3	5.61 hi	6.67	0.28 f	1.05 d	0.19 ghi	0.1
	mean	**12.86 A**	**10.20 C**	**0.69 B**	**2.88 A**	**0.46 B**	**0.25**
Boron (B) ***	B0	*15.93 A*	*14.62 A*	*1.00 A*	*3.64 A*	*0.79 A*	*0.42 A*
	B1	*13.51 B*	*11.62 B*	*0.90 A*	*2.82 B*	*0.43 B*	*0.31 B*
	B2	*11.02 C*	*9.79 C*	*0.51 B*	*2.37 C*	*0.23 C*	*0.21 C*
	B3	*6.61 D*	*7.91 D*	*0.37 C*	*1.19 D*	*0.17 C*	*0.10 D*
R (LSD$_{\alpha = 0.05}$)		1.02	0.63	0.11	0.38	0.59	ns
B (LSD$_{\alpha = 0.05}$)		1.02	0.63	0.11	0.38	0.59	0.03
R×B (LSD$_{\alpha = 0.05}$)		3.55	ns	0.38	1.32	0.20	ns
CV (%)		10.47	6.98	19.52	18.26	17.56	17.76

ns – non-significant (LSD$_{\alpha = 0.05}$)

* There is no difference between the means shown in **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.

applications took place in the range of 0.24-0.28 g. According to boron applications, the highest value was obtained from B0 (0.42 g), and the lowest value was obtained from B3 (0.10 g) – Table 1. It has been indicated that plant weights decreased at high boron concentrations in maize (Çelik et al. 2019). Additionally, it has been reported that seedling dry weight values in wheat vary depending on boron doses (Khan et al. 2021).

Nitrogen balance index

This new index, called the nitrogen balance index, is an indicator of changes in C/N allocation due to N-deficiency rather than a measure of leaf nitrogen content per se (Cartelat et al. 2005). In terms of values of the nitrogen balance index parameters shown in Table 2, the effect of rhizobacteria, boron, and rhizobacteria × boron interaction were found to be statistically significant ($P<0.01$). According to rhizobacteria applications, the highest value was obtained from the R3 application (37.23), and the lowest value (28.82) was obtained from the R1 application, which was not statistically different from R0 and R2 applications. In terms of boron applications, the highest value was obtained from B0 (42.43), and the lowest value was obtained from the B3 application (23.88). The highest value in the rhizobacteria × boron interaction was obtained from R3 × B0 (55.45) – Table 2. Cerovic et al. (2012) stated that the nitrogen balance index was used to determine nitrogen deficiency and change in the plant. In our study, we attribute the decrease in the nitrogen balance index coinciding with the increase in boron doses to the fact that boron prevented nitrogen from being taken up by the plant. Guittonny-Philippe et al. (2015) reported that the nitrogen balance index values decreased due to the increase in heavy metal concentration in *Epilobium hirsutum* plant grown in soil contaminated with heavy metals, while (Nguyen et al. 2019) reported that different rhizobacteria applied to wheat increased the crop's nitrogen uptake from the soil.

Relative water content ratio in leaf tissues

The effects of rhizobacteria, boron doses, and rhizobacteria × boron interaction on the relative water content in leaf tissues were found to be statistically insignificant ($P>0.05$). According to the rhizobacteria applications, the values of the relative water content in the leaf tissues ranged from 87.09 to 88.62 %, while it varying between 86.29-91.07 % according to the boron doses (Table 2). In previous studies, it was reported that toxic boron levels in cucumber decreased the relative water content (Balal et al. 2017), and some boron doses applied to watermelon did not change the relative water content (Hamurcu et al. 2015). And also, it has been reported that the increased chromium metal in the foxtail plant does not cause a statistically significant change in the relative water content of the plant (Duman et al. 2014). The stress of the amount of boron in the soil and taken by the plant is directly related to the genetic stability and defense mechanism of the

Table 2

Effects of boron doses and some rhizobacteria on physiological parameters of purple basil

Applications ***		Nitrogen Balance Index (dualex value)	RWCRLT (%)	RILLT (%)	MSIRLT (%)
Rhizobacteria (R) *	boron (B)				
R0 (control)	B0	32.75 bcde	81.9	16.03 bcd	83.97
	B1	26.20 cde	85.81	14.85 bcd	85.14
	B2	33.65 bcd	81.99	10.05 d	90.62
	B3	26.70 cde	98.67	24.50 b	57.64
	mean	**29.82 B**	**87.09**	**16.36 B**	**79.34**
R1	B0	38.00 bc	90.84	13.89 bcd	86.11
	B1	29.35 cde	84.49	15.66 bcd	84.33
	B2	25.70 de	87.64	20.87 bc	75.3
	B3	22.26 e	88.2	42.56 a	63.03
	mean	**28.82 B**	**87.79**	**23.25 A**	**77.19**
R2	B0	43.53 ab	88.2	14.78 bcd	82.79
	B1	24.00 e	86.34	13.40 cd	86.59
	B2	26.26 cde	87.1	14.37 bcd	85.63
	B3	24.70 e	87.21	14.19 bcd	85
	mean	**29.62 B**	**87.46**	**18.21 B**	**79.14**
R3	B0	55.45 a	87.26	13.99 bcd	83.26
	B1	42.05 b	90.32	13.86 cd	86.14
	B2	29.46 cde	88.46	14.58 bcd	85.42
	B3	21.86 e	89.16	11.09 d	88.91
	mean	**37.20 A**	**88.62**	**12.93 C**	**87.06**
Boron (B) ***	B0	*42.43 A*	*86.87*	*14.22 B*	*85.16 A*
	B1	*30.4 B*	*86.74*	*14.44 B*	*85.55 A*
	B2	*28.77 B*	*86.29*	*14.97 B*	*84.23 A*
	B3	*23.88 C*	*91.07*	*27.11 A*	*67.78 B*
R (LSD$_{\alpha = 0.05}$)		4.09	ns	2.78	8.22
B (LSD$_{\alpha = 0.05}$)		4.09	ns	2.78	8.22
R×B (LSD$_{\alpha = 0.05}$)		14.19	ns	9.63	28.48
CV (%)		12.84	8.61	18.89	12.25

ns – non-significant (LSD$_{\alpha = 0.05}$)

* There is no difference between the means shown in **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. **RWCRLT** – relative water content ratio in leaf tissues, **RILLT** – rate of ion leakage in leaf tissues, **MSIRLT** – membrane stability index rate in leaf tissues plant. It is thought that the reason why the relative water content in the leaf tissues does not change statistically is that the applied boron doses do not reach the toxic dose in basil, or the plant is effective in reducing the boron damage to the defense system.

Rate of ion leakage in leaf tissues

Rhizobacteria, boron doses, and rhizobacteria × boron interaction had a statistically significant 1% effect on the rate of ion leakage in leaf tissues. As seen in Table 2, according to rhizobacteria applications, the highest value was obtained from the R1 application (23.25%), while the lowest value was obtained from R3 treatment (12.93%). According to the boron applications, the highest ion leakage value was obtained from B3 (27.11%), while the lowest value was obtained from the other three applications. As a result of the rhizobacteria × boron interaction, the highest ion leakage value was obtained from R1 × B3 (42.56%) – Table 2. It has been reported that in various environmental stress situations, there is deterioration in the integrity of the cell and membrane permeability, the membrane stability decreases and, as a result, the stress level can be measured by looking at the ion exchange inside and outside the cell (Blokhina et al. 2003). In maize, ion leakage also increased due to increasing boron doses (Kaya et al. 2018), while some rhizobacteria were effective in reducing the damage caused by high temperature to cell membrane stability in wheat (Sarkar et al. 2018), and rhizobacteria, compost and mineral fertilization achieved the same result in wheat. It has been reported that this combination is effective in reducing ion leakage by increasing membrane stability under environmental stress conditions (Kanwal et al. 2017).

Membrane stability index rate in leaf tissues

As regards the membrane stability index rate in leaf tissues, it was determined that the interaction of rhizobacteria and rhizobacteria × boron did not cause statistically significant differences, but boron doses had a significant effect ($P<0.01$). As seen in Table 2, membrane stability index values were found to vary between 77.19% and 87.06% as a result of rhizobacteria applications. According to the boron applications, the highest values (85.16%, 85.55%, and 85.23%) were obtained from the B0, B1, and B2 applications, with no statistical differences between them, while the lowest value (67.78%) was obtained from the B3 application (Table 2). It is known that the toxic level of boron element causes the production of various oxidative components in the plant and, accordingly, the destruction of the cell membrane, decreasing its stability. It has been reported that there is a decrease in membrane stability due to increasing boron doses in potatoes (Eraslan et al. 2007), and the toxic level of boron abrosbed by the plant causes the deterioration of membrane stability and an increase in ion leakage.

Total chlorophyll content

While the effect of rhizobacteria and boron doses on total chlorophyll content was statistically significant at the level of 1%, the effect of rhizobacteria × boron interaction was found to be insignificant (P>0.05). As seen in Table 3, according to the rhizobacteria applications, while the highest total chlorophyll values were obtained from the R2 and R3 applications (20.64 and 20.52), the lowest values were obtained from R0 and R1 applications (18.36 and 17.67). According to boron doses, the highest values (20.01 and 19.80) were obtained from B0 and B1 applications, and the lowest total chlorophyll value was obtained from B2 application (17.91) – Table 3. Song et al. (2019) reported that the element iron is used in the production of chlorophyll and that the toxic level of boron in the plant causes the inhibition of iron uptake, thereby adversely affecting chlorophyll production. Huang et al. (2014) and Kayıhan et al. (2017) stated that increases and decreases in the content of chlorophyll could be seen depending on an excessive amount of boron in the soil. In the study in which the effects of different boron doses on the content of chlorophyll in rice were investigated, there was a decrease in the content of chlorophyll due to the increase in boron doses (Riaz et al. 2021). Additionally, a decrease in the content of chlorophyll and disruptions in the photosynthesis mechanism of the plants grown at toxic boron levels have been reported (Camacho-Cristóbal et al. 2008). It was concluded that inoculation of *Bacillus pumilus* bacteria in rice grown under boron and salt stress causes increases in the content of chlorophyll (Khan et al. 2016).

Total carotenoid content

The effect of rhizobacteria, boron, and rhizobacteria × boron interaction on the total carotenoid content was found to be statistically significant (P<0.01). In rhizobacteria applications, the highest total carotenoid values were obtained from R3 and R2 applications (4.19 and 4.13), while the lowest values were obtained from R1 and R0 applications (3.64 and 3.81) – Table 3. In boron applications, the highest total carotenoid values were obtained from B1 and B3 applications (4.08 and 4.01), and the lowest value was obtained from B2 application (3.76). In the study, it was determined that the highest total carotenoid value (4.60) was obtained from the R3 x B3 application as a result of the interaction of boron and rhizobacteria (Table 3). It has been reported that carotenoids, like many enzymatic and non-enzymatic antioxidants produced in various environmental stress situations in plants, increase due to aggravated adverse environmental conditions (especially in drought) and act to protect the plant (Ullah et al. 2018). In another study, it has been reported that carotenoids increase in plants grown under salt and various heavy metal stress, and that the applied rhizobacteria contribute to the increase in carotenoids (Kamran et al. 2015).

Table 3

Effects of boron doses and some rhizobacteria on biochemical parameters of purple basil

Applications **		Total chlorophyll (μg g^{-1} FW)	Total carotenoid (μg g^{-1} FW)	Total anthocyanin (dualex value)	Total flavanol (dualex value)	MDA (lipid peroxidation) (nmol g^{-1})
Rhizobacteria (R) *	boron (B)					
R0 (control)	B0	19.1	4.05 abc	0.31	0.79	23.31 bcde
	B1	20.84	4.38 ab	0.51	0.99	24.71 bcd
	B2	15.13	3.16 d	0.57	0.75	23.03 bcde
	B3	18.4	3.65 cd	0.58	0.71	27.00 ab
	mean	**18.36 B**	**3.81 B**	**0.48 AB**	**0.80 A**	**24.51 A**
R1	B0	17.24	3.42 cd	0.53	0.75	18.13 de
	B1	18.2	3.64 cd	0.47	0.83	21.16 cde
	B2	17.93	3.98 bc	0.53	0.78	24.52 bcd
	B3	17.34	3.51 cd	0.64	0.7	25.99 bc
	mean	17.67 B	3.64 B	0.54 A	0.76 AB	22.44 AB
R2	B0	23.08	4.11 abc	0.46	0.6	13.72 e
	B1	19.55	4.18 abc	0.52	0.84	17.94 e
	B2	20.15	3.95 bc	0.55	0.69	19.94 de
	B3	19.8	4.30 ab	0.59	0.74	31.46 a
	mean	20.64 A	4.13 A	0.53 A	0.71 B	20.76 BC
R3	B0	20.63	4.12 abc	0.37	0.51	18.67 de
	B1	20.64	4.10 abc	0.34	0.61	18.71 de
	B2	18.45	3.94 bc	0.52	0.61	19.35 de
	B3	22.39	4.60 a	0.51	0.7	22.39 cde
	mean	20.52 A	4.19 A	0.43 B	0.60 C	19.78 C
Boron (B) ***	B0	*20.01 A*	*3.92 AB*	*0.41 B*	*0.65 B*	*18.45 C*
	B1	*19.80 A*	*4.08 A*	*0.45 B*	*0.81 A*	*20.63 BC*
	B2	*17.91 B*	*3.76 B*	*0.54 A*	*0.70 B*	*21.71 B*
	B3	*19.48 AB*	*4.01 A*	*0.57 A*	*0.71 B*	*26.71 A*
R (LSD$_{\alpha = 0.05}$)		1.88	0.28	0.06	0.77	2.62
B (LSD$_{\alpha = 0.05}$)		1.88	0.28	0.06	0.77	2.62
R×B (LSD$_{\alpha = 0.05}$)		ns	0.97	ns	ns	9.09
CV (%)		11.71	8.58	16.21	12.84	14.43

ns – non-significant (LSD$_{\alpha = 0.05}$)

* There is no difference between the means shown in 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.

Total anthocyanin content

While the effect of rhizobacteria applications on the total anthocyanin content was statistically significant at the 5% level, the impact of boron doses was found to be significant at $P<0.01$. The effect of rhizobacteria × boron interaction was found to be insignificant ($P>0.05$). According to the rhizobacteria applications, the highest value was obtained from R1 and R2 (0.54 and 0.53), and the lowest value from the R3 application (0.43). According to boron applications, the highest value was obtained from B3 and B2 (0.57 and 0.54), and the lowest value (0.41 and 0.45) – from B0 and B1 (Table 3). It is reported that anthocyanins increase in various environmental stress conditions, such as presence of heavy metals, and help protect the plant owing to their high antioxidant capacity (Moustaka et al. 2020). Balci et al. (2020) reported that some rhizobacteria applied to the raspberry plant increase the total anthocyanin content. Furthermore, increasing salt doses in black cumin cause increases in the total anthocyanin and total flavanol contents (Golkar et al. 2020).

Total flavanol content

While the effect of rhizobacteria and boron doses on total flavanol amount was statistically significant at the level of 1%, the effect of rhizobacteria × boron interaction was found to be insignificant ($P>0.05$). According to rhizobacteria applications, the highest value was obtained from R0 (0.80), and the lowest value was obtained from R3 (0.60). According to boron doses, the highest total flavanol content was obtained from B1 (0.81), and the lowest content was obtained from the other three applications (Table 3). It is known that the production of flavanols, which contain phenolic compounds and some of the flavonoids, increases in the cytoplasm and endoplasmic reticulum under adverse environmental conditions, and plays a role in reducing the damage caused by free radicals (Ibrahim, Jaafar 2011). In another study, it was reported that the amount of flavanol in beans treated with different doses of cadmium increased with the increasing doses of the metal (El Hocine et al. 2020).

Lipid peroxidation (MDA) content

While the effect of rhizobacteria and boron applications on MDA was statistically significant ($P<0.01$), the impact of the rhizobacteria × boron interaction was significant at the 5% level. According to the rhizobacteria applications, the highest MDA content was obtained from the control group (R0) (24.51), and the lowest content was obtained from the R3 application (19.78). According to boron applications, the highest MDA content was obtained from B3 applications (26.71), and the lowest content was obtained from the control group (B0) (18.45). As a result of the rhizobacteria × boron interaction, the highest content was obtained from the R2 × B3 interaction (31.46) – Table 3. At the toxic level of boron, lipids in the cell membrane undergo oxidation. As a result, malondialdehyde (MDA) production increases, and the level of stress can be determined depending on this increase. It has been reported that the doses of boron applied to beet and the MDA content vary in direct proportion (Song et al. 2019).

CONCLUSIONS

It is of great importance to determine the optimum and toxic levels of boron, an essential micronutrient for plants, needed by cultivated crops, and to identify its adverse symptoms in the plant. In addition, determination of the effectiveness of some beneficial bacteria used to reduce the harmful effects of biotic and abiotic stress conditions in studies conducted on different plants in the context of reducing boron stress is crucial in terms of reducing agricultural damage and providing ecological agriculture. As a result of the study, it was determined that increasing boron doses were toxic towards many parameters of basil, and negative damages were observed, whereas applied rhizobacteria were effective in compensating for toxic boron damages, and *Frateuria aurentia* bacterium was more effective than others. It is essential to examine the effects of different rhizobacteria and their interactions in future studies for the recommended ability of the studied rhizobacteria.

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