

Article

Effects of *Claroideoglossum etunicatum* Fungus on the Growth Parameters of Maize (*Zea mays* L.) Plants under Boron Toxicity and Salt Stress

Mehdi Zarei ^{1,2}, Narges Abdar ¹, Amir Ghaffar Shahriari ^{2,*}, Iman Mirmazloun ^{3,*} and András Geösel ⁴

¹ Department of Soil Science, School of Agriculture, Shiraz University, Shiraz 7144165186, Iran; mehdezarei@shirazu.ac.ir (M.Z.); n.abdar@hafez.shirazu.ac.ir (N.A.)

² Department of Agriculture and Natural Resources, Higher Education Center of Eghlid, Eghlid 7381943885, Iran

³ Department of Plant Physiology and Plant Ecology, Institute of Agronomy, Hungarian University of Agriculture and Life Sciences, Villányi Str. 29-43, 1118 Budapest, Hungary

⁴ Department of Vegetable and Mushroom Growing, Institute of Horticultural Sciences, Hungarian University of Agriculture and Life Sciences, Villányi Str. 29-43, 1118 Budapest, Hungary; geosel.andras@uni-mate.hu

* Correspondence: shahriari.ag@eghli.ac.ir (A.G.S.); mirmazloun.seyediman@uni-mate.hu (I.M.)

Abstract: Soil salinity is an emerging phenomenon threatening arid and semiarid areas due to changing climatic events. Salinity, in combination with other elemental contaminants, can often harm crop performance and productivity. This experiment was conducted to evaluate the mitigating effect of *Claroideoglossum etunicatum*, an arbuscular mycorrhizal fungus (AMF), on combined boron (B) toxicity and salt stress symptoms in maize plants. After the stress and AMF treatments, plants were subjected to a wide range of analyses, such as AMF colonization rates, ion leakage, plant biomass, and concentration of B, phosphorus, sodium, potassium, iron, zinc, copper, and manganese in root and shoot tissues. The results showed that the combined stress did not affect the AMF colonization rate. AMF inoculation significantly increased plant biomass, the K⁺/Na⁺ ratio, and shoot B, sodium, and copper concentrations, but reduced root B concentrations and ion leakage. AMF inoculation slightly increased root dry weight and the sodium, potassium, zinc, copper and Mn contents in shoots under combined B and salinity stress, while AMF reduced the electrolyte leakage in leaves. It is inferred that AMF can ameliorate B toxicity in maize by improving biomass and reducing B concentration in plant tissues. Our research implies that *C. etunicatum* could be a valuable candidate for assisting in the remediation of boron-contaminated and saline soils.

Keywords: arbuscular mycorrhizal fungi; AMF; boron toxicity; salinity stress; combined stress; symbiont fungi



Citation: Zarei, M.; Abdar, N.; Shahriari, A.G.; Mirmazloun, I.; Geösel, A. Effects of *Claroideoglossum etunicatum* Fungus on the Growth Parameters of Maize (*Zea mays* L.) Plants under Boron Toxicity and Salt Stress. *Agronomy* **2024**, *14*, 1013. <https://doi.org/10.3390/agronomy14051013>

Academic Editor: Roberto Barbato

Received: 31 March 2024

Revised: 8 May 2024

Accepted: 9 May 2024

Published: 10 May 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

At trace concentrations, boron (B) plays a vital role as an essential nutrient for plants, serving various functions which are necessary for their growth and development [1]. However, although boron is indispensable at the adequate concentration, excessive levels can lead to toxicity and potentially harm plant health and productivity [1]. An excess of boron (B) and salinity can negatively impact plant growth by altering metabolic processes, inhibiting root cell division, disrupting chlorophyll synthesis in leaves, and impairing photosynthesis [1]. Boron toxicity represents a widespread issue that imposes limitations on plant growth across various regions globally. This phenomenon is most notably observed in North Africa, North America, southern Australia, the Middle East, western Asia, Malaysia, and China, where elevated levels of boron in soils hinder the optimal development of plants [1,2].

Elevated concentrations of boron (10 to 100 mg per kg) frequently occur as a natural phenomenon in soil environments. This accumulation of excess boron in topsoil is primarily attributed to the evaporation of groundwater containing high levels of boron [3].

It is believed that approximately 10% of total soil boron is accessible to plants, and that a bioavailability higher than 5 mg L⁻¹ can cause toxicity to many agricultural crops [4]. Besides salinity and drought stress, excess boron is a prevalent issue in soils of arid and semiarid regions [5]. Additional stresses, such as salinity, may aggravate B toxicity in plants, presenting an obstacle to mitigating the adverse effects of excess boron. An effective strategy for mitigating boron toxicity in plants could be the application of microorganisms associated closely with plant roots [6]. Increasing evidence has proven that arbuscular mycorrhizal fungi (AMF) can assist in the phytoremediation of metal contaminants by changing the spectrum of metal/oids for plant uptake, which could potentially reduce metal toxicity in the host plants [7]. *Bacillus pumilus*, a plant growth-promoting rhizobacterium (PGPR), was effective in mitigating B toxicity in tomato (*L. esculentum* L.) and rice (*Oryza sativa* L.) [8]. *Glomus clarum*, which decreased the uptake of excess boron in wheat (*Triticum durum*), is another AMF [9].

Maize (*Zea mays* L.) stands as a cornerstone crop globally and is crucial for ensuring food security for both humans and animals. However, it faces potential threats from boron toxicity and salinity stress, which can adversely impact its growth and yield, thereby jeopardizing food production systems [10]. The predominant visible symptom observed in maize plants subjected to excessive boron exposure is the development of burns, characterized by chlorotic and/or necrotic patches that appear primarily on the margins and tips of mature leaves [10,11]. Research on the effects of arbuscular mycorrhizal fungi (AMF) on maize plants subjected to both boron toxicity and saline conditions is scarce. The global enhancement of maize productivity hinges largely on effectively managing existing saline soils and preventing the encroachment of salts and soils high in B content [12]. Given that boron toxicity frequently co-occurs with salt stress, the goals of the current work were to investigate whether AMF are able to alleviate B toxicity in maize plants under salt stress. Thus, the key goal of this study was to develop environmentally friendly models, such as plant inoculation with AMF, that may help to prevent the damages caused by B toxicity and salinity stress in maize plants in calcareous soil.

2. Materials and Methods

2.1. Soil Preparation

The soil for this experiment was collected from the 0–30 cm surface horizon of the Colibro series (fine-loamy, carbonatic, hyperthermic Typic Calcustepts) in the Sarvestan region of Fars Province, Iran. The 2 mm sieved and air-dried soil was homogenized by mixing to ensure uniformity. The soil was characterized by the determination of the following mineral elements using the following methods: DTPA-extractable iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) [13]; hot water-soluble B [14,15]; pH [16]; electrical conductivity in saturated soil extract [17]; organic matter content with chromic acid [18]; total nitrogen with the Kjeldahl method [19]; P with the Olsen and Sommers method [20]; available potassium (K) with ammonium acetate [21]; and soil texture with the hydrometer method [22] of silt loam. The physical and chemical properties of the soil are presented in Table 1.

Table 1. Physicochemical analysis of the soil utilized for the inoculation experiment.

Soil Parameters	Measurements
pH in (H ₂ O)	7.68
EC (dS m ⁻¹)	0.6
Organic matter (%)	1.2
Total N (%)	0.05

Table 1. Cont.

Soil Parameters	Measurements
Available K (mg kg^{-1})	293
Available B (mg kg^{-1})	0.25
Available Fe (mg kg^{-1})	2.1
Available Mn (mg kg^{-1})	4.7
Available Zn (mg kg^{-1})	0.6
Available Cu (mg kg^{-1})	0.5
Clay (%)	21.8

2.2. Experimental Setup

This study was conducted in the greenhouse facilities of the Department of Soil Science, Shiraz University, located in Shiraz, Iran ($29^{\circ}43'37''$ N and $52^{\circ}35'16''$ E), in 2022. A factorial experiment was conducted using a completely randomized design (CRD) with at least three replications. The factorial combination of stress levels B and salt was as follows: B = 0 and EC = 0, B = 30 mg kg^{-1} and EC = 0, B = 0 and EC = 8, and B = 30 mg kg^{-1} and EC = 8 dS m^{-1} . Boron was administered in the form of boric acid, which was introduced concurrently with sodium chloride as a solution (Figure 1).

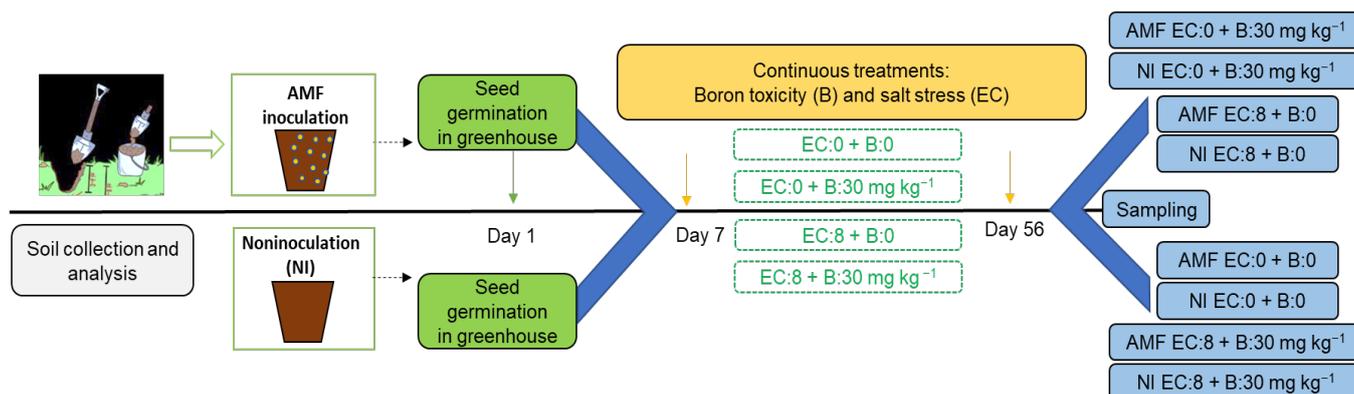


Figure 1. Schematic illustration of the experimental design and the combined stress.

The AMF treatments were inoculation with *C. etunicatum* and noninoculation. Maize seeds (SC 704 single cross) were kindly provided by the Plant Production and Genetics Department of Shiraz University, Iran. This selected cultivar is a single-cross variety which has been cultivated in the region for numerous years, consistently exhibiting significantly higher yields compared to other cultivars. The seeds underwent surface sterilization by immersion in a 0.1% (w/v) KMnO_4 solution for 2 h to eliminate external contaminants. Maize seeds were planted in pots filled with 3 kg soil, at a density of 2 plants per pot. The day/night temperatures were 25–27 $^{\circ}\text{C}$ and 16–17 $^{\circ}\text{C}$, respectively. The average relative humidity was recorded to be 60–70% and a photoperiod of 16 (h) with a photosynthetic photon flux of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were assured for the entire experimental period. Daily irrigation with distilled water was administered to keep the soil in the pots near field capacity. Field capacity at 1/3 bar was determined using a pressure plate apparatus (ATS, Tabriz, Iran) and estimated to be 20%.

The fungal inoculum was composed of sand, spore, mycelia, and colonized root fragments that were obtained from the Department of Soil Science at Shiraz University, Iran. The materials were prepared using a trap culture of *S. vulgare* L. The culture medium consisted of autoclaved soil/sand (1:4, v/v) and a starter culture of *C. etunicatum* (150 g). It was isolated from the rhizospheres of *Veronica rechingeri* growing in the Urumiah–Dokhtar area of Zanjan province of Iran ($36^{\circ} 400' \text{ N}$, $47^{\circ} 200' \text{ E}$). The fungus was characterized for its morphology and genetic identity, which are described in an earlier study by Zarei et al. [23]. The inoculation extent of the AM fungus was approximately 15 spores g^{-1} substrate, and

the root colonization rate was 95%. For the AMF treatment, 100 g of inoculation mixture was applied to each pot.

2.3. Sampling and Analysis

After 8 weeks of growth, the plant shoots and roots were harvested individually and washed with deionized water. Subsequently, shoot and root dry weight and chlorophyll content (measured using a SPAD-502 m) were recorded. Fresh fine roots from the plants were sampled, with equal amounts taken from each plant for assessing the percentage of root colonization [24].

The samples were processed in 8% KOH followed by acidification in 2% HCl and finally stained with lactic–glycerol–royal blue ink. The gridline intersect method was employed to estimate the percentage of root colonization [25]. Electrolyte leakage from the leaves was measured based on the method of Lutts et al. [26]. To determine the shoot and root dry matter, the plant samples were oven-dried at 65 °C for 48 h. The dried samples were pulverized into powder form and stored for subsequent analysis.

The total concentrations of selected nutrients in the plants were quantified using the dry ash method. Prior to filtration, the samples were dissolved in 2 N HCl, after which the concentrations of Fe, Zn, Cu, and Mn were determined by atomic absorption spectrometry (AA-670 Shimadzu, Kyoto, Japan) [27]. The total potassium (K) and sodium (Na) concentrations in the shoots were quantified using a flame photometer (CORNING 405, Gallenkamp, London, UK) [28]. Boron concentrations in shoot and root samples were determined by the azomethine-H colorimetric method [14]. The calculation of elemental uptake was performed according to Formula (1):

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

2.4. Statistical Analysis

Statistical analysis was conducted utilizing SAS 9.2 software (SAS Institute Inc., Madison, WI, USA). Two-way GLM tests were executed to assess the effects of various factors, with all analyses conducted at a 95% confidence interval ($\alpha = 0.05$). Multiple comparison procedures were carried out using Duncan's multiple range test at a significance level of $p < 0.05$, while Pearson's partial correlation was employed to examine the relationships between the measured plant parameters, also at a significance level of $p < 0.05$. GraphPad 9.5 software (GraphPad Software Inc., San Diego, CA, USA) was employed to generate the charts.

3. Results

3.1. Plant Growth

The combined stress of B and salt significantly decreased root dry weight (RDW), which was substantially increased by AMF inoculation (Figure 2b). However, shoot dry weight (SDW) and chlorophyll (CL) did not significantly differ from those of the control plants (Figure 2a,c). Under nonstress conditions, the AMF treatment significantly increased SDW and CL (Figure 2a,c). In contrast, the AMF treatment had no significant effect on RDW under the control (No B) condition (Figure 2b). Under combined stress, AMF significantly increased RDW and CL (Figure 2b,c).

In response to combined stress, observable leaf symptoms emerged. Initially, the leaves exhibited a yellow-green chlorosis. Subsequently, they progressively yellowed from the tips and displayed signs of dehydration. Towards the end of the treatment period, a substantial portion of the leaves turned yellow and wilted, consequently leading to a notable reduction in shoot dry weight (Figure 3).

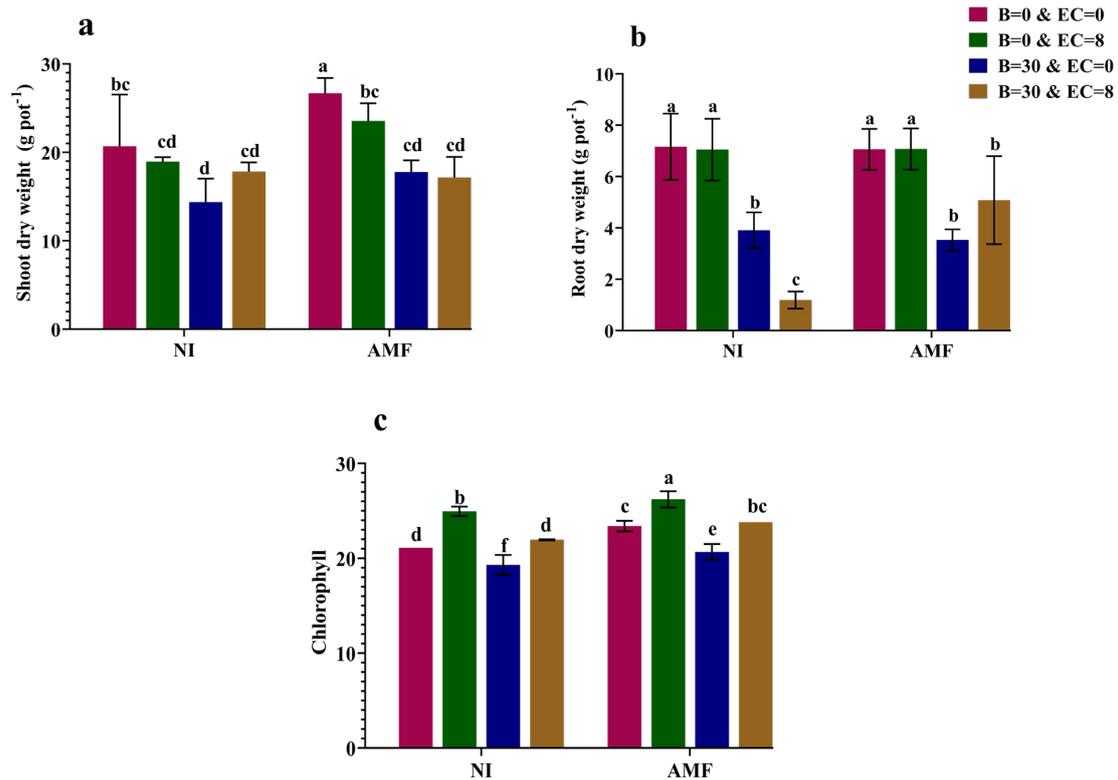


Figure 2. Shoot dry weight (a), root dry weight (b), and chlorophyll content (c) of AMF-inoculated maize plants grown under combined boron ($30 \text{ mg kg}^{-1} \text{ B}$) and salinity stress (8 dS m^{-1}). NI: noninoculation; AMF: *C. etunicatum*; EC, electrical conductivity (dS m^{-1}). Bars with the same letter are not significantly different at $p < 0.05$ according to Duncan's multiple range test.



Figure 3. Typical symptoms of boron toxicity and salinity stress in maize. Exposure to excess boron and salt resulted in yellowish strips on leaves and necrosis of the leaf margins and tips with a subsequent decline in foliage mass.

3.2. Plant B Concentrations and Uptake

The combined B and salinity stress significantly increased B concentration in plant tissue, and B concentration in shoots was much greater than that in roots (Figure 4a,b). Under combined stress, shoot B concentration (SBC) and root B concentration (RBC) significantly decreased in response to the AMF treatment. In contrast, shoot B uptake was significantly lower in the AMF treatment group, but root B uptake was significantly greater in the AMF treatment group than in the noninoculation treatment group (Figure 4c,d). These results

indicate that the AMF treatment alone restricted B accumulation in plants. Excess B in the fertigation solution caused a gradual increase in B accumulation in the leaves with time. A greater B concentration was observed in the treatments with 30 mg L⁻¹ B than in the control plants, which received no B supplementation (Figure 4). AMF also played a fundamental role in the accumulation of B in leaves. Under the B treatment alone, the noninoculated plants had a lower concentration of B in leaf samples compared to the inoculated samples. In contrast, RBC in the inoculated treatments decreased.

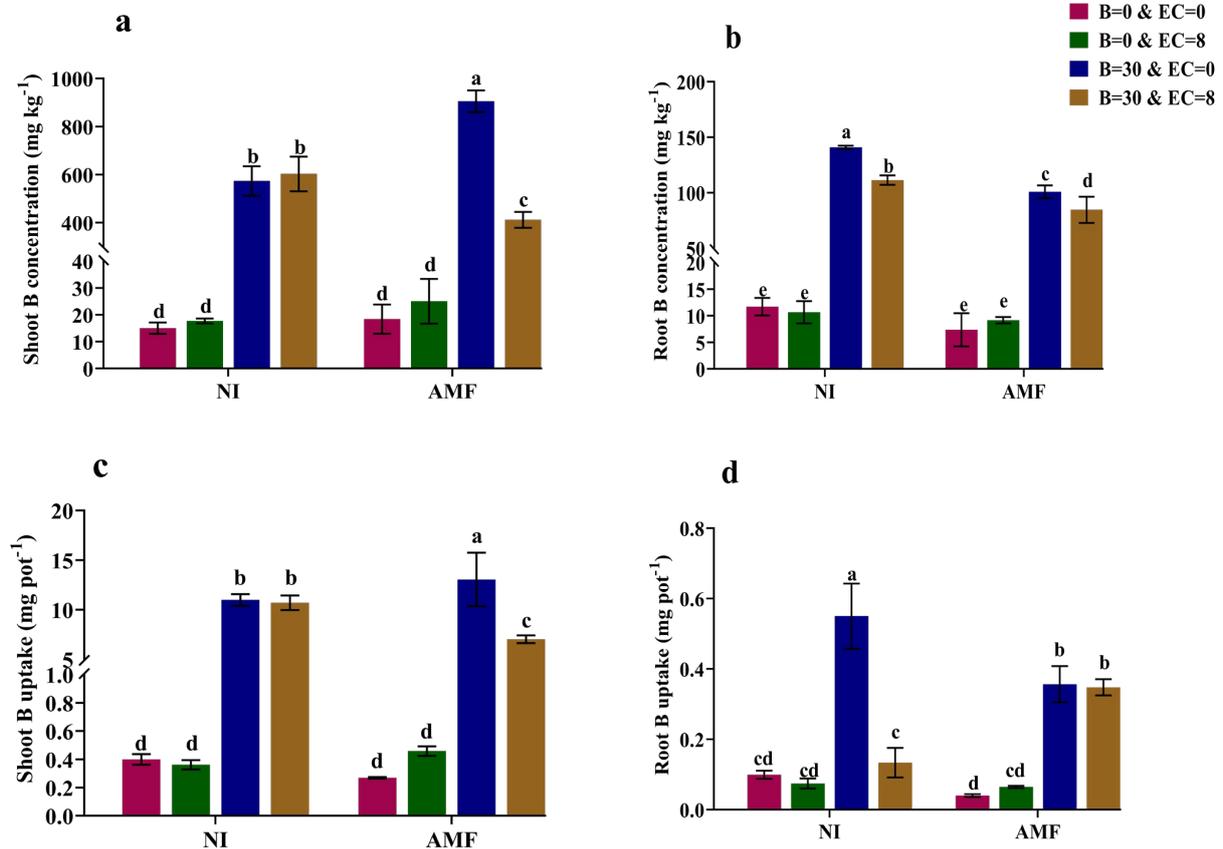


Figure 4. Shoot B concentration (a), root B concentration (b), shoot B uptake (c), and root B uptake (d) of AMF-inoculated maize plants grown under combined boron (30 mg kg⁻¹ B) and salinity stress (8 dS m⁻¹). NI: noninoculation; AMF: *C. etunicatum*; EC, electrical conductivity (dS m⁻¹). Bars with the same letter are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

3.3. Shoot Na and K Concentrations and Uptake

Under nonstress conditions, the AMF treatment did not affect shoot Na⁺ or K⁺ concentrations or uptake (Figures 5a,b and 6). In contrast, the K⁺/Na⁺ ratios in inoculated plants increased (Figure 5c). Compared with the control plants, shoot Na concentration and uptake in plants under the combined stress treatment significantly increased, whereas shoot K content and uptake decreased significantly (Figures 5 and 6). Under joint salinity and B stress, the AMF treatment greatly increased shoot Na and K concentrations and uptake, although the AMF treatment only had a significant effect in these conditions (Figures 5a,b and 6). Under nonstress conditions, AMF did not significantly affect shoot K concentrations. When compared with the control treatment, the combined stress significantly reduced shoot K concentration, whereas AMF significantly increased shoot K concentration (Figure 5b). The K/Na ratios in the shoots were determined based on the Na and K concentration data (Figure 5c). Under nonstress conditions, the AMF treatment significantly affected shoot K/Na ratios. The combined stress significantly influenced tissue K/Na ratio, with its lowest values being detected in the shoots. The AMF treatment slightly enhanced the shoot K/Na ratio, but this did not reach statistical significance (Figure 5c).

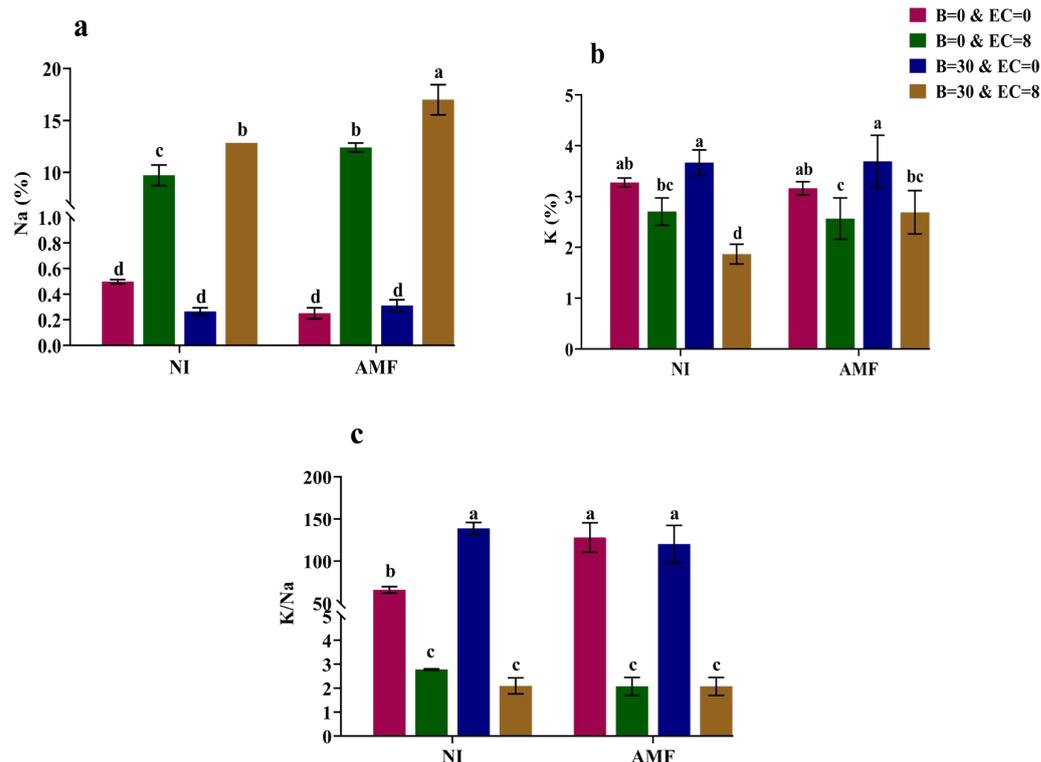


Figure 5. Na concentration (a), K concentration (b), and K/Na ratio (c) in shoots of AMF-inoculated and noninoculated maize plants under combined boron (B) and salinity stress. NI, noninoculation; AMF, *C. etunicatum*; EC, electrical conductivity (dS m^{-1}). Bars with the same letter are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

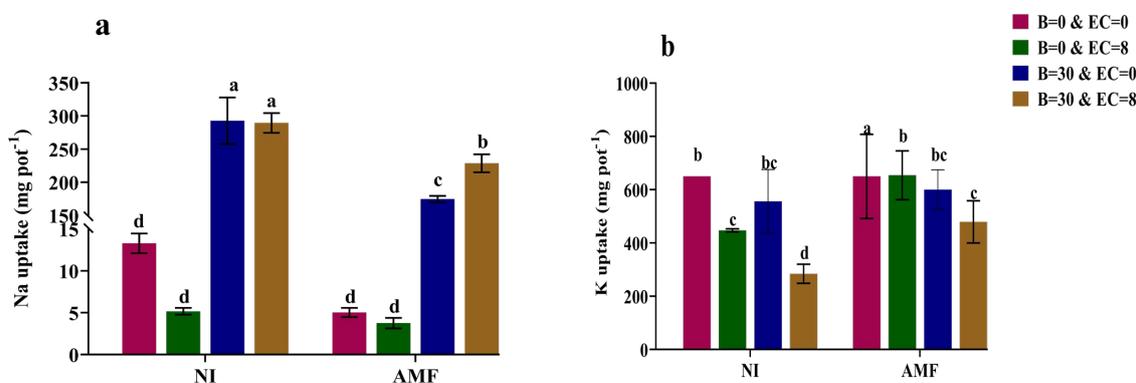


Figure 6. Shoot sodium (Na) uptake (a) and shoot potassium (K) uptake (b) in mycorrhizal and nonmycorrhizal inoculations under combined boron (B) and salinity stress. NI, noninoculation; AMF, *C. etunicatum*; EC, electrical conductivity (dS m^{-1}). Bars with the same letter are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

3.4. Shoot Zn, Cu, Fe, and Mn Concentrations and Uptake

Under nonstress conditions, the AMF treatment did not affect shoot Zn, Fe or Mn concentrations (Figure 7a,c,d). In contrast, shoot Cu concentration in the inoculated plants increased (Figure 7a). Compared with those in the noninoculated treatment, Fe, Zn, Cu, and Mn uptakes in the inoculated group significantly decreased (Table 2). Under the combined stress treatment, shoot Fe concentration and uptake were significantly different from those in the control plants, whereas Zn, Cu, and Mn concentrations in the shoots were not significantly different from those in the control plants (Figure 7 and Table 2). Under combined stress, the AMF treatment substantially increased shoot Zn, Fe, and Mn concentrations, so the AMF treatment had a significant effect (Figure 7).

Table 2. Effects of boron and salinity levels and fungal inoculation on Fe, Zn, Cu, and Mn uptake in maize leaves.

Treatment			Shoot Fe Uptake (mg pot ⁻¹)	Shoot Zn Uptake (mg pot ⁻¹)	Shoot Cu Uptake (mg pot ⁻¹)	Shoot Mn Uptake (mg pot ⁻¹)
EC	B	Microbial				
0	0	No inoculation	0.739 ± 0.07 ab	0.35 ± 0.01 bc	0.0809 ± 0.003 b	0.984 ± 0.11 bc
0	0	Fungus	0.513 ± 0.07 ed	0.234 ± 0.03 de	0.0242 ± 0.004 e	0.802 ± 0.27 cd
EC	B	Microbial				
8	0	No inoculation	0.56 ± 0.07 cd	0.405 ± 0.01 ab	0.495 ± 0.005 d	1.026 ± 0.04 b
8	0	Fungus	0.642 ± 0.08 bcd	0.454 ± 0.07 a	0.0671 ± 0.005 c	1.293 ± 0.003 a
EC	B	Microbial				
0	30	No inoculation	0.597 ± 0.03 cd	0.3 ± 0.05 cd	0.0588 ± 0.01 cd	0.673 ± 0.05 d
0	30	Fungus	0.426 ± 0.09 e	0.241 ± 0.03 de	0.0355 ± 0.01 e	0.389 ± 0.06 e
EC	B	Microbial				
8	30	No inoculation	0.678 ± 0.08 bc	0.337 ± 0.02 bc	0.115 ± 0.01 a	0.829 ± 0.07 bcd
8	30	Fungus	0.838 ± 0.01 a	0.202 ± 0.05 e	0.0315 ± 0.01 e	0.727 ± 0.02 d

Different letters in each column show significantly different values at $p < 0.05$ according to Duncan's multiple range test.

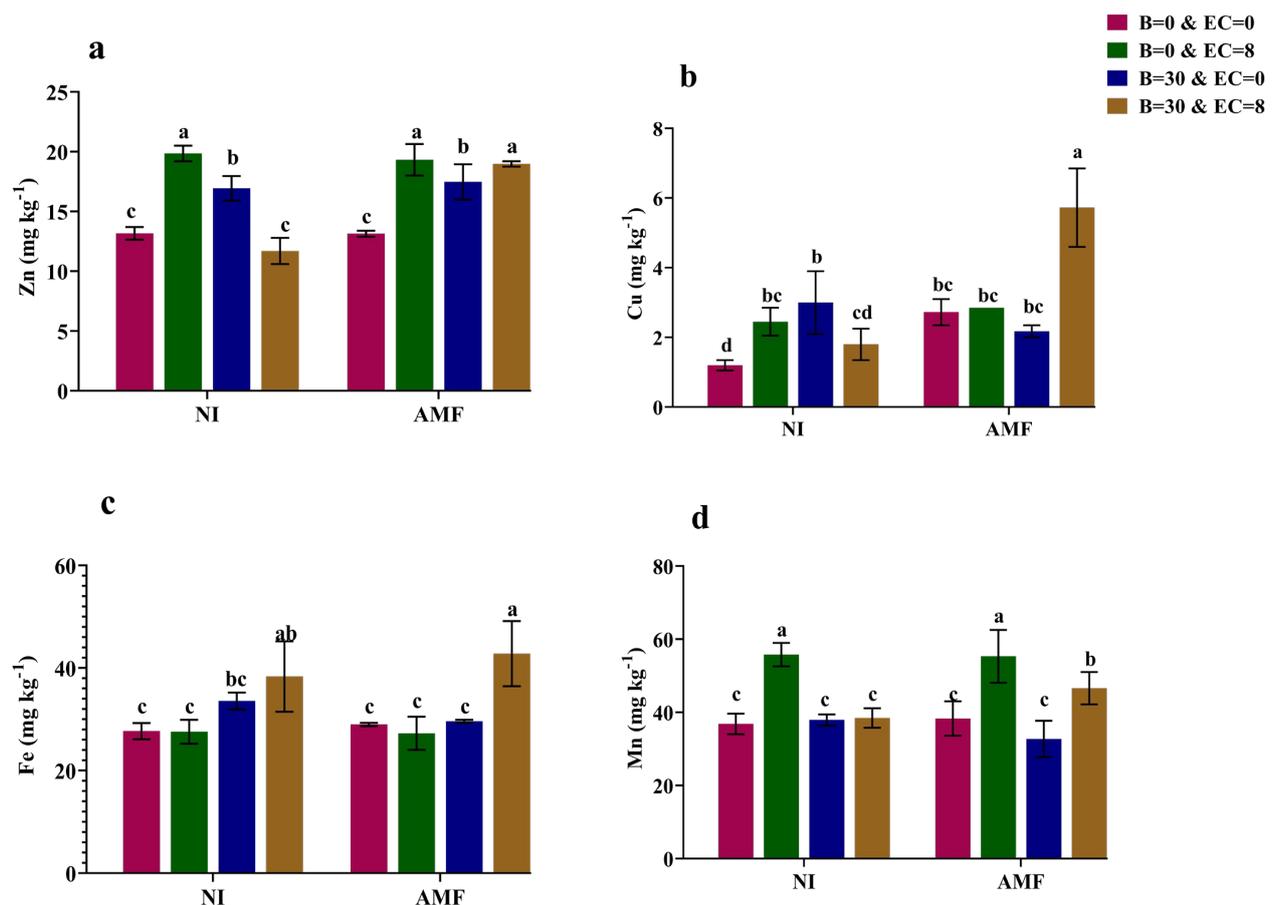


Figure 7. Shoot zinc (Zn) concentration (a), shoot copper (Cu) concentration (b), shoot iron (Fe) concentration (c), and shoot manganese (Mn) concentration (d) under mycorrhizal and nonmycorrhizal inoculation under combined boron (B) and salinity stress. NI, noninoculation; AMF, *C. etunicatum*; EC, electrical conductivity (dS m⁻¹). Bars with the same letter are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

3.5. Ion Leakage

Under nonstress conditions, the AMF treatment did not affect ion leakage (Figure 8a). Ion leakage (IL) did not significantly differ between the plants under the combined stress treatment and the control plants. Under salt stress, IL increased significantly, while the AMF treatment decreased the IL values significantly. Under combined stress, the AMF treatment substantially decreased IL, which shows a significant and positive effect of the AMF treatment (Figure 8a).

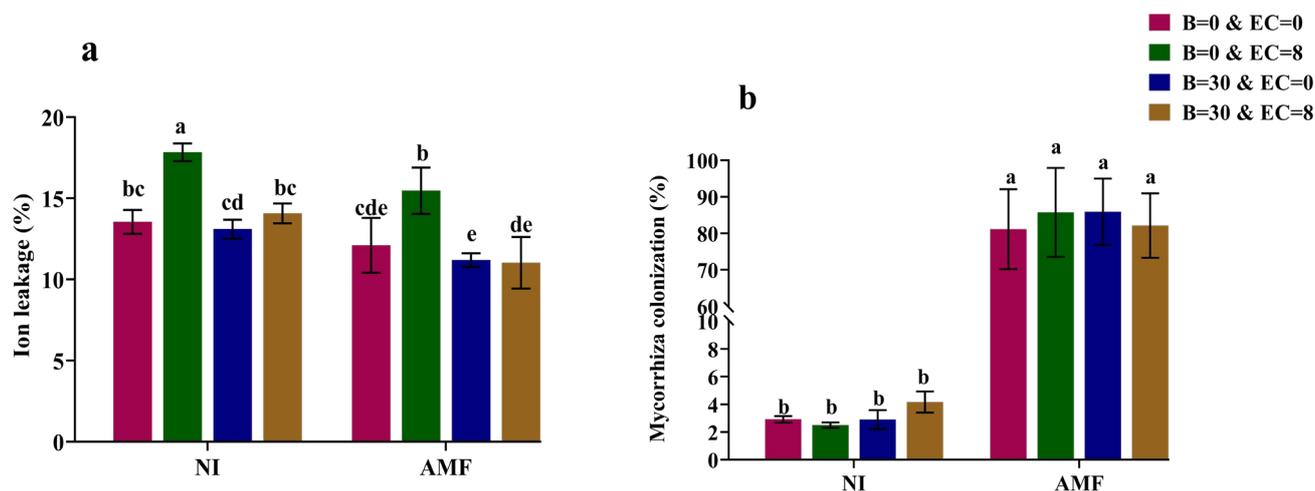


Figure 8. Ion leakage (a) and root colonization (b) under mycorrhizal and nonmycorrhizal inoculation under combined boron (B) and salinity stress. NI, noninoculation; AMF, *C. etunicatum*; EC, electrical conductivity (dS m^{-1}). Bars with the same letter are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

3.6. Mycorrhizal Colonization

In the AMF inoculation treatment, the colonization rate of *C. etunicatum* roots ranged from 3.12 (in noninoculated plants) to 83.72% (in inoculated plants), regardless of the stress treatment. Combined stress did not significantly affect the colonized roots of maize (Figure 8b). Mycorrhizal colonization was minimally detected in noninoculated plants due to the presence of the fungus in the soil microbiota.

4. Discussion

The present study showed that combined B and salt stress significantly inhibited the growth of maize. However, AMF inoculation significantly alleviated the adverse effects of the applied stress.

4.1. Detrimental Effects of Combined B and Salt Stress on Maize Plant Growth and the Influence of AMF Inoculation

The manner in which arbuscular mycorrhizal (AM) fungi colonize plant roots remains poorly understood. It is believed that the excretion of compounds from plant roots significantly enhances the colonization of roots by mycorrhizal fungi, making the identification of these compounds crucial for understanding the mechanisms underlying root mycorrhizal colonization [29]. Despite previous reports and speculations about the negative effects of stress on colonization rates [30], combined B and salt stress did not affect the AMF colonization rate in our experiment (Figure 7b). Under nonstress conditions (control), the AMF treatment significantly increased SDW by 28.91%, while RDW did not change. Liu et al. reported that under nonstress conditions, AMF significantly increased the SDW of *P. tenuiflora* [31]. Under salt stress (8 dS m^{-1}), the AMF treatment had no significant effect on plant dry weight, which is consistent with the observations of Liu et al. [32]. The ameliorating effect of AMF on salt stress was mainly explained by the increase in

nutritional element uptake, water status, osmotic regulation, and antioxidant systems in the host plant [30]. In a study by Yermiyahu et al. [5], B alone reduced root and shoot growth by 29.1 and 8.8%, respectively.

Under combined B and salinity stress, RWD decreased significantly, and the AMF treatment increased RDW by 76.55%, which showed a similar trend to the observations of Liu et al. [31]. Under the applied salinity stress (8 dS m⁻¹), ion leakage (IL) in the leaves of maize plants increased by 31.66%, which was significantly correlated with shoot Na concentrations. Earlier reports showed that salinity stress markedly increased IL in leaves compared with control plants [33]. This increase appeared to be due to plant cell injury and membrane damage caused by salinity stress [34]. Under combined stress, the AMF treatment significantly decreased the IL rate of leaves. Mycorrhizal inoculation significantly reduced the IL of leaves compared with that of plants that had not been inoculated with mycorrhizae [33]. This effect of mycorrhizae may be attributed to their ability to improve nutrient uptake and reduce specific ion effects [34].

4.2. Role of AMF Inoculation and Determination of Plant Nutrient Concentrations

In the present study, high-level B significantly inhibited plant growth. In the 30 mg kg⁻¹ B treatment, SBC and RBC significantly increased in comparison with the control (97.37% and 91.67%, respectively). Root and shoot B concentrations (RBC and SBC) under combined B and salinity stress were lower than those under B stress alone, and the AMF treatment decreased SBC and RBC significantly, by 31.65% and 24.02%, respectively, which is consistent with the observations of Liu et al. [31]. These results indicate that B application increased plant B concentrations and that salt decreased B concentrations in plants inoculated with mycorrhizal fungi. The combined stress of B and salt inhibited the transport of B from the roots to the shoots, indicating that the transpiration stream has an effect on B uptake in plants. It is known that salinity has the potential to decrease the osmotic potential of soil moisture, leading to stomatal closure. Also, Pan et al. reported a 0.25-unit decline in the pH value of maize root tips after inoculation with *C. etunicatum* [35]. This can play a role in the uptake regulation of certain elements or ions. This mechanism may primarily contribute to the inhibition of boron uptake, associated with evapotranspiration, and the limitation of boron transport from the roots to the shoots via the xylem [5,36,37]. Salt stress can also induce ATPase protein production and ATPase activity, which may assist in resistance to B and salt stress [38]. Previous studies have shown that salinity stress can increase Na⁺ accumulation and uptake but inhibits K⁺ accumulation [8]. Shoot potassium concentration significantly decreased by 42.98% under the combined stress treatment in comparison with the control treatment, while it increased in response to the AMF treatment (44.05%). An improved K⁺ uptake under salt stress after AMF inoculation was also reported in rice [6]. Mycorrhizal inoculation enhances K⁺ uptake, which competes with Na uptake to facilitate growth as a result of limited Na⁺ in shoots [39,40].

Under the combined stress of B and salt, the AMF treatment significantly increased Zn, Cu and Mn concentrations but did not affect shoot Fe concentration. Abdar et al. [6] reported greater concentrations of Zn, Cu, Na, and Fe in AMF-colonized plants than in noninoculated plants. It is now quite widely accepted that mycorrhizae can promote the growth of plants by increasing the bioavailability of essential nutrients such as Cu and Zn [3,8,31].

5. Conclusions

The observed seventeen percent increase in total maize biomass following inoculation with *C. etunicatum* implies that the applied mycorrhizal species possesses the ability to ameliorate the challenges posed by boron or/and salinity stress. The mechanisms driving this ameliorative effect primarily involve the regulation of boron uptake and accumulation in the shoot and the mitigation of ion leakage in the leaf. With its demonstrated efficacy, *C. etunicatum* emerges as a promising candidate for enhancing the remediation of soils burdened with excessive levels of boron and salt. These findings not only underscore the

potential of arbuscular mycorrhizal fungi in phytoremediation efforts but also contribute substantial evidence towards the investigation of strategies aimed at alleviating plant boron toxicity using this symbiotic relationship. The inoculation method applied in this experiment can be considered for developing large-scale systems and utilization strategies to harness the potential of AMF in agriculture.

Author Contributions: Conceptualization, Funding acquisition, Data curation, and Formal analysis: M.Z., I.M. and A.G.S.; Investigation, Methodology, and Writing—original draft: N.A., M.Z., A.G.S. and A.G.; Resources, Supervision, Validation, and Writing—review and editing: M.Z., I.M., A.G. and A.G.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Shiraz University (Iran). Open access funding provided by Hungarian University of Agriculture and Life Sciences.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Wang, G.; DiTusa, S.F.; Oh, D.H.; Herrmann, A.D.; Mendoza-Cozatl, D.G.; O'Neill, M.A.; Dassanayake, M. Cross species multi-omics reveals cell wall sequestration and elevated global transcript abundance as mechanisms of boron tolerance in plants. *New Phytol.* **2021**, *230*, 1985–2000. [[CrossRef](#)] [[PubMed](#)]
- Pandey, A.; Khan, M.K.; Hakki, E.E.; Gezgin, S.; Hamurcu, M. Combined boron toxicity and salinity stress—An insight into its interaction in plants. *Plants* **2019**, *8*, 364. [[CrossRef](#)]
- Turhan, A. Interactive effects of boron stress and mycorrhizal (AMF) treatments on tomato growth, yield, leaf chlorophyll and boron accumulation, and fruit characteristics. *Arch. Agron. Soil Sci.* **2021**, *67*, 1974–1985. [[CrossRef](#)]
- Ozturk, M.; Sakcali, S.; Gucel, S.; Tombuloglu, H. Boron and Plants. In *Plant Adaptation and Phytoremediation*; Ashraf, M., Ozturk, M., Ahmad, M.S.A., Eds.; Springer: Dordrecht, The Netherlands, 2010; pp. 275–311.
- Yermiyahu, U.; Ben-Gal, A.; Keren, R.; Reid, R.J. Combined effect of salinity and excess boron on plant growth and yield. *Plant Soil* **2008**, *304*, 73–87. [[CrossRef](#)]
- Abdar, N.; Zarei, M.; Ronaghi, A.M. Mitigation effects of *Rhizophagus intraradices* and *Micrococcus yunnanensis* on boron toxicity in maize (*Zea mays* L.) plant. *J. Plant Nutr.* **2023**, *46*, 3312–3324. [[CrossRef](#)]
- Coninx, L.; Martinova, V.; Rineau, F. Mycorrhiza-assisted phytoremediation. *Adv. Bot. Res.* **2017**, *83*, 127–188.
- Khan, A.; Zhao, X.Q.; Javed, M.T.; Khan, K.S.; Bano, A.; Shen, R.F.; Masood, S. *Bacillus pumilus* enhances tolerance in rice (*Oryza sativa* L.) to combined stresses of NaCl and high B due to limited uptake of Na⁺. *Environ. Exp. Bot.* **2016**, *124*, 120–129. [[CrossRef](#)]
- Sonmez, O.; Aydemir, S.; Kaya, C. Mitigation effects of mycorrhiza on boron toxicity in wheat (*Triticum durum*) plants. *N. Z. J. Crop Hortic. Sci.* **2009**, *37*, 99–104. [[CrossRef](#)]
- Sarafi, E.; Siomos, A.; Tsouvaltzis, P.; Therios, I.; Chatzissavvidis, C. Boron toxicity effects on the concentration of pigments, carbohydrates and nutrient elements in six nongrafted pepper cultivars (*Capsicum annuum* L.). *Indian J. Plant Physiol.* **2018**, *23*, 474–485. [[CrossRef](#)]
- Simón-Grao, S.; Nieves, M.; Martínez-Nicolás, J.J.; Alfosea-Simón, M.; Cámara-Zapata, J.M.; Fernández-Zapata, J.C.; García-Sánchez, F. Arbuscular mycorrhizal symbiosis improves tolerance of *Carrizo citrange* to excess boron supply by reducing leaf B concentration and toxicity in the leaves and roots. *Ecotoxicol. Environ. Saf.* **2019**, *173*, 322–330. [[CrossRef](#)]
- Naz, T.; Alihtar, J.; Iqbal, M.M.; Anwar-ul-Haq, M.; Murtaza, G.; Niazi, N.K.; Dell, B. Assessment of gas exchange attributes, chlorophyll contents, ionic composition and antioxidant enzymes of bread wheat genotypes in boron toxic, saline and boron toxic-saline soils. *Int. J. Agric. Biol.* **2019**, *21*, 1271–1281.
- Lindsay, W.L.; Norvell, W.A. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* **1978**, *42*, 421–428. [[CrossRef](#)]
- Berger, K.C.; Truog, E. Boron determination in soils and plants. *Ind. Eng. Chem. Anal. Ed.* **1939**, *11*, 540–545. [[CrossRef](#)]
- John, M.K.; Chuah, H.H.; Neufeld, J.H. Application of improved azomethine-H method to the determination of boron in soils and plants. *Analy. Lett.* **1975**, *8*, 559–568. [[CrossRef](#)]
- Thomas, G.W. Soil pH and soil acidity. In *Methods of Soil Analysis: Part 3 Chemical Methods*; Wiley: Hoboken, NJ, USA, 1996; Volume 5, pp. 475–490.
- Rhoades, J.; Sparks, D.; Page, A.; Helmke, P.; Loeppert, R.; Soltanpour, P.; Summer, M. *Salinity: Electrical Methods of Soil Analysis Part 3-Chemical Methods Conductivity and Total Dissolved Solids*; Wiley Online Library: Hoboken, NJ, USA, 1996; pp. 417–435.
- Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*; Wiley Online Library: Hoboken, NJ, USA, 1996; Volume 5, pp. 961–1010.

19. Bremner, J.M. Nitrogen-total. In *Methods of Soil Analysis: Part 3 Chemical Methods*; Wiley Online Library: Hoboken, NJ, USA, 1996; Volume 5, pp. 1085–1121.
20. Olsen, S.R.; Sommers, L.E.; Phosphorus, P. *Methods of Soil Analysis, Part 2*; Page, A.L., Ed.; Soil Science Society of America: Madison, WI, USA, 1982; pp. 403–424.
21. Knudsen, D.; Peterson, G.A.; Pratt, P.F. Lithium, sodium, and potassium. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*; Wiley Online Library: Hoboken, NJ, USA, 1983; Volume 9, pp. 225–246.
22. Bouyoucos, G.J. A recalibration of the hydrometer method for making mechanical analysis of soils. *Agro. J.* **1962**, *43*, 434–438. [[CrossRef](#)]
23. Zarei, M.; König, S.; Hempel, S.; Nekouei, M.K.; Savaghebi, G.; Buscot, F. Community structure of arbuscular mycorrhizal fungi associated to *Veronica rechingeri* at the Anguran zinc and lead mining region. *Environ. Pollut.* **2008**, *156*, 1277–1283. [[CrossRef](#)] [[PubMed](#)]
24. Kormanik, P.P.; McGraw, A.C. Qualification of Vesicular Arbuscular Mycorrhizae in Plant Root. In *Method and Principles of Mycorrhizal Research*; Scheneck, N.C., Ed.; American Phytopathological Society: St. Paul, MN, USA, 1982; pp. 37–45.
25. Bañuelos, G.S.; LeDuc, D.; Johnson, J. Evaluating the tolerance of young hybrid poplar trees to recycled waters high in salinity and boron. *Int. J. Phytoremediat.* **2010**, *12*, 419–439. [[CrossRef](#)] [[PubMed](#)]
26. Lutts, S.; Kinet, J.M.; Bouharmont, J. Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Plant Growth Regul.* **1996**, *19*, 207–218. [[CrossRef](#)]
27. Chapman, H.D.; Pratt, P.F. Methods of analysis for soils, plants and waters. *Soil Sci.* **1962**, *93*, 68. [[CrossRef](#)]
28. Page, A.L.; Miller, R.H.; Keeny, D.R. *Methods of Soil and Plant Analysis*; American Society of Agronomy: Madison, WI, USA, 1982.
29. Shen, Y.; Duan, T. The Interaction between Arbuscular Mycorrhizal Fungi (AMF) and Grass Endophyte (*Epichloë*) on Host Plants: A Review. *J. Fungi* **2024**, *10*, 174. [[CrossRef](#)]
30. Jongen, M.; Albadran, B.; Beyschlag, W.; Unger, S. Can arbuscular mycorrhizal fungi mitigate drought stress in annual pasture legumes? *Plant Soil* **2022**, *472*, 295–310. [[CrossRef](#)]
31. Liu, C.; Dai, Z.; Xia, J.; Chang, C.; Sun, H. Combined effect of salt and drought on boron toxicity in *Puccinellia tenuiflora*. *Ecotoxicol. Environ. Saf.* **2018**, *157*, 395–402. [[CrossRef](#)] [[PubMed](#)]
32. Liu, C.; Dai, Z.; Cui, M.; Lu, W.; Sun, H. Arbuscular mycorrhizal fungi alleviate boron toxicity in *Puccinellia tenuiflora* under the combined stresses of salt and drought. *Environ. Pollut.* **2018**, *240*, 557–565. [[CrossRef](#)] [[PubMed](#)]
33. Porcel, R.; Aroca, R.; Ruiz-Lozano, J.M. Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. *Agron. Sustain. Dev.* **2012**, *32*, 181–200. [[CrossRef](#)]
34. Abdar, N.; Zarei, M.; Shahriari, A.G.; Mirmazloum, I. The effect of arbuscular mycorrhizal inoculation and plant growth-promoting rhizobacteria on maize (*Zea mays* L.) under boron toxicity stress. *Not. Bot. Horti. Agrobot.* **2023**, *51*, 13473. [[CrossRef](#)]
35. Pan, G.; Wei, Y.; Zhao, N.; Gu, M.; He, B.; Wang, X. Effects of *Claroideoglossum etunicatum* Fungi Inoculation on Arsenic Uptake by Maize and *Pteris vittata* L. *Toxics* **2022**, *10*, 574. [[CrossRef](#)] [[PubMed](#)]
36. Alharby, H.F.; Nahar, K.; Al-Zahrani, H.S.; Hakeem, K.R.; Hasanuzzaman, M. Enhancing salt tolerance in soybean by exogenous boron: Intrinsic study of the ascorbate-glutathione and glyoxalase pathways. *Plants* **2021**, *10*, 2085. [[CrossRef](#)]
37. Sharma, M.P.; Buyer, J.S. Comparison of biochemical and microscopic methods for quantification of arbuscular mycorrhizal fungi in soil and roots. *Appl. Soil Ecol.* **2015**, *95*, 86–89. [[CrossRef](#)]
38. Lata, C.; Kumar, A.; Sharma, S.K.; Setter, T.L.; Singh, M.; Prasad, K.R.; Yaduvanshi, N.P. Combined effects of excess boron and salinity in wheat varieties differing in tolerance to these stresses. *J. Soil Salin. Water Qual.* **2015**, *7*, 107–114.
39. Saxena, B.; Shukla, K.; Giri, B. Arbuscular Mycorrhizal Fungi and Tolerance of Salt Stress in Plants. In *Arbuscular Mycorrhizas and Stress Tolerance of Plants*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 67–97.
40. Borde, M.; Dudhane, M.; Kulkarni, M. Role of Arbuscular Mycorrhizal Fungi (AMF) in Salinity Tolerance and Growth Response in Plants under Salt Stress Conditions. In *Mycorrhiza-Eco-Physiology, Secondary Metabolites, Nanomaterials*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 71–86.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.