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Research Article

The effect of arbuscular mycorrhizal inoculation and plant growth-promoting rhizobacteria on maize (*Zea mays* L.) under boron toxicity stress

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Abstract

A greenhouse study was conducted to investigate the effect of arbuscular mycorrhiza fungus and plant growth-promoting rhizobacteria on maize (*Zea mays* L.) yield and boron (B) uptake under excessive B concentrations. Four levels of B (0, 10, 20 and 40 mg kg^{-1} soil) and four levels of microbial inoculation (non-inoculation, *Rhizophagus intraradices*, *Micrococcus yunnanensis* and co-inoculation) were applied. In general, elevated B decreased the maize relative yield, plant dry weight, plant height, total leaf area and chlorophyll content. The highest B uptake was recorded in the 20 mg kg^{-1} B treatments, whereas the lowest uptake was in B treatments of 0 and 40 mg B kg^{-1}. Excessive B supply increased the proline content and electrolyte leakage of maize leaves. Microbial inoculation did not change the yield of B-stressed plants. In addition, mycorrhiza inoculation and co-inoculation increased the shoot and root B uptake. Maize plants growing at high B concentrations with or without microbial inoculation showed leaf edge burning and necrosis when compared with control treatments. Microbial inoculation improved the maize yield under B stress up to 20 mg B kg^{-1}. Thus, inoculation with arbuscular mycorrhiza and plant growth promoting rhizobacteria can increase the maize tolerance under B toxicity. Nonetheless, the inoculation of bacterium and arbuscular mycorrhiza had a more alleviative effect when the plants were exposed to excess B.

Keywords: arbuscular mycorrhiza fungus; boron toxicity; growth promoting plant rhizobacteria; maize

Introduction

Boron (B) toxicity limits crop yield and quality in several agricultural areas worldwide, and frequently occurs naturally in alkaline and saline soils together with a low rainfall and poor leaching capacity (Camacho-Cristóbal *et al*., 2008). Landi *et al.* (2019) reported that the maximum permissible concentrations of B in

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irrigation water range from 1-2 mg L^{-1} for semi-tolerant plants (i.e. maize). The major B source in soils is probably the irrigation water (Keles *et al*., 2004). Excess B can disrupt physiological processes (e.g. CO_2 assimilation, photosystem II photochemistry, carbohydrate metabolism, and antioxidant system) in plants and consequently result in visual symptoms such as chlorosis and necrosis of shoots (Reid, 2013; Landi *et al*., 2019). Excess B can also inhibit root growth and consequently reduce water uptake, which will aggravate water stress in arid areas (Aquea *et al*., 2012).

Maize plantations rank third after wheat and rice in the world and provide the greatest percentage of calorie intake in the diet in more than 22 countries (Wu *et al*., 2018; Turhan, 2021). However, the yield still needs to be increased to fulfill the future food demand (Brdar-Jokanović, 2020). Therefore, it is of great importance to enhance the yield and quality of the maize crop. Boron stress is one of the most serious problems globally, affecting the development, yield and quality of maize (Kumar *et al*., 2019). Boron toxicity causes metabolic disorders in the ribose parts bound to adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) due to its damage to the cell wall in plants. It leads to injuries in the dividing and developing cells when bound to RNA, free glucose or ribose in plants. Boron accumulating in a large amount in the leaves of plants also has unwanted effects, like the upsetting of the osmotic order in the direction of the transpiration flow (Yildiztugay *et al*., 2019). The detrimental effects of excess B have been widely reported on agriculture and ecosystems (Stiles *et al*., 2011). The alleviation approaches for B toxicity in plants include the application of additional nutrient elements, plant growth regulators (PGRs), plant growth promoting microbes (PGPMs), lime, water, organic matter, etc. However, our knowledge about the effects and mechanisms of different alleviation approaches is still limited. Thus, the objective of this research is to study the effect of arbuscular mycorrhiza fungus (AMF) and plant growth promoting rhizobacteria (PGPR) for mitigating the damages suffered by maize under B toxicity.

Materials and Methods

Soil preparation

The soil was collected from surface horizon (0-30 cm) from Chitgar series (Fine-loamy, carbonatic, thermic, Typic Calcixerepts) in Sarvestan area of Fars province, I. R. Iran. The 2 mm sieved air-dry soil completely mixed to ensure uniformity. Soil properties were determined (Table 1) as follow: DTPA-extractable iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) (Lindsay and Norvel, 1978), hot water-soluble B (Berger and Truog, 1939; John *et al*., 1975), pH (Thomas, 1996) of 7.68, electrical conductivity in saturated soil extract (Rhoades, 1996), organic matter oxidation with chromic acid (Nelson and Sommers, 1996), total nitrogen with Kjeldahl method (Bremner, 1996), phosphorus (P) (Olsen and Sommers, 1982), available Potassium (K) with ammonium acetate (Knudsen *et al*., 1982), and soil texture with hydrometer method (Bouyoucos, 1962).

Table 1. Physicochemical properties of the soil used for the incubation experiment

Soil parameters	pH	EC (dS m^{-1})	Organic matter (%)	Total N (%)	Available K (mg kg^{-1})	Available B (mg kg^{-1})	Available Fe (mg kg^{-1})	Available Mn (mg kg^{-1})	Available Zn (mg kg^{-1})	Available Cu (mg kg^{-1})
Measurements	7.68	0.6	1.2	0.05	293	0.25	2.1	4.7	0.6	0.5

Microbial inoculants

The fungus inoculum consisted of sand, spores, mycelia, and colonized root fragments was obtained from Department of Soil Science, College of Agriculture, Shiraz University, Shiraz, I. R. Iran. It was prepared using the trap culture of *Sorghum vulgare* L. The culture medium composed of autoclaved soil/sand (<1 mm) (1:4, v/v) and 150 g the starter culture of *Rhizophagus intraradices* GINP101. It was isolated from the

rhizospheres of *Veronica rechingeri* growing in the Urumiah-Dokhtar zone, Zanjan, I. R. Iran (36° 400′ N, 47° 200′E). The fungus was morphologically and molecularly identified, as described in previous studies by Zarei et al. (2008). Inoculum potential of Arbuscular mycorrhiza fungus was approximately 15 spores g^{-1} substrate and 95% root colonization. It was used as 100 g per each pot of the fungal treatments.

The pure bacterium of *Micrococcus yunnanensis* YIM 65004 was obtained from the Department of Soil Sciences Engineering, College of Agriculture & Natural Resources, University of Tehran, Karaj, I. R. Iran (Ghavami *et al*., 2017). The bacterium had the PGP properties including the ability to dissolve the insoluble inorganic and organic phosphates and to produce siderophore (Hosseini *et al*., 2021). It was cultured on nutrient broth (NB) medium and incubated for 24 h at 28 °C. Upon sowing, each seed was inoculated by 2.0 ml *M. yunnanensis* suspension (9×10^7 colony-forming unit (CFU) ml^{-1}) for the treated group.

Experimental setup and analysis

The experiment was carried out in the greenhouse of Department of Soil Science, College of Agriculture, Shiraz University, Shiraz, I. R. Iran in 2019. A factorial experiment based on completely randomized design (CRD) was performed with three replicates. The treatments consisted of four levels of B (0, 10, 20, and 40 mg B kg^{-1} in form of boric acid) and four levels of microbial inoculants (non-inoculation, *R. intraradices*, *M. yunnanensis* and co-inoculation). Maize seeds (single cross SC 704) were provided by Department of Plant Production and Genetics, School of Agriculture, Shiraz University, Shiraz, I. R. Iran. The single cross SC 704 cultivar has been planted in the region for many years, and it has a considerable yield compared to other cultivars. Essential nutrient elements based on soil testing were added to all pots uniformly at the rate of 75 mg N kg^{-1} soil as urea, 25 mg P kg^{-1} soil as $Ca(HPO_2)_2$. Fe, Zn, Mn and Cu was applied at the rate of 10, 5, 10 and 2.5 mg kg^{-1} soil as Fe-EDDHA, $ZnSO_4.7H_2O$, $MnSO_4$. $4H_2O$, $CuSO_4.5H_2O$, respectively before sowing. Besides, urea was again applied at the rate of 75 mg N kg^{-1} 20 days after sowing in a form of top dressing. The seeds were surface sterilized with 5% NaOCl (w/v) solutions (5 min) and then rinsed with distilled water. Disinfected seeds were sown in 3 kg pots containing soil with a density of 2 plants per pot. The day/night temperature was set to 25-27 °C and 16-17 °C, respectively. Average relative humidity of 60-70% and a photoperiod of 16 (h) with photosynthetic photon flux of 800 μmol m^{-2} s^{-1} were provided for growth in the greenhouse. Plants were irrigated daily by distilled water to maintain the pots close to field capacity (FC). FC point at 1/3 bar was measured by a pressure plate apparatus (ATS, Tabriz, Iran) that was equal to 20% (20 g water per 100 g dry soil). After 10 weeks, all the plants were harvested, and their growth parameters (shoot dry weight, root dry weight, height, leaf surface, and chlorophyll (chlorophyll meter (SPAD-502)) content were measured. Fresh fine roots of the plants were sampled and their equal amount were used to determine the percentage of root colonization (Kormanik and McGraw, 1982). They were cleared in 8% KOH, acidified in 2% HCl and stained in lactic–glycerol–royal blue ink. The percentage of root colonization was estimated using the gridline intersect method (Giovannetti and Mosse, 1980). Proline content was measured using a spectrophotometer (PerkinElmer, Lambada 25, USA) at 520 nm on 0.1 g of leaf sample at pre flowering stage according to the method of Bates *et al*. (1973). To measure electrolyte leakage, leaf samples (0.5 g) were washed with distilled water and incubated in tubes containing 10 ml of distilled water for 24 h at 25 °C. The amount of initial EC was then recorded using an EC meter (CMD 500 WPA conductivity meter, Cambridge, UK). The samples were then placed in a hot water bath at 100 °C for 30 min. The second EC was recorded when the sample's temperature reached 25 °C. Electrolyte loss was calculated using the following equation (Lutts *et al*., 1996).

$$\text{Electrolyte leakage} = (\text{First electrical conductivity} / \text{Second electrical conductivity}) \times 100 \quad (1)$$

To determine the maize leaf area, the length and the largest leaf width were measured and then the leaf area was calculated from (2) (Burn *et al*., 1974):

$$A\ (cm^2) = L\ (cm) \times W\ (cm) \times 0.75 \quad (2)$$

Plant samples were homogenized by electric milling and extraction was done by dry digestion method (Jones Jr. *et al*., 1990). Plant B content of the extracts was measured using Azomethine H (Berger and Truog, 1939). The amount of B uptake was calculated by formula 3:

$$\text{Uptake } (\mu g\ pot^{-1}) = \text{Concentration } (\mu g\ g\ \text{dry matter}^{-1}) \times \text{Dry weight } (g\ pot^{-1}) \quad (3)$$

Statistical analysis

Statistical analysis was performed using the SAS 9.2 software. Two-way GLM tests were conducted to determine the influence of different factors. Where effects were significant ($P \leq 0.05$), differences between means were tested for significance using the Duncan multiple range test ($P \leq 0.05$). The chart was drawn with Graph pad 9.5 software.

Results

Plant growth

In the control B treatment, shoot dry weight (SDW), root dry weight (RDW), height (H) and leaf surface (LS) did not change significantly due to the applied microbial inoculations (MI) treatments. AMF and PGPR inoculations resulted in 49.4 and 27.21% more Chlorophyll (Chl) content as compared to the non-inoculation plants. Under B stresses, visible symptoms were observed on plant leaves. Initially, the leaves showed a yellow-green chlorosis. Later, the leaves gradually turned yellow from the tip. At the end of the treatment, most of the leaves became yellow and wilted (Figure 2). B stress significantly decreased the growth parameters which was significantly enhanced by MI treatment (Table 2). B treatment (10 mg B kg^{-1}) along with AMF inoculation significantly increased SDW and plant height (22.03 and 18.37% respectively). B treatment (20 mg B kg^{-1}) and microbial inoculation significantly increased the SDW, RDW and LS which was significantly difference with non-inoculation. In addition, AMF inoculation significantly increased the plants' height by 19.02%. The increased growth of the AMF treated plants was due to the mycorrhization process increasing the growth of leaves, stems and roots in 10 and 20 mg B kg^{-1} treatments (Table 3). However, MI/20 mg kg^{-1} treatments reduced the growth parameters as compared to the control treatment. The total dry biomass results showed that with or without MI, biomass had reduced in of 40 mg B kg^{-1} concentration as shown by the significant differences in the interaction correlation of Microbial × B. MI treatment at the concentration of 10 mg B kg^{-1} showed no significant effect on grow parameters (Table 3).

Table 2. Analysis of variances (ANOVA) for different growth parameters

Character	Boron levels (df =3)	Microbial inoculation (df =3)	Boron levels × Microbial inoculation (df =9)	Error (df =32)
Shoot dry weight	676.54**	12.97**	7.64**	1.95
Root dry weight	92.91**	0.26ns	1.06*	0.38
Height	6730.3**	502.98**	140.97**	64.6
Leafe surface	93184.21**	2705.12**	2326.76**	507.45
Chlorophyll	66.45**	6.21ns	20.18**	3.04
Shoot B uptake	53781372**	4405864.3**	175005.3**	375022.9
Root B uptake	140125.94**	16086.35**	61606.51**	1139.56
Electrolyte leakage	50.05**	34.26**	23.44**	3.45
Proline	4.79ns	186.05*	136.56**	42.86
Root colonization	2.47ns	26321.95**	27.11ns	40.26

* Significant at p=0.05; ** significant at p=0.01.

Table 3. Effect of boron levels and microbial inoculation on shoot and root dry weight, plant height, leaf area and chlorophyll content of maize leaves

Treatment		Shoot dry weight (g)	Root dry weight (g)	Height (cm)	Leafe surface (cm^2)	Chlorophyll (mg g^{-1} FW)
B	Microbial					
0	No inoculation	17.81^{ab}	7.19^{a}	91.66^{bc}	238.3^{ab}	17.75^{e}
0	Bacterium	16.50^{bc}	6.58^{a}	94.23^{bc}	253.62^{ab}	22.58^{bcd}
0	Fungus	19.21^{a}	6.88^{a}	$96.66a^{bc}$	248.67^{ab}	19.4^{def}
0	Co-inoculation	15.79^{bc}	6.42^{ab}	95.75^{bc}	210.87^{b}	26.52^{a}
B	Microbial					
10	No inoculation	16.02^{bc}	5.28^{c}	94.33^{bc}	259.63^{a}	18.85^{ef}
10	Bacterium	15.53^{bc}	5.40^{bc}	101.83^{abc}	236.63^{ab}	21.25^{cde}
10	Fungus	19.55^{a}	4.97^{c}	111.66^{a}	262.8^{a}	19.11^{ef}
10	Co-inoculation	14.88^{c}	4.51^{cd}	103.83^{abc}	237.08^{ab}	19.92^{def}
B	Microbial					
20	No inoculation	10.41^{d}	2.12^{e}	89.5^{c}	173.22^{c}	26.57^{a}
20	Bacterium	14.58^{c}	3.84^{d}	96.5^{abc}	229.97^{ab}	20.98^{cdef}
20	Fungus	14.7^{c}	2.39^{e}	106.23^{ab}	268.27^{a}	25.3^{ab}
20	Co-inoculation	15.36^{bc}	3.88^{d}	101.33^{abc}	247.97^{ab}	23.56^{abc}
B	Microbial					
40	No inoculation	1.27^{e}	0.25^{f}	51.83^{d}	83.35^{d}	19.01^{ef}
40	Bacterium	0.72^{e}	0.19^{f}	31.66^{e}	20.1^{e}	19.05^{ef}
40	Fungus	1.34^{e}	0.44^{f}	61.75^{d}	89.81^{d}	18.07^{ef}
40	Co-inoculation	1.22^{e}	0.38^{f}	61.83^{d}	60.29^{d}	18.12^{ef}

Plant B uptake

Individual B stress significantly increased both root and shoot B uptake. Besides, MI had significant effect on both root and shoot B uptake. The highest root and shoot B uptake were in 20 mg B kg^{-1} treatment in AMF inoculated plants. MI treatment at 20 mg kg^{-1} B availability significantly increased both the root and shoot B content (Figure 1). While at 10 mg kg^{-1} B treatment, MI significantly reduced the B uptake by roots when compared with the non-inoculation treatment (Figure 1 b). However, MI did not show any significant effect on shoot B uptake. Thus, the accumulation of B in leaf was progressive, without reaching a stationary state during the entire experimental period. The mycorrhizae also played a fundamental role in the accumulation of B in the leaves. All of the applied B treatment, the AMF inoculation, and co-inoculation resulted in the highest shoot B uptake as compared to non-inoculation plants. Nevertheless, PGPR inoculation decreased the shoot B uptake but not significantly in comparison with control plants. In addition, when comparing the uptake of B according to tissue, the following order was observed (from greater to lower concentration): shoot > root, and this order maintained in AMF inoculation, co-inoculation and PGPR inoculation treated plants (Figure 1).

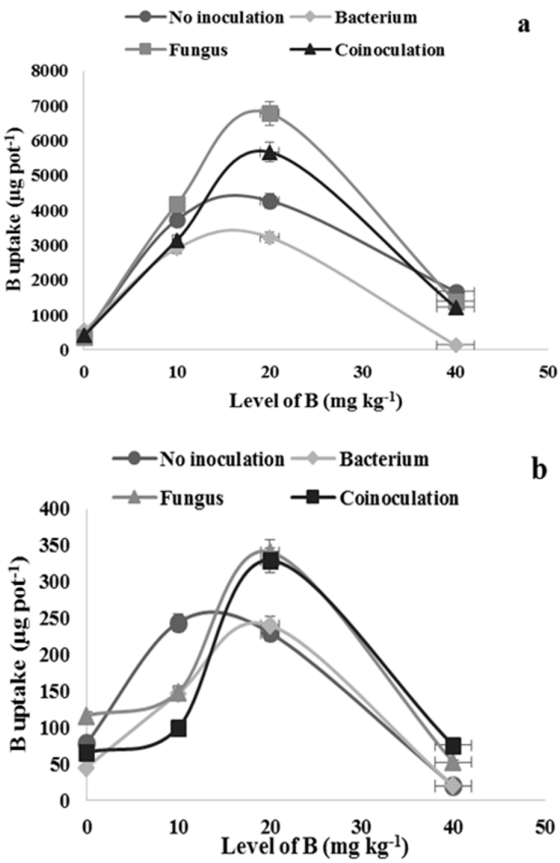

Figure 1. Shoot boron uptake (a), and root boron uptake (b) of maize plants grown under microbial and non-microbial inoculations under different levels of boron (B)

No inoculation: controls; bacterium: inoculated with *M. yunnanensis*; fungus: inoculated with *R. intraradices*; co-inoculation: inoculated with *M. yunnanensis* and *R. intraradices*. Values are presented as mean ± SD for three replicates.

Proline and electrolyte leakage

Under non-stress conditions, microbial inoculation did not affect the electrolyte leakage (Table 4). Under B stresses (0 to 20 mg kg^{-1}), the electrolyte leakage showed no significant difference when compared to the control plant. With 40 mg kg^{-1} B treatment, the electrolyte leakage increased significantly. MI of B treatment 40 mg kg^{-1}, electrolyte leakage decreased significantly in comparison with control plants. Thus, the inoculation of *R. intraradices*, *M. yunnanensis* and co-inoculation showed a significant effect (Table 4).

The leaf proline concentration did not change significantly with the excess B treatments (Table 4). Under non-stress conditions, microbial inoculation did not affect the proline content. With 40 mg B kg^{-1}, concentration the proline content increased but not significantly when compared to control plants. *M. yunnanensis* inoculations of B treatment at 40 mg kg^{-1}, concentration decreased the proline content significantly in comparison with control plants. In contrast, no significant difference was observed among the AMF inoculations and co-inoculation with non-inoculation (Table 4).

Mycorrhizal colonization

In AM-inoculated treatments, mycorrhizal colonization of *R.intraradices* on roots ranged from 2.73 to 85.71%, regardless of the stress conditions. Mycorrhizal colonization of co-inoculation AMF and PGPR was higher than inoculation with AMF with no significant difference. The B stress did not affect significantly the

colonization of maize roots. Mycorrhizal colonization was not detected in non-inoculated treatments (Table 4).

Table 4. Simple correlation between measured parameters

Treatment		Shoot B uptake	Root B uptake	Electrolyte leakage	Proline	Root colonization
B	Microbial					
0	No inoculation	369.3^{gh}	78.47^{def}	14.72^{bcde}	37.87^{abcd}	4.16^{c}
0	Bacterium	546.6^{gh}	45.45^{ef}	11.58^{e}	40.25^{abcd}	2.90^{c}
0	Fungus	350.7^{gh}	116.31^{cd}	11.75d^{e}	28.17^{d}	85.9^{ab}
0	Co-inoculation	403.6^{gh}	65.73^{ef}	12.26^{bcde}	40.45^{abcd}	82.13^{ab}
B	Microbial					
10	No inoculation	3729^{cde}	244.05^{b}	13.66^{bcde}	38.33^{abcd}	2.5^{c}
10	Bacterium	29064^{c}	164.79^{c}	12.31^{bcde}	30.34^{d}	2.92^{c}
10	Fungus	4182^{cd}	148.84^{c}	12.06^{cde}	33.68^{cd}	81.14^{ab}
10	Co-inoculation	3142.8^{de}	99.95^{cde}	12.10^{cde}	45.55^{abc}	85.73^{ab}
B	Microbial					
20	No inoculation	4272.25^{c}	229.89^{bc}	12.47^{bcde}	39.04^{abcd}	2.96^{c}
20	Bacterium	3230.5^{cde}	240.89^{b}	15.85^{b}	27.97^{d}	2.51^{c}
20	Fungus	6776.9^{a}	340.66^{a}	11.99^{cde}	47.42^{ab}	85.7^{ab}
20	Co-inoculation	5666.0^{b}	329.09^{a}	13.34^{bcde}	37.37^{abcd}	85.13^{ab}
B	Microbial					
40	No inoculation	1668.6^{f}	20.47^{f}	24.57^{a}	48.63^{a}	3.53^{c}
40	Bacterium	135.3^{h}	19.93^{f}	11.95^{cde}	30.71^{d}	2.6^{c}
40	Fungus	1454.78^{de}	52.54^{f}	15.52^{b}	35.67^{bcd}	77.2^{b}
40	Co-inoculation	1213.9^{fgh}	75.52de^{f}	15.52^{bcde}	36.46^{abcd}	89.86^{a}

Figure 2. B toxicity symptoms in maize. Typical symptoms of toxicity from exposure to excess boron are yellowing of leaf margins and tips and sometimes between veins. Symptoms progress to necrosis (browning) and premature drop of foliage

Discussion

Effect of microbial inoculation on B uptake and plant grow

There was a significant interaction effect on shoot B uptake between B treatments and MI ($P < 0.01$) (Table 2). B uptake in shoots ranged from 135.3 to 6776.9 μg pot^{-1} (Table 3). The highest shoot B uptake was with the highest B treatments (20 mg B kg^{-1} and AMF inoculation), whereas the lowest concentrations were

in treatment 40 mg B kg^{-1} with PGPR inoculation. Data showed that AMF inoculation and co-inoculation significantly increased shoot B uptake (Table 4).

There was a significant interaction effect on root B uptake between B treatments and MI ($P < 0.01$) (Table 2). Maize root B uptake varied from 19.93 to 340.66 μg pot^{-1} in inoculated plants (Table 3). Increasing B concentrations increased root B content and MI decreased the root B uptake except in 20 and 40 mg kg^{-1} treatment (Table 4). In general, B uptake is high in plant shoot compared to root because they are transferred from roots to shoot and stored in the aerial tissues. Similar results were reported by Sonmez *et al*. (2009). High B tolerance of plants could be related to the capacity of the plants to accumulate more B in the root and a less amount of B being transported to the shoot (Aftab *et al*., 2011). The mechanism behind this phenomenon might be due to the fact that B is generally transported by the transpiration stream and accumulates at the site of evapotranspiration (Oertli and Kohl, 1961). Thus, B-induced damages occur in the aerial parts of the plants (leaves, stems, buds and/or fruits) (Landi *et al*., 2019). Therefore, B tolerance is commonly associated with the ability to restrict the transport of B from root to shoot and thus maintain low B concentrations in plant shoot (Sutton *et al*., 2007). AMF fungus, *Glomus clarum* (Sonmez *et al*., 2009), and a PGPR, *Bacillus pumilus* (Khan *et al*., 2016), were both considered to reduce B concentrations in plant shoot via the restriction of translocation. However, in a study using two AMF species, *Funneliformis mosseae* and *Claroideoglomus etunicatum*, no restriction of B translocation from root to shoot was found (Liu *et al*., 2018). Excess B can also inhibit root growth and consequently reduce water uptake, which will aggravate water stress in dry areas (Aquea *et al*., 2012). The decrease in shoot B concentration may be partly a "dilution effect" caused by the increase of plant biomass. The dilution effect has been considered as a possible mechanism for AMF enhancing the tolerance of *Puccinellia tenuiflora* to B toxicity (Liu *et al*., 2018). In other word, the dilution effect due to increased biomass or yield may be the most immediate method to mitigate B toxicity.

Goldbach (1997) reported that B moves into plants during active transpiration across a concentration gradient and once in the plant, it moves readily through the xylem in the transpiration stream and accumulates at the point where water is lost through stomata in the leaf. Therefore, it is not surprising to find a higher B concentration in shoots than roots. Princi *et al*. (2016) also reported high shoot B concentrations compared to root B concentrations. Sonmez *et al*. (2009) reported that all B treatments significantly increased shoot B uptake whereas mycorrhiza inoculation decreased shoot B uptake. The highest uptakes were at the highest B treatments whereas the lowest ones were in the control treatments. Mycorrhiza inoculation significantly decreased shoot B uptake at each level of B. Reduced uptake occurred whether B was applied or not. With 0, 3, and 6 mg B L^{-1} application, there was a 47%, 34%, and 47% reduction in uptake, respectively with mycorrhiza fungi (Sonmez et al., 2009).

Increasing B concentrations with or without MI decreased relative yield of maize (Table 3). Mycorrhiza inoculation significantly improved relative yield in 10 mg B kg^{-1}, those increases in relative yield of B stressed-plants were significant. With 40 mg B kg^{-1} application, there was no difference between those with and without MI (Table 3). The only treatment in which relative yield was improved by mycorrhiza treatments was with 10 mg B kg^{-1} application. root colonization was not changed by B toxicity (Table 3).

Maize growing at high B concentrations (10, 20 and 40 mg B kg^{-1}) with or without MI showed leaf edge burning and necrosis compared with the control treatments. Increasing shoot B concentration in maize increased the symptoms of B toxicity. Toxicity symptoms were less visible on plants inoculated with mycorrhiza compared with non-inoculated plants (Figure 2). Similar results were found by Sonmez *et al*. (2009). Contrary to our results, Manchanda and Sharma (1991) reported that increasing B toxicity symptoms in wheat occurred in all treatments having similar or low total shoot B concentrations. Results from our study showed that increasing shoot B concentration increased B toxicity symptoms. Mycorrhiza inoculation alleviated B toxicity symptoms although it increased plant shoot B concentrations and uptake. The reason for that is unknown. The prevention mechanism of mycorrhiza on the adverse effects of B toxicity on plants needs to be studied in more detail.

Effect microbial inoculation on leaf proline and electrolyte leakage

There was a significant interaction effect on leaves proline concentration between B treatments and MI ($P < 0.01$) (Table 2). At 40 mg B kg^{-1} treatment, leaf proline content significantly decreases by *Rhizophagus intraradices* and *Micrococcus yunnanensis* inoculations, although there were no significant differences between AMF/40 mg kg^{-1} and PGPR/40 mg kg^{-1} (Table 4). Interestingly, some studies have reported that the PGRs decreased plant proline contents, which was increased by excess B (Gunes *et al*., 2007; Soylemezoglu *et al*., 2009). The authors also sustained that a decline in leaves proline content, important for ROS detoxification, could contribute to greater lipid peroxidation under B excess (Molassiotis *et al*., 2006). Thus, a greater concentration of proline in the leaves signifies a lesser B toxicity. The results obtained in this study showed that AMF and PGPR inoculations are more tolerant than non-inoculated plants under excess B, as the concentration of proline hardly changed in the latter, but significantly decreased in the former (Yusuf *et al*., 2011; Surgun *et al*., 2016).

There was a significant interaction effect on electrolyte leakage between B treatments and MI ($P < 0.01$) (Table 2). Application of 40 mg B kg^{-1} increased the electrolyte leakage significantly in comparison with control plants. While MI significantly decreased the electrolyte leakage when compared to the non-inoculation treatments (Table 4). Celikkol Akcay *et al*. (2016) observed the B toxicity in shoot tissues as early as exposure as manifested in several stress indicators including electrolyte leakage, malondialdehyde and H_2O_2 levels. B toxicity has been reported to damage plant cell membranes through lipid peroxidation, which may induce an increase in membrane permeability. Since passive transport across the cell membrane is a primary mechanism of B uptake in plants (Cervilla *et al*., 2012), the increase of membrane permeability may result in more B absorption. The increase of membrane permeability induced by excess B has been observed in some studies by the determination of tissue electrolyte leakage (Gunes *et al*., 2007; Eraslan *et al*., 2008).

Conclusions

In general, addition of boron increased shoot and root boron uptake. Microbial inoculations increased the boron uptake of plants. Additions of boron decreased the relative maize yield, but relative yield was not affected by mycorrhizal infection except where boron was applied at 10 mg kg^{-1}. Maize plants growing at high boron concentrations (10, 20 and 40 mg kg^{-1}) with or without microbial inoculations showed leaf edge burning and necrosis when compared to the control treatments. However, those toxicity symptoms were more pronounced in treatments of non-inoculation. Controversial results on the effects of microbial inoculations on boron toxicity are reported in literature. Therefore, there is a need for more detailed studies on this subject.

Authors' Contributions

Conceptualization, Funding acquisition, Data curation and Formal analysis: MZ, IM and AGS; Investigation, Methodology, Writing - original draft: NA, MZ and AGS; Resources, Supervision, Validation, and Writing - review and editing: MZ, IM and AGS.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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