








Article

Biofertilizer-Based Zinc Application Enhances Maize Growth, Gas Exchange Attributes, and Yield in Zinc-Deficient Soil

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Citation: Saboor, A.; Ali, M.A.; Ahmed, N.; Skalicky, M.; Danish, S.; Fahad, S.; Hassan, F.; Hassan, M.M.; Brestic, M.; EL Sabagh, A.; et al. Biofertilizer-Based Zinc Application Enhances Maize Growth, Gas Exchange Attributes, and Yield in Zinc-Deficient Soil. *Agriculture* **2021**, *11*, 310. <https://doi.org/10.3390/agriculture11040310>

Academic Editors: Laura Ercoli and Eric Blanchart

Received: 20 February 2021

Accepted: 29 March 2021

Published: 2 April 2021

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Abstract: Zinc (Zn) concentration in soil varies from deficient to toxic. Its deficiency, as well as toxicity, through imbalanced application and cropping in industrial sites can reduce maize growth and yield. Therefore, balanced Zn application is necessary to save resources and achieve optimum growth and yield in maize. Arbuscular mycorrhizal fungi (AMF) can provide tolerance to the host plant against Zn-induced stress. Inoculation with AMF helps in regulating the uptake of Zn and enhances the growth and yield of crops. Different application rates of Zn (0, 20, 40, 60, 80, 100, and 120 mg Zn kg^{−1}) were applied with inoculation with AMF (AM) and without AMF (NM). Results showed that root colonization was 45% higher in AMF-inoculated plants than non-inoculated plants. A significant increase in plant height (15%), number of leaves (35.4%), cob weight (4.39%), 1000-grain weight (10.5%), and biological yield (42.2%) signified the efficacious functioning of Zn20 + AM over sole inoculation with AM. We also observed that AMF inoculation with Zn20 helped to improve photosynthesis, transpiration, and stomatal conductance. Furthermore, both Zn20 + AM and Zn20 + AM significantly increased total soluble protein compared with AM. Higher application rates of zinc, i.e., Zn80 and Zn120, induced Zn toxicity with (AM) and without (NM) AMF. In conclusion, Zn20 + AM is an effective treatment to achieve better growth and maize yield without Zn deficiency or toxicity.

Keywords: arbuscular mycorrhizal fungi; maize; zinc; gas exchange attributes; morphological attributes; yield

1. Introduction

Maize (*Zea mays* L.) is the third most cultivated crop and a member of the Poaceae (Gramineae) family. In Pakistan, it is normally cultivated in spring and autumn [1]. The Punjab and Khyber Pakhtunkhwa (KPK) provinces are major maize producers in Pakistan, accounting for about 97% of total production [2]. Maize is considered an exhaustive

nutrient crop as it takes a high amount of nutrients from the soil. Unfortunately, soils in Pakistan cannot supply a sufficient quantity of nutrients [3–5], especially zinc (Zn), to fulfill plant demands and improve crop yield [6–8].

Zinc is considered the most important micronutrient for normal and healthy plant growth [3,4,6,9]. It is a structural component or cofactor of various enzymes involved in many biochemical processes. In plants, it is involved in photosynthesis, carbohydrate metabolism, protein metabolism, pollen formation, auxin metabolism, maintenance of membrane integrity, and induction of tolerance against various stresses [10]. The total concentration of Zn in soil ranges from 10 to 300 mg Zn kg^{−1} soil, and the average is 55 mg Zn kg^{−1} soil. However, most Zn forms complexes with soil colloids and organic matter (OM), reducing Zn bioavailability for plants. Therefore, a very small amount of Zn is normally available for plant uptake.

Zinc deficiency in soil is a global problem. Arid and semi-arid soils are usually deficient in Zn due to the high immobilization of Zn. Furthermore, high soil pH (8.0 to more than 8.5) drastically reduces soil Zn bioavailability. Soils in Pakistan mostly exhibit high soil pH, high CaCO₃, and low OM content; therefore, Zn deficiency is a major issue in most regions [11]. High input of phosphatic fertilizers also reduces Zn bioavailability due to the formation of insoluble Zn phosphates in soil. The soil microbial application technique is considered the best among the various available methods to achieve optimum plant growth and yield [6,8,12–21]. Commonly used organic amendments [22–24] and soil microbial techniques include the application of plant growth-promoting bacteria [25–28], Zn-solubilizing bacteria, and arbuscular mycorrhizal fungi. Arbuscular mycorrhizal fungi colonize the plant roots and form a symbiotic relationship with plants by providing nutrients, water, and protection from pathogens [29]. More than 90% of terrestrial plants benefit from this symbiosis [30]. Their contribution in increasing the uptake of nutrients (P, Zn, Fe, Cu, and K) from the soil system to plants is well known [14]. Arbuscular mycorrhizal fungi (AMF) also mediates the toxic effects of metals (Zn) and exerts a protective effect to promote plant growth under metal-polluted conditions [31]. It has been observed that AMF-inoculated plants produced more biomass as compared to non-inoculated plants. Increased biomass is directly related to improved plant nutrition due to increased root colonization rate [32].

AMF contribution to Zn uptake was quantified by Jansa et al. [33]. After that, Watts-Williams et al. [34] quantified the total Zn uptake and mycorrhizal contribution and plant dependency on *Rhizophagus irregularis*. In wheat and barley, *R. irregularis* contributed to up to 24.3% and 12.7% of Zn uptake, respectively [14]. Therefore, the current study was conducted to explore the potential benefits of AMF on maize growth and yield attributes under different Zn application rates. The study aimed to assess the performance of AMF under Zn deficiency and toxicity conditions. It was hypothesized that AMF can help in decreasing the application rate of Zn. AMF can also play an important role in the improvement of growth, gas exchange attributes, and yield of maize.

2. Materials and Methods

2.1. Experimental Soil and Location

A completely randomized design (CRD) pot experiment was conducted at the research area of the Department of Soil Science, Bahauddin Zakariya University, Multan, Pakistan. Extractable zinc was deficient (0.52 mg Zn kg^{−1}) in soil collected from the Chak 5 Faiz location (latitude 29.9° N and longitude 71.5° E).

2.2. Experimental Soil Characterization

Air-dried samples of soil of 2-mm mesh size were mixed with sand in an 8:2 ratio, termed soil. After mixing, soil was analyzed for various physicochemical properties. It contained 39% saturation, 54% water-holding capacity, 0.14 dSm^{−1} ECe [35], 8.82 pH [36], 6.5 mg kg^{−1} available P [37], 126 mg kg^{−1} extractable K [38], 0.43% organic matter [39], and 0.0215% total N [40].

2.3. Pot Preparation by Addition of Zn and AMF

Non-draining pots were filled with 20 kg soil inoculated with 5 g mycorrhizal inoculum Clonex® (Root Maximizer; 5711 Enterprise Drive, Lansing, MI, USA) having 158 propagule gram^{-1} . *Glomus* species were the major constituents of the inoculum. In AMF-controlled pots (M^-), Topsin M (Thiophanate Methyl 70% wettable powder (WP)) was applied at 50 mg kg^{-1} soil for reducing AMF root colonization. Six Zn doses (20, 40, 60, 80, 100, and 120 mg Zn kg^{-1} soil in ZnSO_4 form) were applied along with a control treatment without Zn addition. The treatments are referred to as Zn0, Zn20, Zn60, Zn80, Zn100, and Zn120.

2.4. Moisture Maintenance and Fertilizer Addition

The moisture content in each pot was maintained at 60% of the total WHC on a weight basis [41], and the recommended doses of N and K with half P fertilizers were applied at the rate of 227.24:71.63:91.39 kg ha^{-1} .

2.5. Seeds Sowing and Thinning

Maize variety YH 1898 was used in this study. Two weeks after germination, plants were thinned to two plants per pot and were irrigated until maturity.

2.6. Morphological Attributes

At the time of maturity (R6 = milk line no longer evident, black layer formed; end of mass gain; maximum dry weight is attained), plants were harvested. Plant agronomic parameters such as the number of leaves were counted, and plant height (cm) and stem girth (cm) were measured using a measuring tape before harvesting in three replications.

2.7. Yield Parameters

At maturity (115 days after sowing), the aboveground biomass of plants in all pots was destructively sampled and the weights of grains and residues were determined. The number of leaves was counted manually and cob length was noted by using a standard measuring tape.

2.8. Gas Exchange Attributes

Stomatal conductance, photosynthetic rate, and transpiration rate were measured at the tasseling stage (V12–V14; 45 days after germination when leaves' growth was active) with constant light intensity of a photosynthesis device ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$), CO_2 concentration (400 μmol), and airflow (500 $\mu\text{mol s}^{-1}$). When photosynthesis reached a steady state, the measurement was recorded by an LCI- SD Ultra Compact Photosynthesis System® between 10:30 and 11:30 a.m. at a saturating intensity of light [16,42,43].

2.9. Total Soluble Protein (TSP)

Composite leaf samples (V12–V14; 45 days after germination when leaves' growth was active) were stored at -80°C in a freezer (Panasonic Japan; MDF-U55V-PE). For enzyme extraction, samples were vortexed and then centrifuged in potassium phosphate buffer (pH 4). Bradford reagent was added, and absorbance was measured using an ELISA plate at a wavelength of 595 nm [44].

2.10. AMF Colonization Determination/Measurement

Roots were harvested for AMF root colonization assessment by the gridline intersect method [45]. Briefly, roots were washed in 10% KOH solution, and trypan blue staining was used to observe arbuscular mycorrhizal colonization in root tissues by following the method developed by Phillips and Hayman [46].

2.11. Statistical Analysis

Data were statistically analyzed by using a two-way analysis of variance (ANOVA) [47]. The difference of treatment means was examined by the least significance difference (LSD) test using a 5% level of significance using Statistical Package Statistix 8.1 (Analytical Software, 2105 Miller Landing Rd Tallahassee, FL, USA) and Origin 2021. Pearson correlation and a principal component analysis were conducted using Origin 2021 (OriginPro, Version 2021. OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Root Colonization

Inoculation of AMF significantly ($p \leq 0.05$) increased the colonization percentage irrespective of soil Zn level up to 60 mg Zn kg⁻¹ application; however, higher application resulted in decreased colonization percentage. Non-inoculated (NM) maize roots showed a maximum of 45% root AMF colonization, as shown in Figure 1. Conversely, AMF-inoculated (AM) maize plant roots showed higher AMF colonization (Figure 1). Colonization percentage was significantly ($p \leq 0.05$) high, with an average of 45% in AMF-inoculated plants irrespective of AMF inoculation, and Zn application reduced the AMF colonization rate after 20 mg Zn kg⁻¹ application. A more drastic effect of Zn application was observed in Z0 + NM plants.

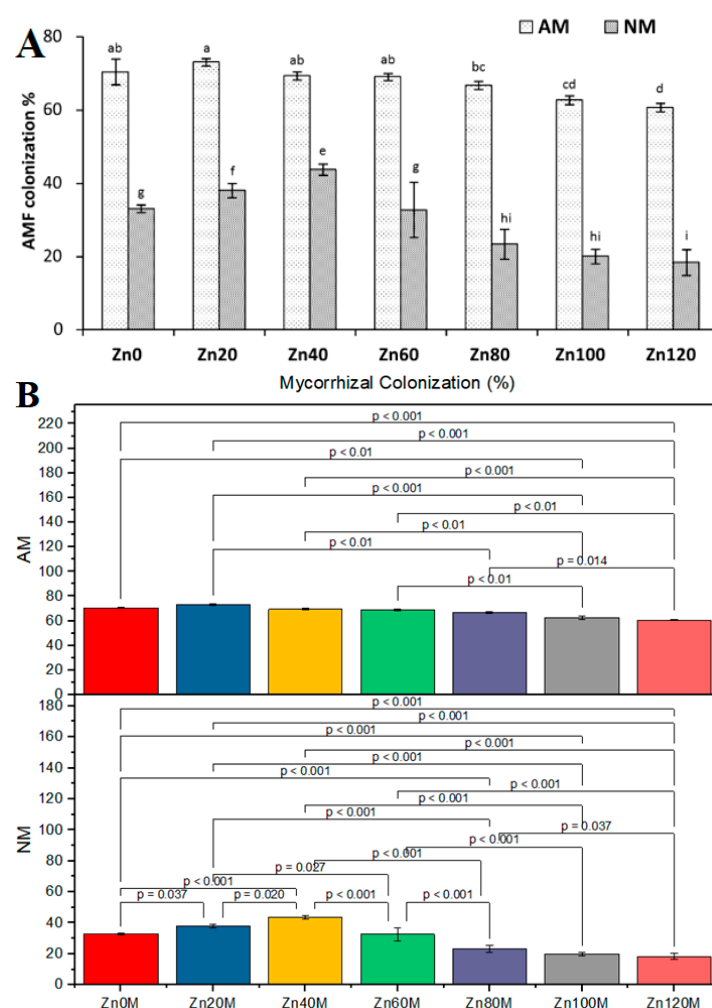


Figure 1. Effect of different application rates of Zn on arbuscular mycorrhizal fungi (AMF) colonization with and without AMF. Means are average of 3 replicates. Different values on graph (A) bars show a significant difference at $\alpha \leq 0.05$. Values on graph (B) bars are p -values computed using Fisher's least significance difference (LSD) test; $\alpha \leq 0.05$.

3.2. Plant Height

Application of different levels of Zn with (AM) and without AMF (NM) significantly affected the maize plant height. The treatments Zn20 + AM and Zn40 + AM differed significantly compared to Z0 + AM and Z0 + NM in terms of plant height (Table 1; Figure 2). No significant change was observed among Zn20 + NM and Z0 + AM, but Zn20 + NM was significantly different to Z0 + NM. However, Zn40 + NM remained significantly better than Z0 + AM. The treatments Zn80 + AM, Zn100 + AM, and Zn120 + AM significantly affected plant height compared to Z0 + AM. The same trend was also noted among Zn80 + NM, Zn100 + NM, and Zn120 + NM compared to Z0 + NM. The maximum increase of 15% and 25% in plant height was observed in Zn20 + AM compared to Z0 + AM and Z0 + NM, respectively.

Table 1. Effect of different application rates of Zn with (AM) and without AMF (NM) on maize growth attributes. Values are means of three replicates. Different letters show significant difference at $p \leq 0.05$; LSD test.

Treatment	Plant Height (cm)		Stem Girth (cm)		No. of Leaves	
	AM	NM	AM	NM	AM	NM
Control (Zn0)	160c	147d	2.7ab	2.6bc	9.3c	8.3de
Zn20	184a	158c	2.8a	2.7cd	12.6a	9.3c
Zn40	182ab	176b	2.6b	2.5b	12.0a	11.0b
Zn60	149d	136e	2.5d	2.4e	9.1cd	8.5cde
Zn80	138ef	118gh	2.4e	2.3f	9.0cd	8.0ef
Zn100	132ef	113h	2.3f	2.1g	8.3de	7.3fg
Zn120	124g	111fh	2.2g	1.9h	7.3fg	7.0f
AM		***		***		***
Zinc		***		***		***
AM*Zn		*		***		**

* ($p \leq 0.005$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

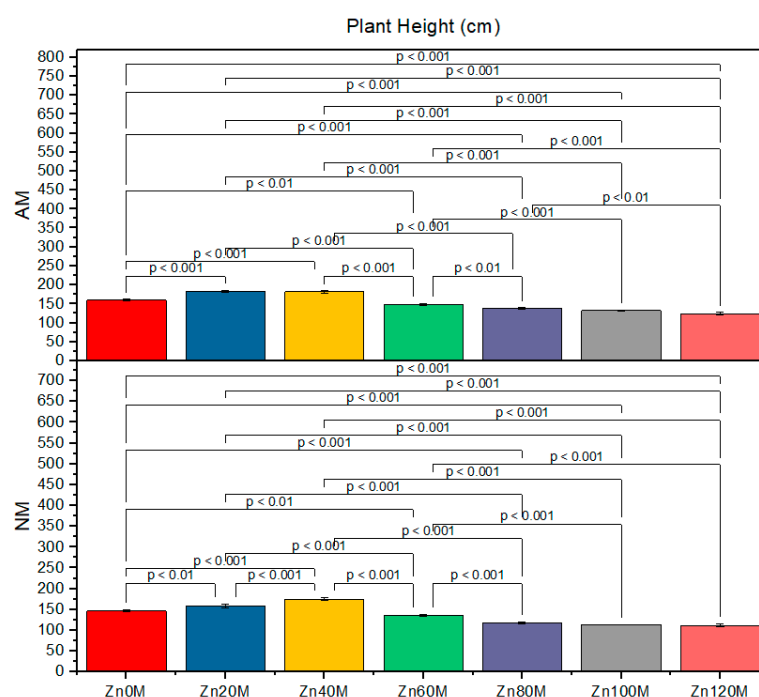


Figure 2. Effect of different application rates of Zn on maize plant height with and without AMF. Means are average of 3 replicates. Different values on bars are p -values computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.3. Stem Girth

Zinc application with (AM) and without AMF (NM) significantly affected maize stem girth. The treatment Zn20 + AM was significantly different compared to Z0 + NM for increased stem girth (Table 1; Figure 3). No significant change was observed among Zn20 + AM, Zn40 + AM, and Z0 + AM for stem girth. The treatments Zn80 + AM, Zn100 + AM, and Zn120 + AM significantly affected stem girth compared to Z0 + AM. The same trend was also noted among Zn80 + NM, Zn100 + NM, and Zn120 + NM compared to Z0 + NM. The maximum increase of 7.69% in stem girth was observed in Zn20 + AM over Z0 + NM.

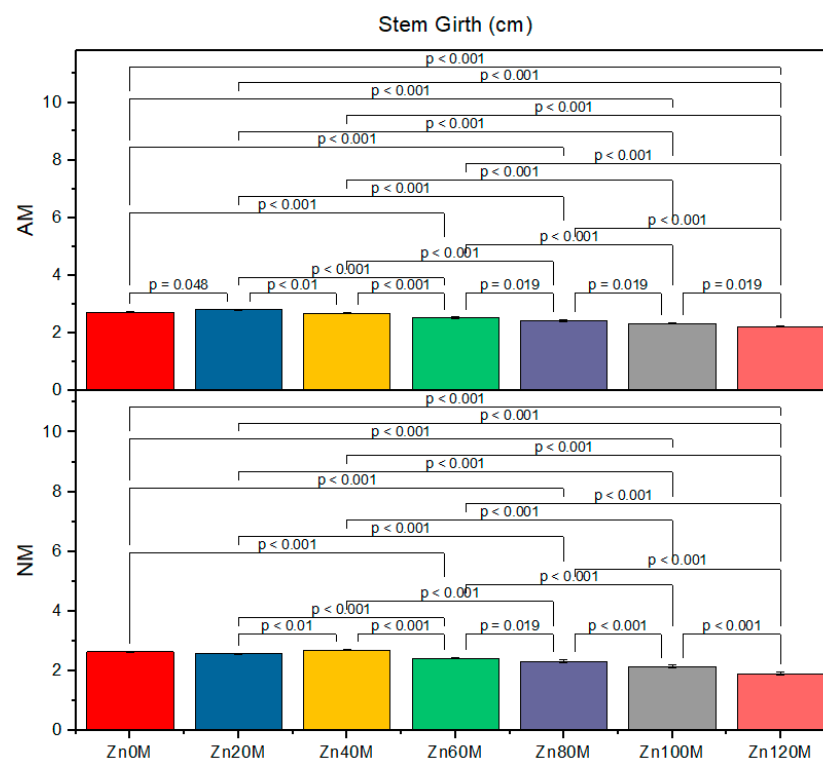


Figure 3. Effect of different application rates of Zn on maize stem girth with and without AMF. Means are average of 3 replicates. Different values on bars are *p*-values computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.4. Number of Leaves

The interaction of Zn application and AMF inoculation significantly affected the number of maize leaves. Application of Zn20 + AM and Zn40 + AM increased the leaf number compared to Z0 + AM and Z0 + NM (Table 1; Figure 4). A significant change in the number of leaves was also observed among Zn20 + AM and Zn40 + AM compared to Z0 + AM. Treatments Zn100 + AM and Zn120 + AM significantly affected the number of leaves compared to Z0 + AM. The same trend was also observed among Zn100 + NM and Zn120 + NM compared to Z0 + NM. The maximum increase of 35.4% and 51.8% in the number of leaves was recorded in Zn20 + AM over Z0 + AM and Z0 + NM, respectively.

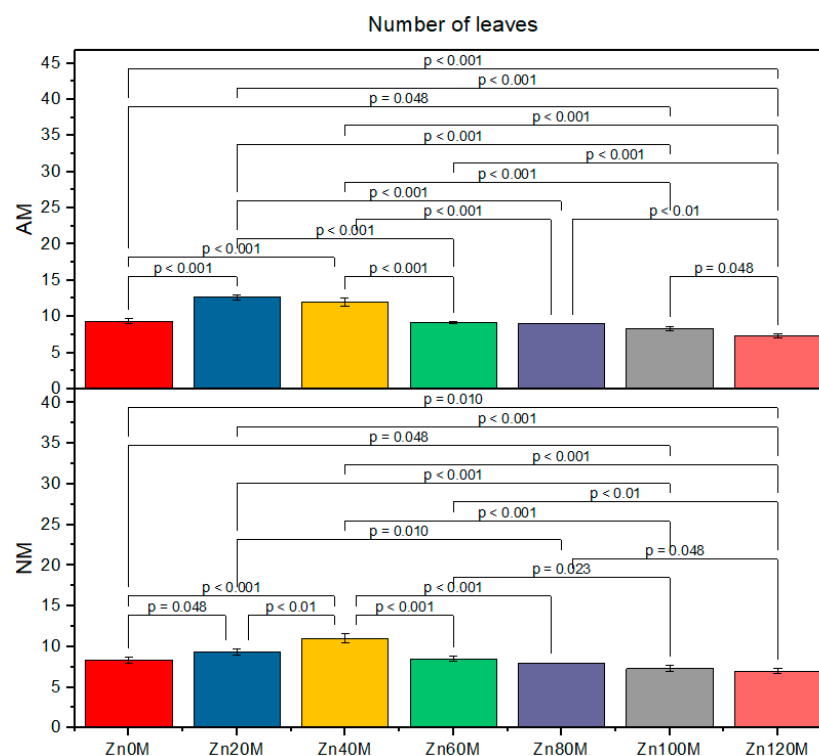


Figure 4. Effect of different application rates of Zn on maize number of leaves with and without AMF. Means are average of 3 replicates. Different values on bars are p -values were computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.5. Cob Weight

The effect of the interaction of Zn dose with AMF inoculation significantly affected cob weight of maize. Cob weight differed between Zn20 + AM and Z0 + NM (Table 2; Figure 5). Cob weight was significantly higher with Zn20 + NM and Zn40 + NM compared with Z0 + NM. A significant change in cob weight was also observed among Zn20 + AM, Zn40 + AM, and Zn60 + AM treatments. Zn80 + AM, Zn100 + AM, and Zn120 + AM significantly affected cob weight compared to Z0 + AM. The same trend was also observed among Zn80 + NM, Zn100 + NM, and Zn120 + NM compared to Z0 + NM. The maximum increase of 4.39% and 12.8% in cob weight was recorded in Zn20 + AM over Z0 + AM and Z0 + NM, respectively.

Table 2. Effect of different application rates of Zn with and without AMF on growth attributes of maize. Values are means of three replicates. Different letters show significant difference at $p \leq 0.05$; LSD.

Treatment	Cob Weight (g)		Cob Length (cm)		1000-Grain Weight (g)		Biological Yield (t/ha)	
	AM	NM	AM	NM	AM	NM	AM	NM
Control (Zn0)	227.6bc	210.6d	16.5a	15.2ef	152d	138g	4.17c	3.79d
Zn20	232.6ab	222.6c	20.8a	16.4d	168a	143f	5.93a	4.30c
Zn40	237.6a	227.6bc	17.7c	19.5b	163b	158c	5.21ab	5.06b
Zn60	224.6c	214.6d	15.5e	14.5f	158c	158c	5.00c	4.88e
Zn80	211.6d	201.6e	15.5e	14.5f	138g	146e	4.76ef	4.03gh
Zn100	203.3e	176g	15.5e	14.5f	132h	122i	3.78ef	16.13h
Zn120	190f	139h	14.5f	13.4g	128hi	117j	3.72fg	3.82h
AM	***		***		***		***	
Zinc	***		***		***		***	
AM*Zn	***		***		***		*	

* ($p \leq 0.005$), *** ($p \leq 0.001$).

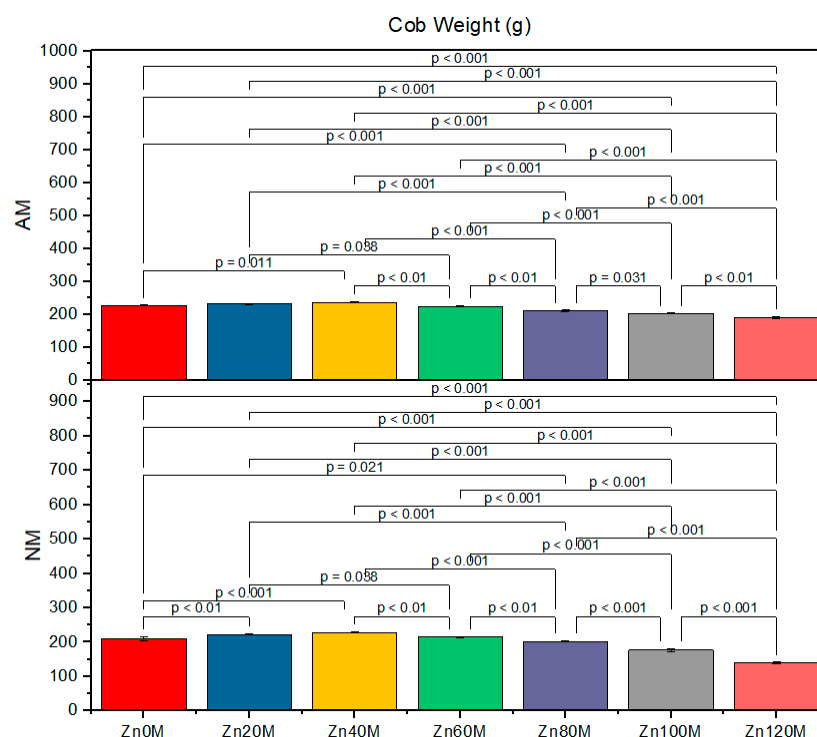


Figure 5. Effect of different application rates of Zn on maize cob weight with and without AMF. Means are average of 3 replicates. Different values on bars are p -values computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.6. Cob Length

The interaction of Zn application with AMF inoculation significantly affected cob length of maize. The treatment Zn20 + AM significantly increased cob length over Z0 + NM (Table 2; Figure 6). The addition of Zn20 + NM and Zn40 + NM differed significantly for cob length from Zn0 + NM. A significant decrease was also noted among Zn40 + AM, Zn60 + AM, Zn80 + AM, and Zn100 + AM over Z0 + AM for cob length. Treatments Zn60 + NM, Zn80 + NM, Zn100 + NM, and Zn120 + NM also showed a significant decline in cob length compared to Z0 + NM. The maximum increase of 36.8% in cob length was observed in Zn20 + AM over Z0 + NM.

3.7. 1000-Grain Weight

Zn application and AMF inoculation significantly affected the 1000-grain weight of maize. The addition of Zn20 + NM, Zn40 + NM, Zn60 + NM, and Zn80 + NM significantly enhanced the 1000-grain weight of maize compared with Zn0 + NM (Table 2; Figure 7). A significant decrease was observed among Zn80 + AM, Zn100 + AM, and Zn120 + AM over Zn0 + AM for 1000-grain weight. The treatments Zn100 + NM and Zn120 + NM also showed a significant decline in 1000-grain weight over Z0 + NM. The maximum increase of 10.5% and 21.7% in 1000-grain weight was observed in Zn20 + AM over Z0 + AM and Z0 + NM, respectively.

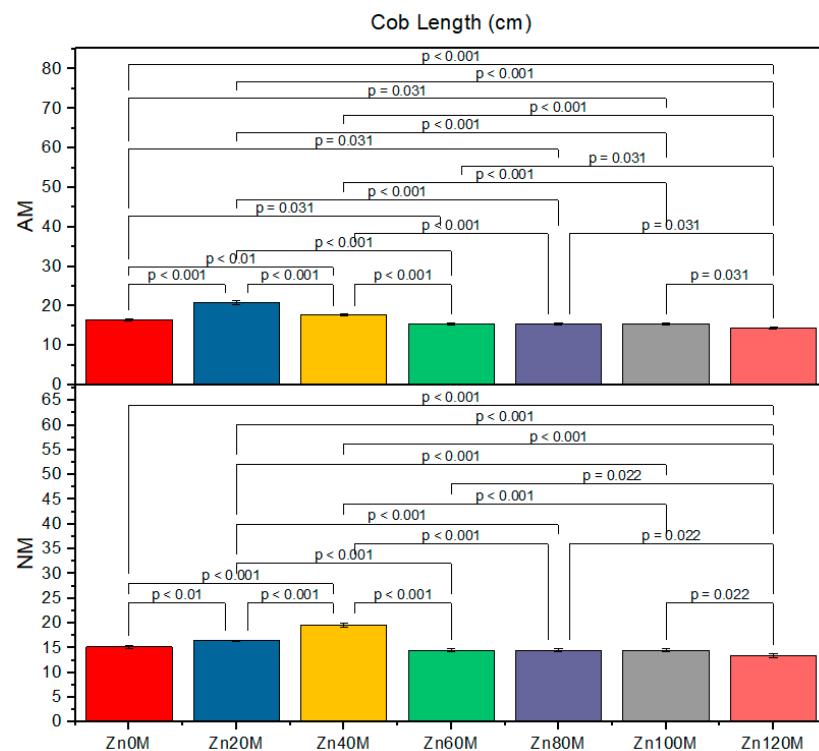


Figure 6. Effect of different application rates of Zn on maize cob length with and without AMF. Means are average of 3 replicates. Different values on bars are p -values computed using Fisher's LSD test; $\alpha \leq 0.05$.

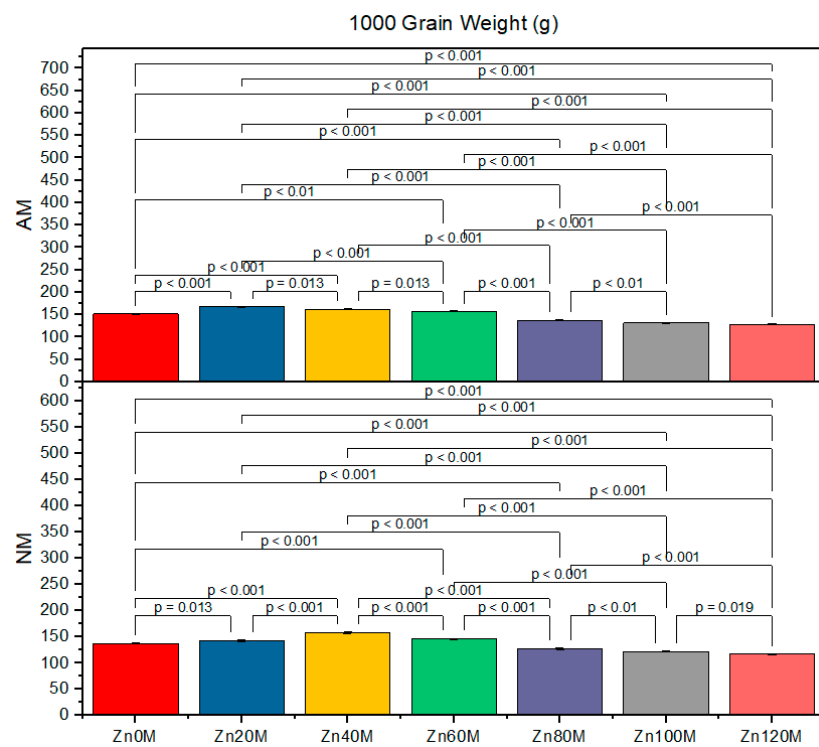


Figure 7. Effect of different application rates of Zn on maize 1000-grain weight with and without AMF. Means are average of 3 replicates. Different values on bars are p -values computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.8. Grain Yield

Zinc application and AMF inoculation significantly affected maize grain yield. The treatments Zn20 + NM and Zn40 + NM significantly enhanced the grain yield compared with Z0 + NM (Table 2; Figure 8). A significant decline in grain yield was observed when Zn80 + AM, Zn100 + AM, and Zn120 + AM were applied compared to Z0 + AM. The treatments Zn60 + NM, Zn80 + NM, Zn100 + NM, and Zn120 + NM also led to a significant decline in biological yield compared to Z0 + NM. The maximum increase of 42.2% and 56.5% in maize biological yield was observed in Zn20 + AM over Z0 + AM and Z0 + NM, respectively.

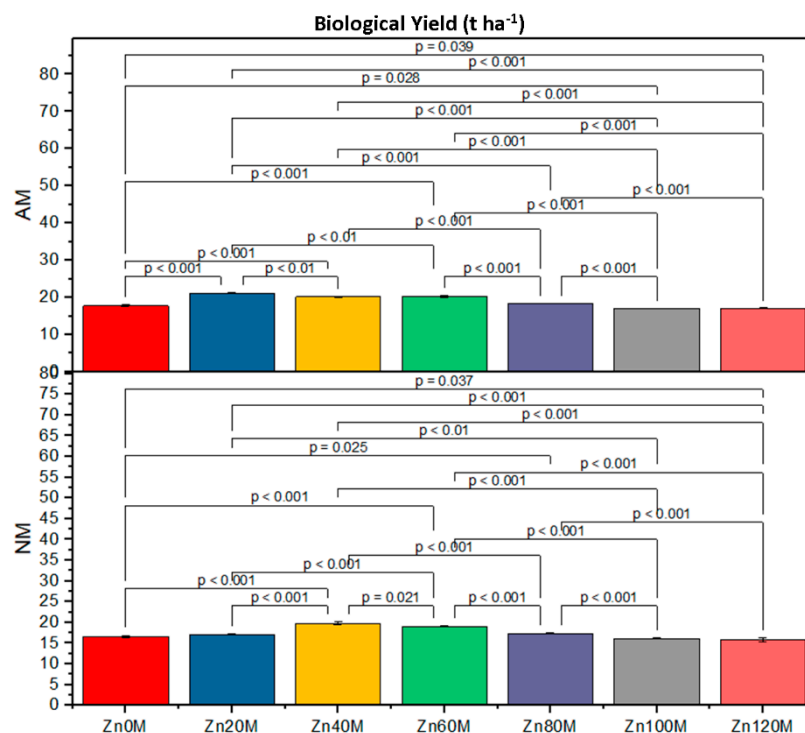


Figure 8. Effect of different application rates of Zn on maize biological yield with and without AMF. Means are average of 3 replicates. Different values on bars are p -values computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.9. Photosynthetic Rate

Zinc application and AMF inoculation significantly affected the photosynthetic rate at the 45th day after germination. All the application rates of Zn significantly increased the photosynthetic rate in maize compared to Z0 + AM (Figure 9). The treatments Zn20 + NM, Zn40 + NM, Zn60 + NM, and Zn80 + NM also significantly enhanced the photosynthetic rate of maize compared to Z0 + NM. The treatment Zn120+NM caused a significant decline in photosynthetic rate compared to Z0 + NM.

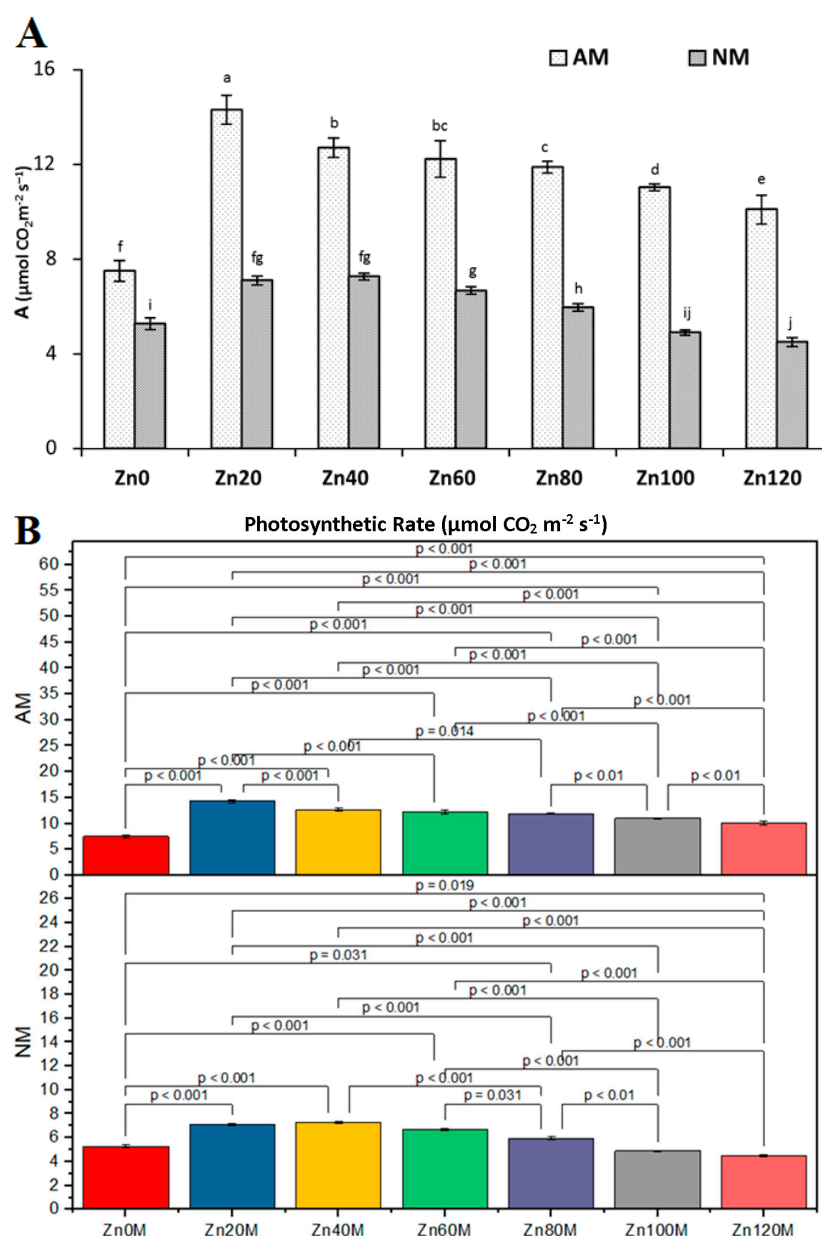


Figure 9. Effect of different application rates of Zn on the photosynthetic rate of maize with and without AMF. Means are average of 3 replicates. Different values on graph (A) bars show a significant difference at $\alpha \leq 0.05$. Values on graph (B) bars are p -values computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.10. Transpiration Rate

Application of variable Zn rates with (AM) and without AMF (NM) led to significantly different results for maize transpiration rate at the 45th day after germination. All the application rates of Zn20 + AM, Zn40 + AM, Zn60 + AM, Zn80 + AM, Zn100 + AM, and Zn120 + AM significantly increased transpiration rate in maize over the Z0 + AM treatment (Figure 10). The treatments Zn20 + NM, Zn40 + NM, Zn60 + NM, and Zn80 + NM also enhanced the transpiration rate significantly in maize over Z0 + NM. The treatment Zn120 + NM caused a significant decrease in transpiration rate compared to Z0 + NM.

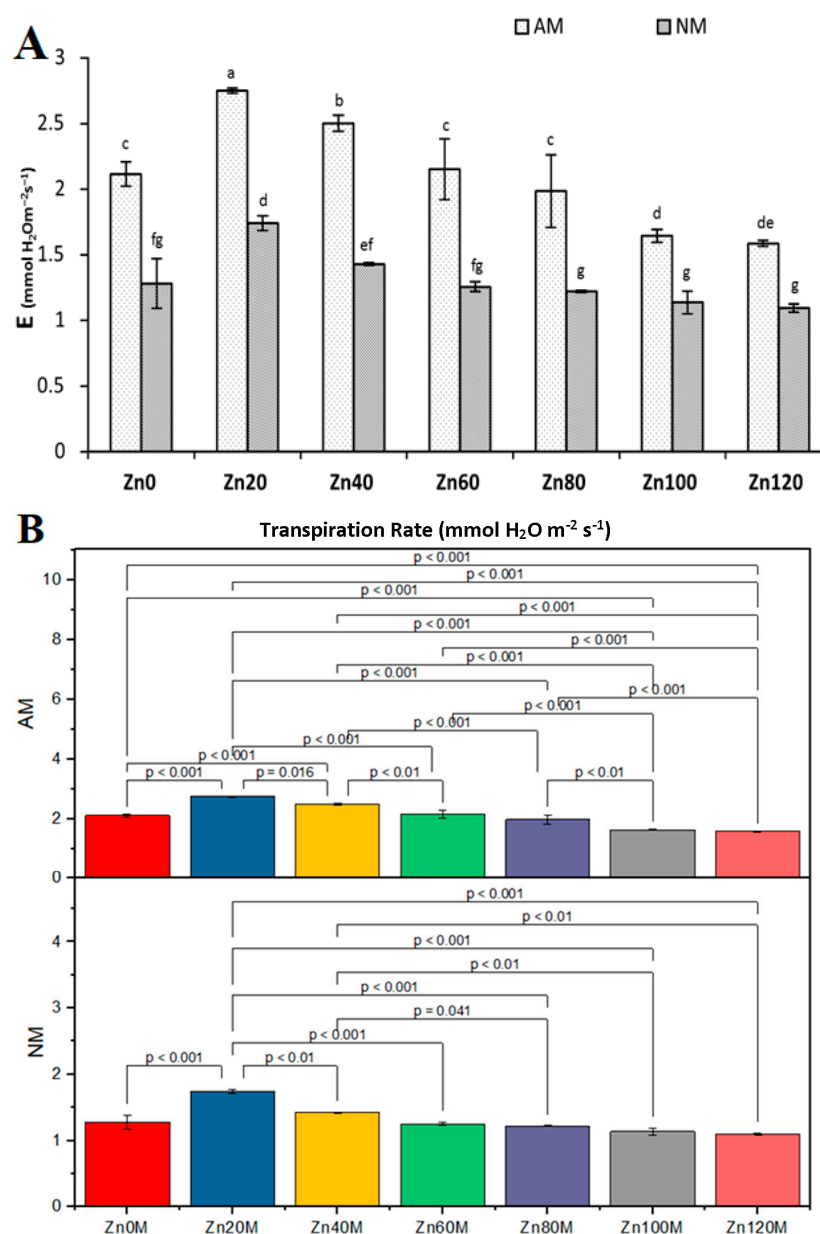


Figure 10. Effect of different application rates of Zn on the transpiration rate of maize with and without AMF. Means are average of 3 replicates. Different values on graph (A) bars show a significant difference at $\alpha \leq 0.05$. Values on graph (B) bars are p -values computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.11. Stomatal Conductance

Application of variable Zn rates with (AM) and without AMF (NM) led to significantly different results for maize's stomatal conductance at the 45th day after germination. The addition of Zn20 + AM significantly enhanced stomatal conductance in maize compared to Z0 + AM (Figure 11). It was noted that stomatal conductance was affected in the Zn60 + AM, Zn80 + AM, Zn100 + AM, and Zn120 + AM treatments compared to Z0 + AM. The treatment Zn20 + NM also enhanced stomatal conductance significantly in maize compared to NM. Application of Zn80 + NM, Zn100 + NM, and Zn120 + NM caused a significant decrease in stomatal conductance compared to Z0 + NM.

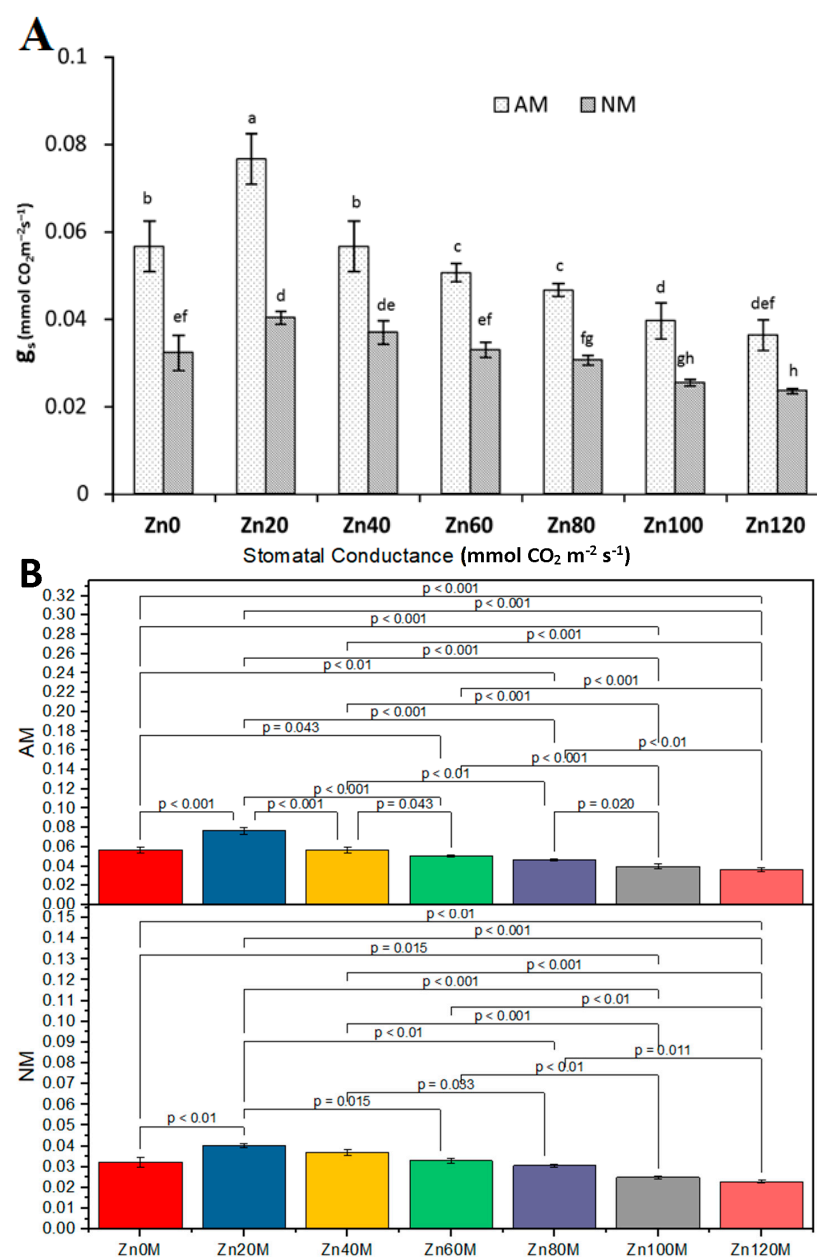


Figure 11. Effect of different application rates of Zn on stomatal conductance of maize with and without AMF. Means are average of 3 replicates. Different values on graph (A) bars show a significant difference at $\alpha \leq 0.05$. Values on graph (B) bars are p -values computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.12. Total Soluble Protein Concentration in Leaves

Zinc application and AMF inoculation significantly affected the total soluble protein concentration in maize leaves. The addition of Zn20 + AM and Zn40 + AM significantly improved the total soluble protein content in maize compared to Zn0 + AM (Figure 12). It was observed that the total soluble protein content was affected in the Zn100 + AM and Zn120 + AM treatments compared to Z0 + AM. The treatments Zn20 + NM and Zn40 + NM also enhanced total soluble protein significantly in maize more than Z0 + NM did. The addition of Zn80 + NM, Zn100 + NM, and Zn120 + NM caused a significant decline in maize total soluble protein compared to Z0 + NM.

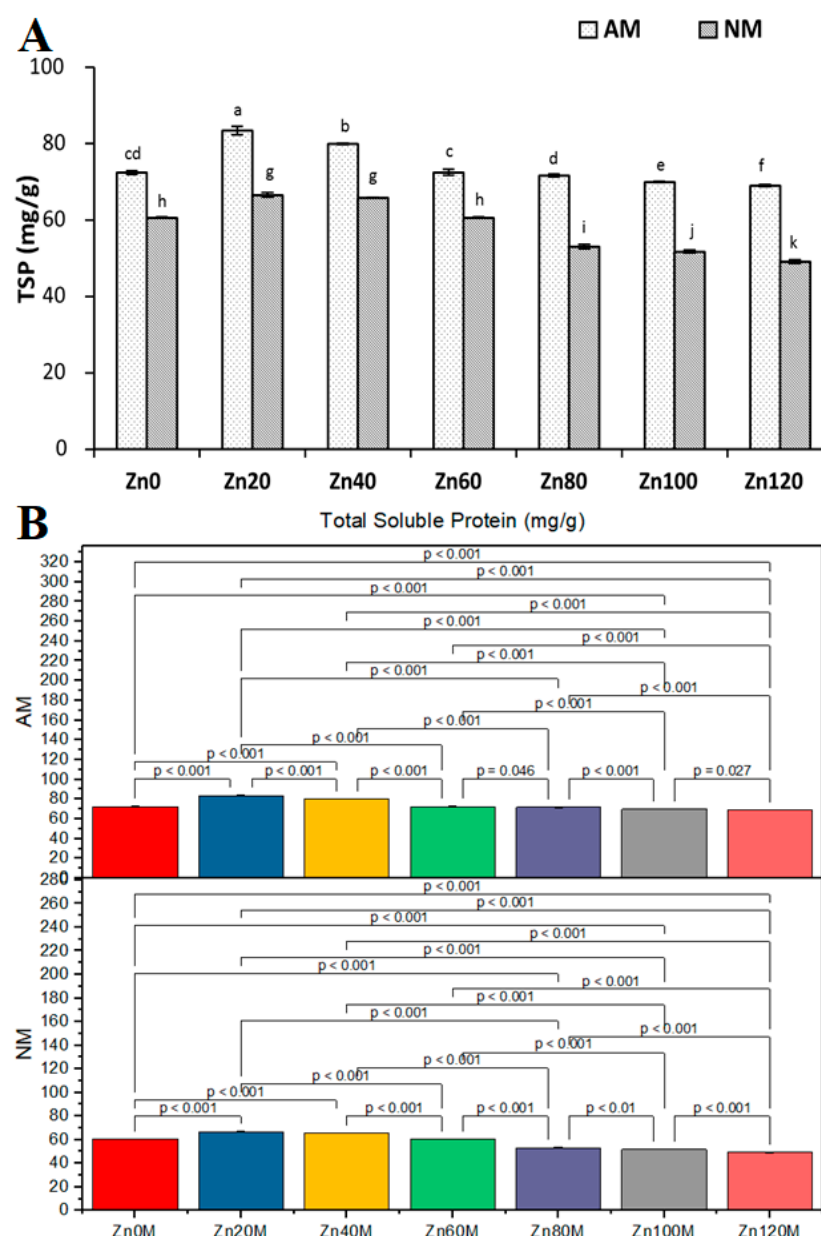


Figure 12. Effect of different application rates of Zn on the total soluble protein (TSP) contents of maize with and without AMF. Means are average of 3 replicates. Different values on graph (A) bars show a significant difference at $\alpha \leq 0.05$. Values on graph (B) bars are p -values computed using Fisher's LSD test; $\alpha \leq 0.05$.

3.13. Pearson Correlation and Principal Component Analysis

Pearson correlation showed that maize growth attributes were positively and significantly correlated with gas exchange attributes and yield components were affected by Zn application rate irrespective of AMF application (Figure 13). Principal component analyses showed that stomatal conductance, transpiration rate, total soluble protein, and biological yield were more closely associated with Zn20 (Figure 14B). Photosynthetic rate and AMF colonization were more closely associated with Zn60. All growth, yield, and gas exchange attributes were more closely associated with AMF (Figure 14B).

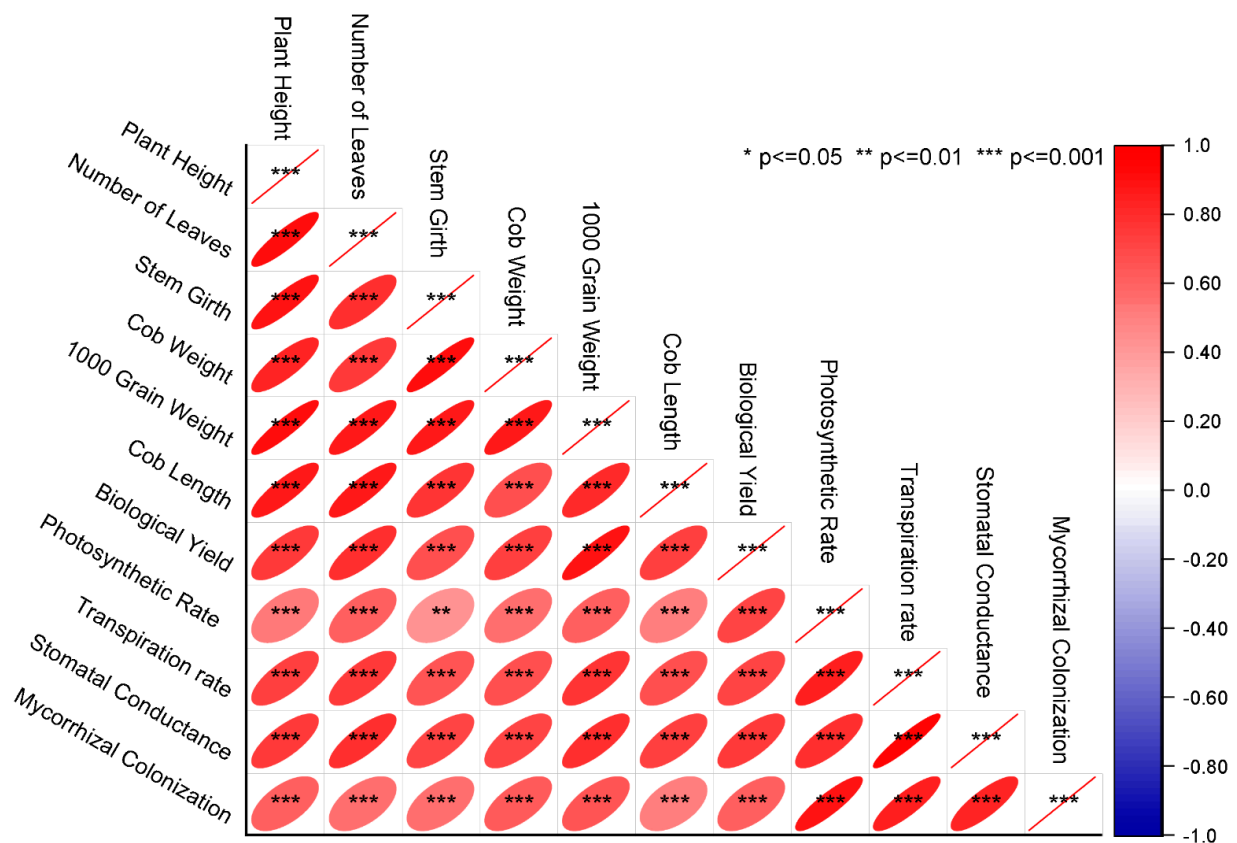


Figure 13. Pearson correlation for maize growth, yield, and gas exchange attributes affected by different application rates of Zn with and without AMF.

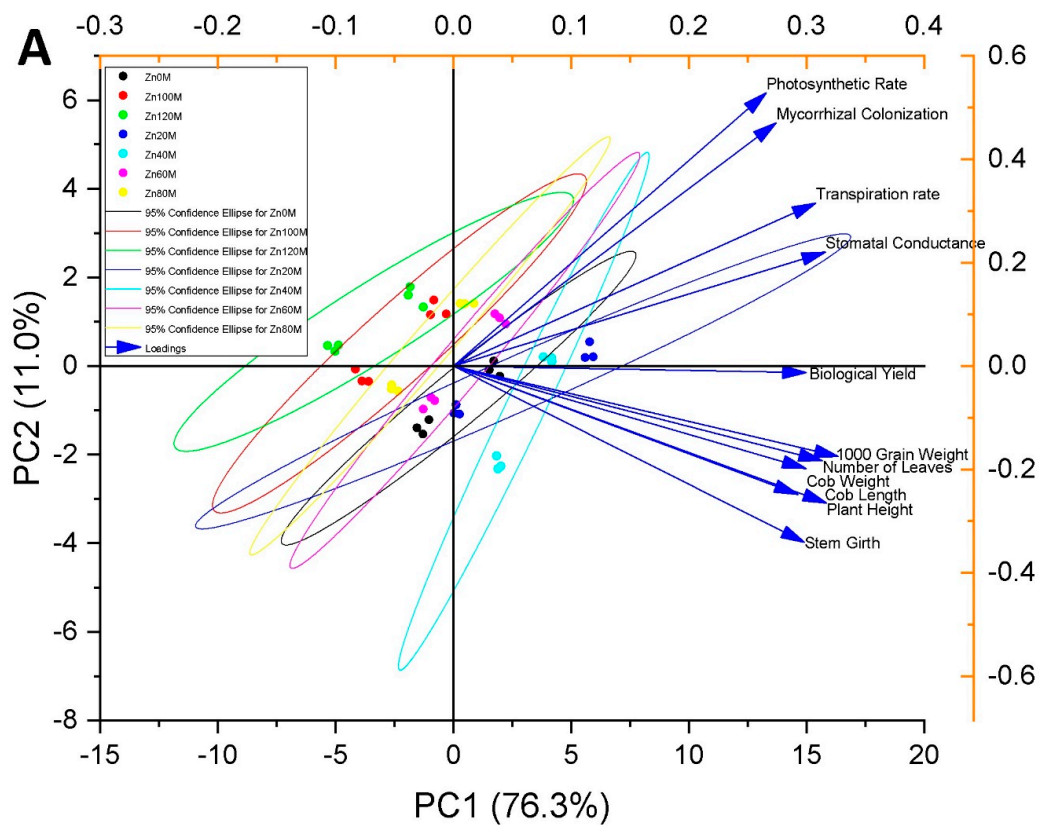


Figure 14. Cont.

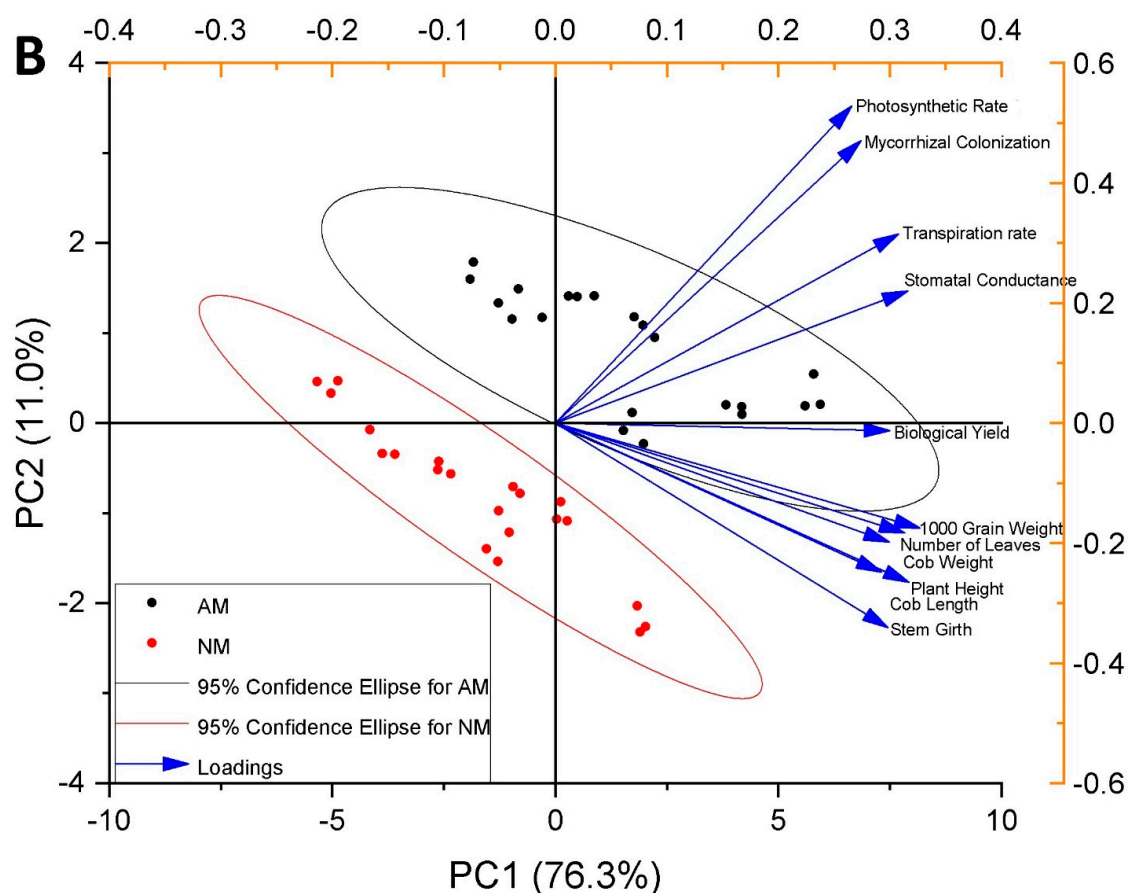


Figure 14. Principal component analysis for maize growth, yield, and gas exchange attributes affected by different application rates of Zn with and without AMF. (A) Different levels of Zn as a grouping component. (B) No AMF (NM) and AMF (AM) as a grouping component.

4. Discussion

Colonization of roots by AMF varies with Zn application and increases with the increase in the rate in Zn-deficient soil [48,49]. Conversely, high Zn (80–120 mg Zn kg⁻¹) supply reduced the AMF root colonization percentage of maize, and these results are in agreement with those of Watts-William et al. [50], Wang et al. [31], and Coccina et al. [14]. Lingua et al. [51] observed that at high soil Zn concentration, AMF colonization was suppressed owing to reduced hyphal growth and prevention of spore production and germination. High Zn concentration in roots mediates two kinds of responses, i.e., fungal and plant responses. Alteration in fungal response includes reduced spore germination, spore life length, and density of spores, growth of hyphae, and appressoria formation [52]. In plant responses, high Zn upregulates the plant defense system, alters the hormonal balance, and, most importantly, it alters the root exudates' composition [53,54]. Irrespective of AMF inoculation, high Zn supply negatively affects the plant growth parameters and yield [31,50]. Inoculated plants showed improved maize growth. AMF have a nutrient threshold level below which they upregulate nutrient transporter genes, and above this threshold, they impart a protective effect on plants and increase plant growth [55]. A detailed study by Paneque et al. [56] showed that AMF inoculation increased plant yield by 20%. A similar result was also documented by Zhang et al. [57], whereby inoculation of *R. irregularis* increased yield by 17%, and in another study of wheat, a 24% increase in grain yield was observed with high Zn application [14]. Another study also showed high yield in *R. irregularis*-inoculated plants at 100 and 500 mg Zn kg⁻¹ application [58]. AMF colonization rate was positively correlated with plant height, stem width, and cob weight and length. According to Monnet et al. [59], Zn deficiency adversely affects plant

growth by minimizing photosynthesis and damaging chloroplast thylakoids and light-harvesting complex (LHC) proteins, leading to disorganization and disintegration of the chloroplast membrane. In our study, reduced plant height, stem girth, number of leaves, and other growth attributes were due to poor Zn uptake. Coccina et al. [14] described that improved growth due to Zn application is associated with increased Zn uptake by the plant. Application of Zn in deficient soil increased photosynthesis, transpiration, and stomatal conductance rate [60]. It might be due to an increase in total soluble protein (TSP) contents, which was also observed in the current study.

Our results are also supported by those of Liu et al. [61]. They documented an increased photosynthetic efficiency by increasing chlorophyll a and b concentrations due to Zn application in Zn-deficient soil [61]. Zinzala et al. [60] showed that many physiological parameters were negatively affected above 80 mg Zn kg⁻¹ soil application due to Zn toxicity [60]. We observed a reduction in the net photosynthetic rate (A) and transpiration rate (E) due to reduced stomatal conductance. However, no visual symptoms of Zn toxicity were observed in our study [62]. Sagardoy et al. [63] argued that Zn toxicity reduced the stomatal conductance (gs) severely (76%), which limits other related physiological and chemical processes [63]. They also found that excess Zn led to alteration of the physical structure of mesophyll cells and stomata. Reduced photosynthesis was also due to the restricted activity of carbonic anhydrase enzyme [63]. According to Tobin [64], Zn is a crucial part of carbonic anhydrase (CA) in chloroplasts and cytoplasm [65]. CA activity becomes restricted under limited Zn uptake [65,66]. CA activity also controls CO₂ diffusion in cell lipid phase to chloroplasts during photosynthesis [67,68]. According to Sharma et al. [69], CA activity restriction under Zn deficiency decreased stomatal conductance and transpiration rate.

Vassilev et al. [62] studied excess Zn in plant tissues, which reduced Mg²⁺ uptake, replaced Mg atoms, and disrupted chlorophyll structure and decreased photosynthetic rate [62]. Inoculation with AMF improved the total soluble protein content in maize [70,71]. The results of our study are in accordance with those of Nguyen et al. [71]. In soil with low Zn, AMF increases carbonic anhydrase enzyme activity and enhances photosynthesis. In contrast, in soil with high Zn, AMF imparted a protective effect on plants by mitigating the Zn toxicity effect [71]. In this study, the results showed that AMF-inoculated plants improved the plant physiological response with high soil Zn compared to non-inoculated plants, and the results are in line with those of Wang et al. [31].

5. Conclusions

The application of Zn20 + AM proved to be the most effective one for improving maize growth attributes cultivated in Zn-deficient conditions. A significant increase in the yield attributes of maize through the addition of Zn20 + AM validated the efficacious functioning of the combined use of Zn and AM in terms of photosynthetic rate, gas exchange attributes, and stomatal conductance. Higher application rates of Zn, i.e., 80, 100, and 120, induced adverse effects on maize growth, yield, and gas exchange attributes with AM and NM. It is recommended to apply Zn20 + AM to achieve better growth and yield of maize in Zn-deficient soils.

Author Contributions: Conceptualization, A.S. and M.A.A.; methodology, M.A.A.; software, S.D.; validation, S.D., M.A.A., and N.A.; formal analysis, A.S.; investigation, A.S.; resources, M.A.A.; data curation, A.S.; writing—original draft preparation, M.S., M.M.H., M.A.A., S.D., R.D., S.F., and F.H.; writing—review and editing, M.B., A.E.S., M.A.A., S.D., R.D., and S.F.; supervision, M.A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Bahauddin Zakariya University, Multan, Pakistan, and Higher Education Commission (HEC), Islamabad, Pakistan. The authors are also very highly grateful to Taif University Researchers Supporting Project number (TURSP-2020/143), Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to Taif University Researchers Supporting Project number (TURSP-2020/143), Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

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