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Regulation of Phosphorus and Zinc Uptake in Relation to Arbuscular Mycorrhizal Fungi for Better Maize Growth

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Abstract: Zinc (Zn) is an important micronutrient for plants, whose deficiency in alkaline soils creates hurdles in the achievement of optimum crop growth. Moreover, overuse of phosphorus (P) fertilizers often causes Zn immobilization in the soil. The employment of arbuscular mycorrhizal fungi (AMF) could be potentially environmentally friendly technology in this regard. Therefore, a pot experiment was conducted to assess the beneficial role of AMF (*Glomus* species) on maize under low and high P and Zn levels. Seven levels of Zn (0, 20, 40, 60, 80, 100 and 120 mg Zn kg^{-1} soil $ZnSO_4 \cdot 7H_2O$) and three levels of P (0, 14.5, 29 and 58 kg ac^{-1} as single superphosphate) were applied with (M+) and without AMF (M−). The results showed that a high application rate of Zn (100 and 120 mg Zn kg^{-1} soil) restricted P translocation in plants and vice versa. Moreover, the nutritional status of mycorrhizal plants (AM) was better than non-mycorrhizal (NM) plants. AM plants showed a maximum positive response at 20 mg Zn kg^{-1} soil, or 29 kg P ac^{-1}. In response to 20 mg Zn kg^{-1} soil, root colonization was maximum, which enhanced the maize nutrient concentration in shoots. In conclusion, AMF inoculation (M+) with P (29 kg ac^{-1}) and Zn (20 mg kg^{-1}) is efficacious for improving maize's growth and nutrition. More investigations are suggested at the field level under different agroclimatic zones to ascertain whether P (29 kg ac^{-1}) or Zn (20 mg kg^{-1}) with AMF is the best treatment for maize growth optimization.

Keywords: phosphorus; zinc; mycorrhizae; optimization application rates; maize

1. Introduction

Mineral nutrients are pillars of agriculture that contribute significantly to the establishment of soil fertility [1]. Plants need optimum macro and micronutrients for sustainable productivity [2–4]. Zinc (Zn) is one of the most important micronutrients. It is required in a minute quantity [2,5–10] but positively influences yield, fruit set, and fruit quality [11,12]. It is also involved in carbonic anhydrase activity, carbohydrate metabolism and maintenance of membrane integrity, regulation of auxin and protein production, and synthesis of pollen grains [12–15].

According to one survey, calcareous soils are mostly deficient in Zn [16]. Moreover, most Pakistani soils are Zn deficient due to their high soil pH, high $CaCO_3$ content, and lower organic matter (OM) contents [17]. Zinc concentrations vary from soil to soil depending upon several factors including parental material, climate, crops, fertilizer input, and soil quality. Soil pH alters the availability of Zn to a great extent; raising the pH from 5 to 8 reduced the available Zn concentration in soil solution. Alkaline soil pH lowers the availability of Zn and forms carbonates ($ZnCO_3$) and hydroxides ($Zn(OH)_2$) of Zn, which are generally precipitated in soil [18]. Soil Zn availability is also influenced by nutrient-nutrient interactions i.e., with phosphorus (P) [15].

Phosphorus is required in all parts of plants during all vegetative and reproductive phases. It is an essential component of phospholipids, deoxyribonucleic acid, ribonucleic acid, and adenosine triphosphate/diphosphate/monophosphate. It also regulates cell signaling processes and phosphorylation [19]. Uptake of P is highly affected by root growth due to its immobility in the soil medium [20–24]. Its deficiency is most common during the early growth of plants and under cold soil conditions [19,25].

Zinc and P can interact to form insoluble complexes like $Zn_3(PO_4)_2$, which decreases their availability for plants. The application of high levels of P yields Zn deficiency symptoms in plants [9]. It further reduces the translocation of Zn towards the upper parts of plants [26]. An increase in P application accelerated plant growth, and thus, Zn accumulation was reduced in plant tissues. It is unclear whether P application directly reduced Zn uptake or if high P levels reduced AMF root colonization [27].

On the other hand, biofertilizers are effective to overcome this problem [28–32]. Arbuscular mycorrhizal fungi form a symbiosis with 90% plant roots [33]. This symbiosis can enhance the mobilization of immobile nutrients in soil [34], leading to better uptake of nutrients such as P, Cu, Fe, Zn in plants [27,35,36]. Most AMF inoculated plants use two major pathways for the uptake of nutrients:

1. Direct uptake via the root epidermis, or
2. Use of fungal structures (arbuscules) made by AMF [37].

An increase in the surface area of roots by AMF symbiosis also facilitates optimum uptake of nutrients from the soil [38]. In addition to Zn deficiency, soils near mines and industrial sites often have high concentrations of Zn (even to toxic levels). Under such conditions, AMF can impart a protective effect on the plant [36,39] by increasing Zn accumulation in deficiency or reduced Zn uptake in toxicity [36].

Despite the antagonistic interaction of Zn and P, AMF can regulate the uptake and accumulation of both nutrients. This study addresses the knowledge gap regarding optimum Zn and P application rates for maize with and without AMF. The current study was undertaken to explore the best application rate combination of Zn and P for maize with and without AMF. It is hypothesized that low application rates of P and Zn might be better without disturbing the growth of maize inoculated with AMF.

2. Materials and Methods

2.1. Experimental Site

The current study was conducted in the research area of the Department of Soil Science, Bahauddin Zakariya University Multan, Pakistan.

2.2. Soil Collection and Analysis

Zn deficient (0.52 mg Zn kg^{-1}) soil was collected from Chak 5 Faiz Multan region latitude 29.9 °N and longitude 71.5 °E. After collection, the soil was ground and then air-dried. Later, 2 mm mesh size soil was combined with sand at 8:2 ratio. The mixing of the soil with sand facilitates the extraction of roots from the soil. When the collected soil was analyzed for its chemical properties, it was found to contain 39% saturation, 54% water holding capacity, 1.40 dSm^{-1} EC [40], 8.82 pH [41], 6.5 mg kg^{-1} P [42], 126 mg kg^{-1} K [43], 0.43 % organic matter [44] and 15 mg kg^{-1} N [45].

2.3. Pot Preparation and Treatments

Non-draining mud pots (10 inches wide, 45 inches depth) were filled with 10 kg of soil inoculated with 5 g of mycorrhizal inoculum. Our mycorrhizal inoculum had predominantly *Glomus* species along with 9 propagules (include the spores, hyphal fragments and root portions) of *Gigaspora albida* (Clonex® Root Maximizer; Bustan, Toronto, Canada). Thus, the inoculum used in this study was predominantly rich in *Glomus* species. In AMF control pots (M), Topsin M (Thiophenate methyl 70% wettable powder (WP)) was applied at 50 mg kg^{-1} soil to inhibit AMF root colonization. Seven levels of Zn were used as follows: 0, 20, 40, 60, 80, 100, and 120 mg Zn kg^{-1} soil (applied as $ZnSO_4 \cdot 7H_2O$) along with control [36,46] and four levels of P (0, 14.5 kg, 29 kg and 58 kg/acre was applied) using single superphosphate (SSP) as a source along with control treatments [8,10,47]. All of the treatments were applied in a completely randomized design (CRD) following three replicates. The details of three factorial amendment applications are provided in detail in Table 1. The moisture content in each pot was maintained at 60% of the total water holding capacity (WHC). The recommended doses for maize production of N, K (92 and 37 kg $acre^{-1}$), and three P levels (14.5, 29, and 58 kg $acre^{-1}$) were applied according to the Punjab government, 2018. Maize cultivar YH 1898 was used as a test crop. Five seeds were sown in each pot and after two weeks of germination; three plants in each pot were maintained. These plants were irrigated till the flowering stage.

Table 1. Treatment chart.

Zn Level	**Control (P0)**		**P1**		**P2**		**P3**	
(mg/kg)	**M+**	**M−**	**M+**	**M−**	**M+**	**M−**	**M+**	**M−**
Zn 0	Z0P0M1	Z0P0M0	Z0P1M1	Z0P1M0	Z0P2M+	Z0P2M−	Z0P3M+	Z0P3M−
Zn 20	Z1P0M1	Z1P0M0	Z1P1M1	Z1P1M0	Z1P2M+	Z1P2M−	Z1P3M+	Z1P3M−
Zn 40	Z2P0M1	Z2P0M0	Z2P1M1	Z2P1M0	Z2P2M+	Z2P2M−	Z2P3M+	Z2P3M−
Zn 60	Z3P0M1	Z3P0M0	Z3P1M1	Z3P1M0	Z3P2M+	Z3P2M−	Z3P3M+	Z3P3M−
Zn 80	Z4P0M1	Z4P0M0	Z4P1M1	Z4P1M0	Z4P2M+	Z4P2M−	Z4P3M+	Z4P3M−
Zn 100	Z5P0M1	Z5P0M0	Z5P1M1	Z5P1M0	Z5P2M+	Z5P2M−	Z5P3M+	Z5P3M−
Zn 120	Z6P0M1	Z6P0M0	Z6P1M1	Z6P1M0	Z6P2M+	Z6P2M−	Z6P3M+	Z6P3M−

P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; M+ = AMF, M− = No AMF.

2.4. Measurement of Physiological Traits

2.4.1. Chlorophyll Contents

Fresh 3rd or 4th leaf samples (0.5 g) were collected and homogenized in 80% of 10 mL acetone. Homogenized solution was left at 4 °C overnight to ensure complete extraction. After extraction, the homogenized solution was centrifuged at 10,000 rpm for 5 min at 4 °C. Finally, the absorption of the supernatant was measured (UV–1800, Shimadzu, Cole-Parmer, IL, USA) at 470 nm, 646 nm, and 663 nm. Calculation of chlorophyll and carotenoid contents was done as described by Arnon [48].

2.4.2. Gaseous Exchange Traits

Stomatal conductance, photosynthetic rate, and transpiration rate were measured at the tasseling stage (VT) of maize by a constant light intensity photosynthesis device (1500 µmol m^{-2} s^{-1}), CO_2 amount (400 µmol) and airflow (500 µmol s^{-1}). Upon attaining steady-state, measurements were recorded using a LCi-SD Ultra-Compact Photosynthesis System® (ADC BioScientific Ltd., Hoddesdon, UK).

2.4.3. Total Soluble Protein

Leaf samples were collected to make a composite sample and stored at −80 °C. Enzyme extraction was done using a potassium phosphate buffer (pH 4). Finally, Bradford reagent was added, and the absorbance was measured, using an ELISA plate reader, at a wavelength of 595 nm [49].

2.5. Morphological Characteristics

Maize plants were harvested at maturity (R6). Morphological traits of the plants i.e., the number of leaves were counted manually, whereas a measuring tape was used to measure plant height (cm) and stem girth (cm). The fresh weight of the plant was measured using an analytical balance.

2.6. Quantification of AMF Colonization

Roots were harvested and washed with a 10% KOH solution. For staining, trypan blue stain was used to observe the colonization of arbuscular mycorrhizal fungi in root tissues of maize [50].

2.7. Measurement of Nutrient Contents in Plant and Soil

After harvesting, shoot and root samples were collected, oven-dried, and ground to form homogeneous samples. Samples, 0.1 g, were digested in 2 mL of H_2SO_4 (98%) and H_2O_2 (30%). Digested samples were filtered with Whatmann filters and then subjected to Zn quantification by atomic absorption spectrophotometer. Phosphorus quantification was performed by the malachite green method [51]. Post soil was sampled for extractable DTPA-Zn and Olsen-P determination using the methods described by Lindsay and Norvell [52] and Kuo [53].

2.8. Statistical Analysis

Data were statistically analyzed by using a two-way analysis of variance (ANOVA). The difference of treatment means was analyzed by LSD test using a 5% level of significance with the program Statistix 8.1 (Analytical Software, Tallahassee, FL, USA). Pearson correlation was used for determining the correlation between parameters [54].

3. Results

3.1. Mycorrhizal Colonization

The mycorrhizal colonization percentage was significantly ($p \leq 0.05$) decreased at 58 kg ac^{-1} P application, and the maximum colonization was observed at 29 kg ac^{-1} P application. Application of P increased the colonization percentage up to 29 kg ac^{-1}; above this level of P application, the colonization rate declined. Irrespective of P application, Zn stress (deficiency or toxicity) negatively affected the root AMF colonization percentage, as shown in Figure 1. In Zn deficient soil, the Zn supply increased AMF colonization, but at high Zn supply, it severely reduced AMF colonization (Figure 1). At 58 kg/ac P, although plant growth increased in AM plants, it decreased plant growth as a reduction in AMF root colonization. Statistical analysis showed that AMF root colonization is directly correlated with plant growth (Table 2). The highest level of P (58 kg/ac) and Zn (120 mg/kg) significantly decrease AMF colonization, while the medium level of P (29 kg/ac) and low level of Zn (20 mg/kg) facilitate AMF colonization.

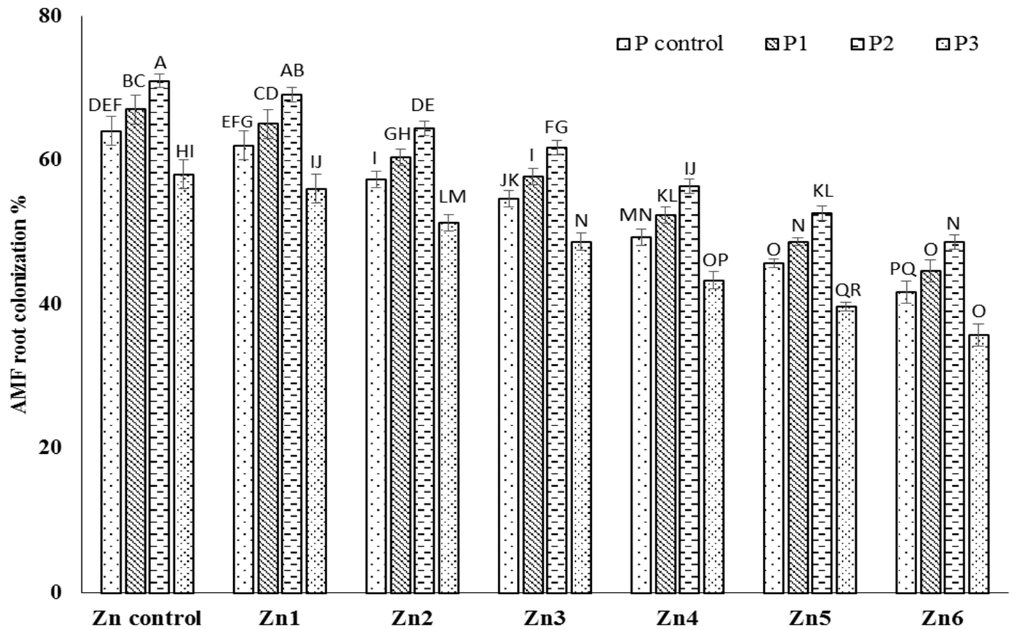

Figure 1. Arbuscular mycorrhizal fungi (AMF) root colonization percentage at harvest of AM maize plants grown under various P (0–full recommended dose) and Zn (0–120 mg Zn/kg soil) application rates. Mean values are presented with ± standard error (SE). Different letters on the bars indicate significant differences at $p \leq 0.05$. P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; Zn control = 0, Zn1 = 20, Zn2 = 40, Zn3 = 60, Zn4 = 80, Zn5 = 100, and Zn6 = 120 mg Zn kg^{-1}.

Table 2. ANOVA summary of various measured parameters.

Factors	AMF	Gs	A	E	Stem Girth	Plant Height	Fresh Weight	Shoot P	Root P	Shoot Zn	Root Zn	Chl a	Chl b	Total Chl	TSP
Mycorrhiza	***	**	***	***	***	***	***	***	***	***	***	***	***	***	***
Phosphorous	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Zinc	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
M × P	**	***	***	***	***	***	***	***	***	***	***	***	***	***	***
M × Zn	***	***	***	**	***	***	***	***	***	***	***	***	***	***	***
P × Zn	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***
M × P × Zn	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***

Factors in analysis were mycorrhiza (M), phosphorous (P) and zinc (Zn). Interactions and main effects were presented with M × P, M × Zn, P × Zn and M × P × Zn. ** $p < 0.01$; *** $p < 0.0001$. TSP = Total soluble protein; Chl = Chlorophyll; Gs = Stomatal conductance; E = Transpiration rate; A = Photosynthetic rate.

3.2. Chlorophyll Contents

There was a significant improvement in the chlorophyll contents with the inoculation of AMF, as shown in Figure 2A–C. Zn deficiency reduced the synthesis of chlorophyll and 20 mg kg^{-1} Zn application increased chlorophyll contents by ~9% compared to the control. Similarly, chlorophyll a and b concentrations increased to 7% and 13.3%, respectively, with 20 mg kg^{-1} Zn application. Zn application up to 40 mg kg^{-1} increased the chlorophyll content (Figure 2), although the effect was decreased upon further application of Zn. Application of P increased the production in chlorophyll up to 60% in Zn deficient soil. The maximum level of chlorophyll was observed at 20 mg kg^{-1} Zn as it increased the total chlorophyll content by 62% compared with the control. The efficiency of P fertilization on increasing the chlorophyll content was reduced with high levels of added Zn, as shown in Figure 2. At 120 mg kg^{-1} Zn application, only 38% more chlorophyll content was observed. AMF inoculation increased the chlorophyll content in maize compared to non-mycorrhizal plants. Mycorrhizal plant response was predominantly observed in treatments receiving 29 kg/ac P application at 20 mg/kg than other treatments (Figure 2).

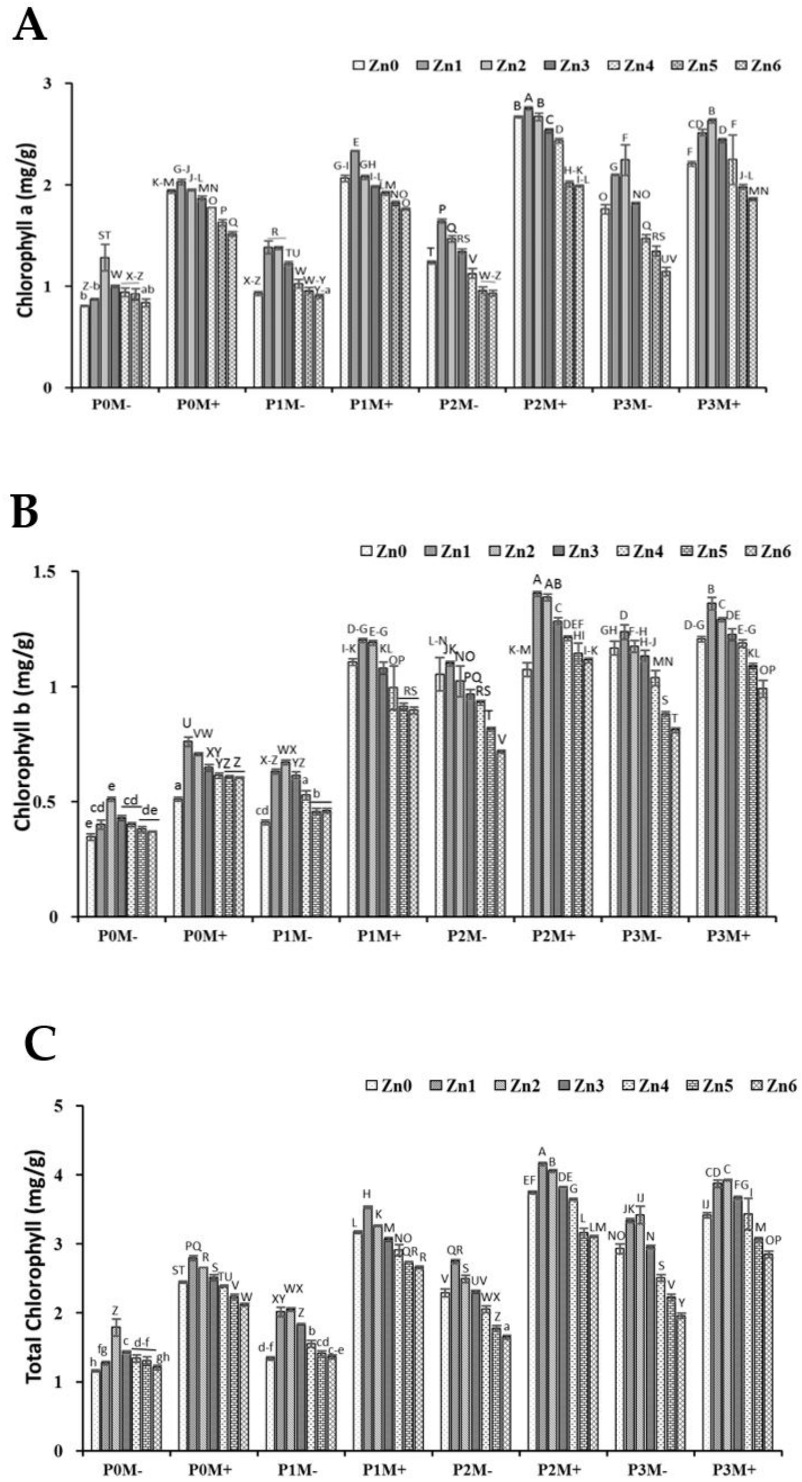

Figure 2. Chlorophyll a (**A**), b (**B**), and total chlorophyll content (**C**) in the maize leaves grown under various P (0, quarter, half and full recommended dose) and Zn (0, 20, 40, 60, 80, 100 and 120 mg Zn/kg soil) application rates under AM and NM conditions. Mean values are presented ± standard error (SE). Different letters on the bars indicate significant differences at $p \leq 0.05$. Capital letters indicate the highest values. After completely using uppercase letters up to Z, lower case letters are used to show the decrease of mean values. P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; Zn control = 0, Zn1 = 20, Zn2 = 40, Zn3 = 60, Zn4 = 80, Zn5 = 100, and Zn6 = 120 mg Zn kg^{-1}.

3.3. Total Soluble Protein

Similar results were observed regarding TSP in maize (Figure 3). In AM plants, 20 mg kg^{-1} Zn application provided maximum results whereas, in NM plants, 40 mg kg^{-1} Zn application provided higher TSP contents. It has also been observed that the highest level of P caused a significant decrease of TSP over control at Zn4, Zn5, and Zn6. In contrast, TSP remained significantly higher at 58 kg/ac P compared to the values at Zn0, Zn1, Zn2, and Zn3.

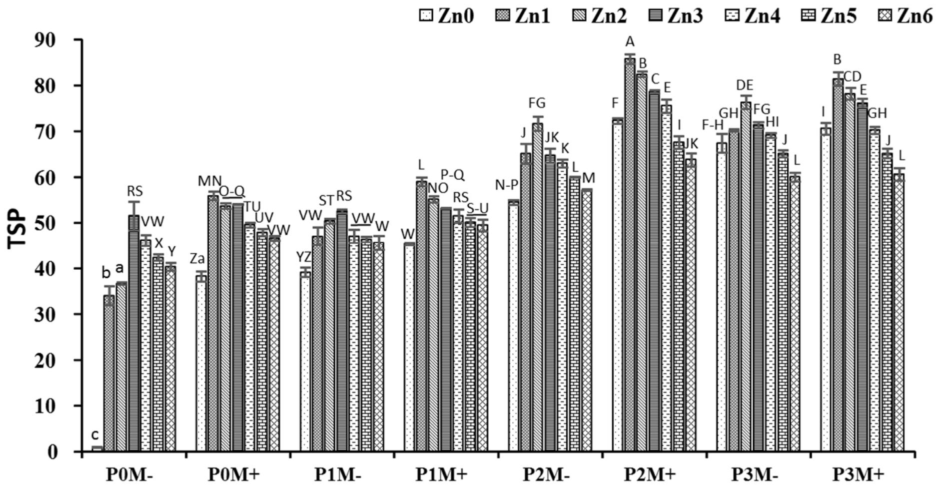

Figure 3. Total soluble protein in maize grown under various P (0, quarter, half, and full recommended dose) and Zn (0, 20, 40, 60, 80, 100 and 120 mg Zn/kg soil) application rates under AM and NM conditions. Mean values are presented ± standard error (SE). Different letters on the bars indicate significant differences at $p \leq 0.05$. Capital letters indicate the highest values. After completely using upper case letters up to Z, lower case letters are used to show the decrease of mean values. P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; Zn control = 0, Zn1 = 20, Zn2 = 40, Zn3 = 60, Zn4 = 80, Zn5 = 100, and Zn6 = 120 mg Zn kg^{-1}.

3.4. Morphological Attributes

As expected, deficiency of Zn and P reduced plant growth, as clearly demonstrated by measurements of plant height, fresh weight, and stem girth. Application of P increased the growth of maize in all NM plants, but in AM plants, a full dose of P reduced the growth compared with a half dose (Table 3). A remarkable reduction in plant growth was observed in control P treatments when high soil Zn was applied (Table 3). However, at high levels of Zn with full P application, not much difference was reported in the fresh weight of plants (Table 3). Maximum fresh weight (3.2 kg), plant height (88.3 cm) and stem girth (3.26 cm) were observed in AM plants at 20 mg kg^{-1} Zn along with 29 kg ac^{-1} P. Although stem girth was not affected significantly, other traits (plant height and weight) were strongly influenced by mycorrhizal inoculation at various Zn and P application rates. Under P deficient soil, high Zn supply worsens plant growth by severely reducing plant growth (Tables 3–5).

Table 3. Plant height (cm) and stem diameter (cm) of AMF inoculated (AM) and non-inoculated (NM) plants grown under various P (0 to full recommended dose) and Zn (0–120 mg Zn/kg soil) application rates.

Zn (mg/kg)	Plant Height (cm)							
	$P_{control}$		P1		P2		P3	
	NM	AM	NM	AM	NM	AM	NM	AM
$Zn_{control}$	$62.3 \pm 1.5^{\text{W–Y}}$	$75 \pm 1^{\text{W–Y}}$	$62.5 \pm 0.5^{\text{W–Y}}$	$76.6 \pm 0.6^{\text{E–G}}$	$64.6 \pm 0.6^{\text{U–X}}$	$76.3 \pm 1.1^{\text{F,G}}$	$66.6 \pm 1.5^{\text{R–U}}$	$76.3 \pm 5.8^{\text{F,G}}$
Zn20	$63.3 \pm 1.1^{\text{V–Y}}$	$76.6 \pm 1.5^{\text{E–G}}$	$65.8 \pm 1^{\text{S–V}}$	$84.3 \pm 0.6^{\text{B}}$	$70.6 \pm 1.1^{\text{M–P}}$	$88.3 \pm 2^{\text{A}}$	$75 \pm 0.7^{\text{G–L}}$	$74.3 \pm 3^{\text{G–J}}$
Zn40	$64.3 \pm 3.5^{\text{U–X}}$	$75.3 \pm 3^{\text{G–I}}$	$68.5 \pm 1.3^{\text{P–S}}$	$82.8 \pm 0.3^{\text{B,C}}$	$72 \pm 4^{\text{L–O}}$	$83.6 \pm 0.6^{\text{B,C}}$	$75.3 \pm 1.5^{\text{G–I}}$	$72.6 \pm 1.1^{\text{I–N}}$
Zn60	$65 \pm 2^{\text{T–W}}$	$75 \pm 1^{\text{G–K}}$	$64.5 \pm 0.8^{\text{U–X}}$	$79 \pm 1^{\text{D–E}}$	$70.6 \pm 1.1^{\text{M–P}}$	$83.3 \pm 1.5^{\text{B,C}}$	$72.5 \pm 1.5^{\text{J–N}}$	$72.3 \pm 2.5^{\text{K–N}}$
Zn80	$63 \pm 1^{\text{W–Y}}$	$74 \pm 0^{\text{G–L}}$	$62.8 \pm 3^{\text{W–Y}}$	$75.6 \pm 0.7^{\text{G,H}}$	$70.3 \pm 1.5^{\text{N–Q}}$	$81.4 \pm 1.2^{\text{C,D}}$	$70.6 \pm 1.1^{\text{M–P}}$	$67.6 \pm 1.5^{\text{M–P}}$
Zn100	$62.3 \pm 1.1^{\text{W–Y}}$	$72.8 \pm 0.7^{\text{I–N}}$	$62 \pm 0^{\text{X,Y}}$	$73.3 \pm 1.5^{\text{H–M}}$	$68 \pm 0^{\text{P–S}}$	$79.3 \pm 0.6^{\text{D,E}}$	$69.3 \pm 0.6^{\text{O–R}}$	$71.6 \pm 2.5^{\text{L–O}}$
Zn120	$59 \pm 2.6^{\text{Z}}$	$72 \pm 1.15^{\text{K–N}}$	$61 \pm 1^{\text{Y,Z}}$	$72 \pm 0.29^{\text{L–O}}$	$64 \pm 1^{\text{U–X}}$	$76.3 \pm 0.6^{\text{F,G}}$	$66 \pm 0^{\text{S–V}}$	$70.3 \pm 0.6^{\text{N–Q}}$

Mean values are presented ± standard error (SE). Different letters show significant difference at $p \leq 0.05$. Capital letters indicate the highest values. After completely using upper case letters up to Z, lower case letters are used to show the decrease of mean values. AMF inoculated (AM); non-inoculated (NM); P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; Zn control = 0, Zn1 = 20, Zn2 = 40, Zn3 = 60, Zn4 = 80, Zn5 = 100, and Zn6 = 120 mg Zn kg^{-1}.

Table 4. Stem diameter (cm) of AMF inoculated (AM) and non-inoculated (NM) plants grown under various P (0 to full recommended dose) and Zn (0–120 mg Zn/kg soil) application rates.

Zn (mg/kg)	Stem Diameter (cm)							
	$P_{control}$		P1		P2		P3	
	NM	AM	NM	AM	NM	AM	NM	AM
$Zn_{control}$	$2.01 \pm 0.03^{\text{Z–c}}$	$2.35 \pm 0.05^{\text{J–N}}$	$2.06 \pm 0.03^{\text{W–a}}$	$2.39 \pm 0.01^{\text{H–K}}$	$2.21 \pm 0.05^{\text{V–Y}}$	$2.40 \pm 0.02^{\text{G–J}}$	$2.28 \pm 0.1^{\text{N–P}}$	$2.04 \pm 0.05^{\text{X–b}}$
Zn20	$2.2 \pm 0.08^{\text{Q–U}}$	$2.4 \pm 0.04^{\text{G–I}}$	$2.31 \pm 0.03^{\text{K–O}}$	$2.57 \pm 0.02^{\text{D,E}}$	$2.36 \pm 0.02^{\text{I–M}}$	$3.26 \pm 0.04^{\text{A}}$	$2.4 \pm 0.01^{\text{G–J}}$	$2.4 \pm 0.05^{\text{G–J}}$
Zn40	$2.3 \pm 0.05^{\text{L–P}}$	$2.47 \pm 0.25^{\text{F,G}}$	$2.3 \pm 0.01^{\text{L–P}}$	$2.52 \pm 0.02^{\text{E,F}}$	$2.4 \pm 0.02^{\text{G–J}}$	$3.06 \pm 0.05^{\text{B}}$	$2.4 \pm 0.01^{\text{G–J}}$	$2.26 \pm 0.11^{\text{O–R}}$
Zn60	$2.1 \pm 0.05^{\text{V–Y}}$	$2.29 \pm 0.01^{\text{M–P}}$	$2.25 \pm 0.05^{\text{O–R}}$	$2.45 \pm 0.01^{\text{F–H}}$	$2.3 \pm 0.05^{\text{L–P}}$	$2.89 \pm 0^{\text{C}}$	$2.38 \pm 0^{\text{H–L}}$	$2.27 \pm 0.02^{\text{N–Q}}$
Zn80	$2.08 \pm 0.07^{\text{V–Z}}$	$2.2 \pm 0^{\text{P–T}}$	$2.12 \pm 0.02^{\text{U–X}}$	$2.4 \pm 0.01^{\text{G–I}}$	$2.23 \pm 0.02^{\text{P–S}}$	$2.63 \pm 0.02^{\text{D}}$	$2.3 \pm 0.02^{\text{K–O}}$	$2.15 \pm 0.05^{\text{S–V}}$
Zn100	$1.98 \pm 0.02^{\text{b,c}}$	$2.18 \pm 0^{\text{R–U}}$	$2 \pm 0.05^{\text{Y–b}}$	$2.3 \pm 0.02^{\text{K–P}}$	$2.12 \pm 0.02^{\text{U–X}}$	$2.5 \pm 0.02^{\text{E,F}}$	$2.19 \pm 0^{\text{Q–U}}$	$2.03 \pm 0.05^{\text{Y–b}}$
Zn120	$1.7 \pm 0.05^{\text{e}}$	$2 \pm 0^{\text{a–c}}$	$1.82 \pm 0.16^{\text{d}}$	$2.14 \pm 0.04^{\text{T–W}}$	$1.95 \pm 0.05^{\text{c}}$	$2.3 \pm 0.01^{\text{L–P}}$	$2.01 \pm 0.02^{\text{Z–c}}$	$1.96 \pm 0.02^{\text{b,c}}$

Mean values are presented ± standard error (SE). Different letters show significant difference at $p \leq 0.05$. Capital letters indicate the highest values. After completely using upper case letters up to Z, lower case letters are used to show the decrease of mean values. AMF inoculated (AM); non-inoculated (NM); P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; Zn control = 0, Zn1 = 20, Zn2 = 40, Zn3 = 60, Zn4 = 80, Zn5 = 100, and Zn6 = 120 mg Zn kg^{-1}.

Table 5. Fresh weight (kg) of AMF inoculated (AM) and non-inoculated (NM) plants grown under various P (0 to full recommended dose) and Zn (0–120 mg Zn/kg soil) application rates.

Zn (mg/kg)	Fresh Weight (kg)							
	$P_{control}$		P1		P2		P3	
	NM	AM	NM	AM	NM	AM	NM	AM
$Zn_{control}$	$0.07 \pm 0.001^{\text{Z,a}}$	$0.14 \pm 0.01^{\text{P,Q}}$	$0.09 \pm 0.003^{\text{X,Y}}$	$0.16 \pm 0.003^{\text{L–N}}$	$0.11 \pm 0.005^{\text{U–W}}$	$0.26 \pm 0.1^{\text{E,F}}$	$0.18 \pm 0.003^{\text{P,Q}}$	$0.23 \pm 0.02^{\text{H}}$
Zn20	$0.08 \pm 0.003^{\text{Y,Z}}$	$0.21 \pm 0.005^{\text{I}}$	$0.11 \pm 0.005^{\text{U–W}}$	$0.24 \pm 0.02^{\text{G,H}}$	$0.12 \pm 0.0^{\text{S–V}}$	$0.32 \pm 0.02^{\text{A}}$	$0.196 \pm 0.002^{\text{N–P}}$	$0.3 \pm 0.007^{\text{A}}$
Zn40	$0.11 \pm 0.002^{\text{T–W}}$	$0.18 \pm 0.001^{\text{J,K}}$	$0.13 \pm 0.001^{\text{Q–S}}$	$0.22 \pm 0.01^{\text{I}}$	$0.14 \pm 0.002^{\text{O–Q}}$	$0.3 \pm 0.01^{\text{B}}$	$0.22 \pm 0.003^{\text{J,K}}$	$0.28 \pm 0.005^{\text{C,D}}$
Zn60	$0.10 \pm 0.002^{\text{K,L}}$	$0.17 \pm 0.008^{\text{R–U}}$	$0.12 \pm 0.002^{\text{J}}$	$0.19 \pm 0.005^{\text{Q–T}}$	$0.13 \pm 0.001^{\text{B,C}}$	$0.29 \pm 0.003^{\text{B,C}}$	$0.21 \pm 0.001^{\text{K–M}}$	$0.27 \pm 0.002^{\text{C,D}}$
Zn80	$0.09 \pm 0.003^{\text{X,Y}}$	$0.15 \pm 0.006^{\text{WX}}$	$0.10 \pm 0.003^{\text{L–N}}$	$0.16 \pm 0.01^{\text{R–U}}$	$0.12 \pm 0.003^{\text{D,E}}$	$0.27 \pm 0.002^{\text{D,E}}$	$0.19 \pm 0.002^{\text{N–P}}$	$0.25 \pm 0.02^{\text{F,G}}$
Zn100	$0.04 \pm 0.009^{\text{b}}$	$0.12 \pm 0.007^{\text{Z,a}}$	$0.07 \pm 0.001^{\text{Q–S}}$	$0.13 \pm 0.005^{\text{W,X}}$	$0.10 \pm 0.003^{\text{Q,R}}$	$0.19 \pm 0.01^{\text{Q,R}}$	$0.17 \pm 0.01^{\text{Q–S}}$	$0.17 \pm 0.01^{\text{R–V}}$
Zn120	$0.03 \pm 0.001^{\text{b}}$	$0.10 \pm 0.001^{\text{a}}$	$0.06 \pm 0.001^{\text{U–W}}$	$0.11 \pm 0.005^{\text{X,Y}}$	$0.09 \pm 0.001^{\text{T–W}}$	$0.17 \pm 0.01^{\text{T–W}}$	$0.14 \pm 0.01^{\text{W,X}}$	$0.16 \pm 0.01^{\text{V–X}}$

Mean values are presented ± standard error (SE). Different letters show significant difference at $p \leq 0.05$. Capital letters indicate the highest values. After completely using upper case letters up to Z, lower case letters are used to show the decrease of mean values. AMF inoculated (AM); non-inoculated (NM); P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; Zn control = 0, Zn1 = 20, Zn2 = 40, Zn3 = 60, Zn4 = 80, Zn5 = 100, and Zn6 = 120 mg Zn kg^{-1}.

3.5. Gaseous Exchange Traits

The maximum photosynthesis rate (21.4 μmol CO_2 g^{-1}) was observed in AM plants at 20 mg Zn kg^{-1} with half the recommended dose of P. The current study showed that while Zn and P deficiency lower the photosynthesis rate, their application positively enhanced the photosynthesis process (Table 2). However, a high Zn supply decreased photosynthesis, irrespective of P application. The association of AMF with maize roots significantly increased the photosynthesis rate, irrespective of P and Zn supply. On average, AM plants improved their photosynthesis rate by more than 70% compared with NM plants (Table 6). Phosphorous and Zn supply significantly increased the transpiration rate of maize, irrespective of AMF inoculation (Table 6). The increased transpiration rate reflected the importance of Zn and P in plant physiological processes, as their deficiency lowered the transpiration rate. Zinc supplied up to an optimum rate disturbed the transpiration rate process. The transpiration rate (3.72 µmol H_2O m^{-2} s^{-1}) and gaseous exchange rate (0.163 mmol m^{-2} s^{-1}) were maximal from 20–40 mg Zn kg^{-1} at 29 kg ac^{-1} P (in AM maize) and 58 kg ac^{-1} P (in NM maize). Inoculation of AMF improved in all Zn and P rates, but the maximum rate was observed at 20 mg Zn kg^{-1} with 29 kg ac^{-1} P. As soil Zn supply increased from 20 mg kg^{-1}, physiological processes further reduced significantly.

Table 6. Photosynthesis rate, transpiration rate, and stomatal conductance rate of AMF inoculated (AM) and non-inoculated (NM) plants grown under various P (0 to full recommended dose) and Zn (0–120 mg Zn/kg soil) application rates. Mean values are presented ± standard error (SE). Different letters show significant difference at $p \leq 0.05$. Capital letters indicate the highest values. After completely using upper case letters up to Z, lower case letters are used to show the decrease of mean values.

P Application Rates	Zn Application Rates	Photosynthesis Rate		Transpiration Rate		Stomatal Conductance	
P (kg/ac)	Zn (mg/kg)	NM	AM	NM	AM	NM	AM
$P_{control}$	$Zn_{control}$	1.03 ± 0.06 ^{d}	2.5 ± 0.1 ^{a}	0.92 ± 0.06 $^{b–d}$	1.19 ± 0.11 ^{XY}	0.021 ± 0.00 $^{S–W}$	0.04±0.01 $^{M–O}$
	Zn 20 mg/kg	2.23 ± 0.05 ^{ab}	7.53 ± 0.20 ^{LM}	1.33 ± 0.05 ^{UV}	1.47 ± 0.04 ST	0.030 ± 0.001 $^{O–S}$	0.55 ± 0.008 $^{I–K}$
	Zn 40 mg/kg	3.4 ± 0.1 ^{Y}	6.83 ± 0.20 ^{OP}	1.10 ± 0.01 ^{YZ}	1.33 ± 0.01 ^{UV}	0.015 ± 0.001 $^{U–Y}$	0.019 ± 0.001 $^{T–X}$
	Zn 60 mg/kg	1.96 ± 0.05 ^{b}	5.24 ± 0.05 ^{RS}	0.98 ± 0.01 $^{a–c}$	1.13 ± 0.04 $^{X–Z}$	0.012 ± 0.001 $^{W–Z}$	0.016 ± 0.001 $^{U–Y}$
	Zn 80 mg/kg	1.53 ± 0.05 ^{c}	4.92 ± 0.02 $^{S–U}$	0.92 ± 0 $^{b–d}$	1.13 ± 0.01 ^{ab}	0.093 ± 0.005 ^{DE}	0.014 ± 0.001 $^{U–Y}$
	Zn 100 mg/kg	0.99 ± 0.01 ^{d}	3.3 ± 0.1 ^{YZ}	0.88 ± 0.01 ^{cd}	0.99 ± 0.17 ^{bc}	0.071 ± 0.001 ^{G}	0.012 ± 0.001 $^{W–Z}$
	Zn 120 mg/kg	0.24 ± 0.03 ^{e}	2.53 ± 0.11 ^{a}	0.71 ± 0.01 ^{e}	0.96 ± 0.030 ^{d}	0.010 ± 0.001 $^{X–Z}$	0.010 ± 0 $^{X–Z}$
P1	$Zn_{control}$	3.55 ± 0.12 ^{XY}	4.63 ± 0.07 ^{UV}	1.38 ± 0.01 ^{TU}	1.52 ± 0.03 ^{S}	0.04 ± 0.001 ^{LM}	0.06 ± 0.01 $^{H–J}$
	Zn 20 mg/kg	4.6 ± 0.1 ^{UV}	6.75 ± 0.05 ^{OP}	1.52 ± 0.10 ^{S}	1.88 ± 0.03 ^{Q}	0.063 ± 0.003 $^{G–I}$	0.084 ± 0.001 ^{EF}
	Zn 40 mg/kg	4.24 ± 0.05 ^{VW}	5.46 ± 0.11 ^{R}	1.48 ± 0.01 ST	1.72 ± 0.01 ^{R}	0.017 ± 0.001 $^{T–Y}$	0.023 ± 0.001 $^{Q–U}$
	Zn 60 mg/kg	3.9 ± 0.1 ^{WX}	5.10 ± 0.16 $^{R–T}$	1.32 ± 0.07 $^{U–W}$	1.42 ± 0.03 $^{S–U}$	0.011 ± 0 $^{W–Z}$	0.013 ± 0.002 $^{V–Z}$
	Zn 80 mg/kg	3.16 ± 0.15 ^{TU}	4.72 ± 0.05 ^{YZ}	1.22 ± 0.03 ^{WX}	1.34 ± 0.01 ^{U}	0.06 ± 0 $^{G–I}$	0.009 ± 0.001 ^{YZ}
	Zn 100 mg/kg	2.96 ± 0.05 ^{Z}	4.26 ± 0.15 ^{VW}	1.16 ± 0.04 $^{X–Z}$	1.23 ± 0.01 $^{V–X}$	0.051 ± 0.002 ^{JK}	0.008 ± 0 ^{YZ}
	Zn 120 mg/kg	1.83 ± 0.15 ^{bc}	3.16 ± 0.05 ^{YZ}	1.06 ± 0.05 ^{Za}	1.09 ± 0.01 ^{YZ}	0.032 ± 0.003 $^{M–R}$	0.003 ± 0.001 ^{Z}
P2	$Zn_{control}$	9.8 ± 0.52 ^{I}	11.20 ± 0.80 ^{G}	2.66 ± 0.16 ^{IJ}	2.81 ± 0.10 ^{GH}	0.071 ± 0.001 ^{G}	0.1 ± 0 ^{D}
	Zn 20 mg/kg	17.13 ± 0.11 ^{C}	21.49 ± 0.429 ^{A}	3.22 ± 0.08 ^{C}	3.72 ± 0.07 ^{A}	0.12 ± 0.01 ^{C}	0.163 ± 0.02 ^{A}
	Zn 40 mg/kg	12.2 ± 0.25 ^{F}	18.23 ± 1 ^{B}	3.06 ± 0.20 ^{DE}	3.5 ± 0.09 ^{B}	0.090 ± 0.001 $^{D–F}$	0.146 ± 0.02 ^{A}
	Zn 60 mg/kg	8.3 ± 0.1 ^{K}	10.4 ± 0.51 ^{H}	2.98 ± 0.02 ^{EF}	3.21 ± 0.06 ^{C}	0.065 ± 0.004 ^{GH}	0.083 ± 0.005 ^{F}
	Zn 80 mg/kg	7.49 ± 0.45 ^{LM}	8.86 ± 0.05 ^{J}	2.85 ± 0.03 ^{G}	3.15 ± 0.04 ^{CD}	0056 ± 0.004 $^{H–K}$	0.063 ± 0.005 $^{G–I}$
	Zn 100 mg/kg	6.6 ± 0.1 ^{OP}	7.26 ± 0.11 ^{MN}	2.3 ± 0.13 ^{N}	2.65 ± 0.05 ^{IJ}	0.033 ± 0.002 $^{M–P}$	0.04 ± 0.002 $^{M–O}$
	Zn 120 mg/kg	4.94 ± 0.22 $^{S–U}$	5.96 ± 0.15 ^{Q}	1.85 ± 0.06 ^{Q}	2.22 ± 0.07 ^{O}	0.022 ± 0.001 $^{R–V}$	0.033 ± 0.006 $^{M–Q}$
P3	$Zn_{control}$	8.90 ± 0.10 ^{J}	10.2 ± 0.1 ^{HI}	2.54 ± 0.05 ^{KL}	2.74 ± 0.02 ^{HI}	0.041 ± 0.001 ^{LM}	0.06 ± 0.003 $^{H–J}$
	Zn 20 mg/kg	14.3 ± 0.20 ^{E}	18.2 ± 0.1 ^{B}	2.68 ± 0.09 ^{I}	2.98 ± 0.01 ^{EF}	0.051 ± 0.001 ^{JK}	0.073 ± 0.003 ^{G}
	Zn 40 mg/kg	10.3 ± 0.43 ^{H}	15 ± 0.05 ^{D}	2.72 ± 0.030 ^{HI}	2.88 ± 0.01 ^{FG}	0.040 ± 0.001 $^{L–N}$	0.05 ± 0.01 ^{KL}
	Zn 60 mg/kg	7.33 ± 0.32 $^{L–N}$	8.33 ± 0.32 ^{K}	2.44 ± 0.005 ^{LM}	3.08 ± 0.07 ^{DE}	0.035 ± 0.005 $^{M–P}$	0.040 ± 0.004 ^{LM}
	Zn 80 mg/kg	6.5 ± 0.1 ^{P}	7.7 ± 0.17 ^{L}	2.38 ± 0.01 ^{MN}	2.88 ± 0.01 ^{FG}	0.030 ± 0.002 $^{N–S}$	0.037 ± 0.006 $^{M–O}$
	Zn 100 mg/kg	5.4 ± 0.26 ^{R}	6.94 ± 0.06 ^{NO}	2.04 ± 0.05 ^{P}	2.56 ± 0.04 ^{JK}	0.026 ± 0.002 $^{P–T}$	0.032 ± 0.003 $^{M–R}$
	Zn 120 mg/kg	3.16 ± 0.15 ^{YZ}	5.26 ± 0.30 ^{RS}	1.86 ± 0.02 ^{Q}	2.06 ± 0.15 ^{P}	0.012 ± 0.002 $^{W–Z}$	0.021 ± 0.001 $^{S–W}$

Mean values are presented ± standard error (SE). Different letters show significant difference at $p \leq 0.05$. Capital letters indicate the highest values. After completely using upper case letters up to Z, lower case letters are used to show the decrease of mean values. AMF inoculated (AM); non-inoculated (NM); P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; Zn control = 0, Zn1 = 20, Zn2 = 40, Zn3 = 60, Zn4 = 80, Zn5 = 100, and Zn6 = 120 mg Zn kg^{-1}.

3.6. Nutrient Contents in Plants and Soil

Phosphorous concentration increased significantly as P application rates increased in AM and NM plants under all Zn concentrations (Table 2). Analysis of the P concentration showed a significant interaction between mycorrhizal inoculation and P addition (Table 2). Phosphorous accumulation in the shoot was higher in all AM plants, whereas high P addition reduced the P concentration in the shoots of AM plants. Maximum P accumulates at 29 kg ac^{-1} application. Irrespective of Zn application rates, AM plants had better P uptake than NM plants. There was also a two-way interaction reported in Zn addition and P addition. Although under control P treatments, Zn application increased the P uptake in plants, however, as Zn concentrations range towards toxic levels, they negatively affected P uptake (Table 7).

Table 7. Shoot and root P of AMF inoculated (AM) and non-inoculated (NM) plants grown under various P (0 to full recommended dose) and Zn (0–120 mg Zn/kg soil) application rates. Mean values are presented ± standard error (SE). Different letters denote the significant differences among treatments. Lower case letters show a decrease in the values of a particular attribute.

Zn (mg/kg)	**Shoot P (mg/kg)**							
	$P_{control}$		**P1**		**P2**		**P3**	
	NM	**AM**	**NM**	**AM**	**NM**	**AM**	**NM**	**AM**
$Zn_{control}$	543.2 ± 2.7^{w}	981 ± 1^{q}	1851 ± 5.2^{i}	2107 ± 6^{b}	4093 ± 6^{U}	6120 ± 5^{C}	5130 ± 10^{H}	$4213 \pm 15^{Q,R}$
Zn20	620.4 ± 0.5^{u}	1356 ± 47^{k}	2015.3 ± 4.5^{f}	2259 ± 10^{Y}	4114.8 ± 7.2^{S}	6277 ± 2.7^{A}	5419 ± 9^{G}	4422 ± 2.5^{N}
Zn40	734.4 ± 4.9^{r}	1273 ± 24^{l}	2136 ± 3.6^{a}	2173 ± 6.6^{Z}	4197.6 ± 2.5^{R}	6149 ± 11^{B}	5549 ± 6^{F}	4326 ± 15^{O}
Zn60	708.4 ± 7.1^{s}	1213 ± 5.7^{m}	2083 ± 8.8^{c}	2136 ± 2.8^{a}	4073.3 ± 9.4^{T}	5816 ± 11^{D}	5321 ± 11.5^{H}	4264 ± 2.5^{P}
Zn80	643.3 ± 7.6^{t}	1042 ± 7.5^{o}	$2008 \pm 7.3^{f,g}$	2062 ± 0.0^{d}	3965 ± 13.2^{V}	5631 ± 10^{E}	5263 ± 3.2^{I}	4222 ± 7.5^{Q}
Zn100	590.9 ± 9.3^{v}	1005 ± 6^{p}	1972 ± 9.1^{h}	2036 ± 10^{e}	3932 ± 7.5^{W}	5241 ± 27^{J}	5193 ± 11.2^{K}	$4109 \pm 10^{S,T}$
Zn120	543.4 ± 3^{w}	1139 ± 9.2^{n}	1853.8 ± 5.4^{j}	1994 ± 7.8^{g}	3803 ± 6.5^{X}	4511 ± 8.5^{M}	5027 ± 4.9^{L}	3980 ± 7.7^{V}
				Root P (mg/kg)				
$Zn_{control}$	663.3 ± 8.3^{t}	1636 ± 9.4^{k}	1587 ± 6.5^{l}	2632 ± 17.5^{R}	2918 ± 17.5^{N}	4762 ± 3^{B}	3920 ± 20^{K}	$2729 \pm 14^{P,Q}$
Zn20	696.6 ± 6.1^{s}	1806 ± 5.8^{h}	1880 ± 16^{g}	2746 ± 3.6^{P}	2985 ± 3.6^{M}	4788 ± 2.6^{A}	4214 ± 2.6^{G}	2807 ± 6^{O}
Zn40	810.6 ± 3.7^{o}	1722 ± 2.5^{i}	2240 ± 6.1^{Y}	2518 ± 6.6^{U}	2820 ± 6.6^{O}	4614 ± 5.2^{C}	4144 ± 5.2^{H}	2717 ± 12^{Q}
Zn60	780.3 ± 2.5^{p}	1645 ± 5.5^{j}	2212 ± 2.6^{Z}	2440 ± 1^{W}	2615 ± 1^{S}	4545 ± 9.8^{D}	4004 ± 9.8^{I}	2640 ± 7^{R}
Zn80	761.1 ± 4.5^{q}	$1643 \pm 4.5^{j,k}$	2167 ± 12^{b}	2188 ± 1.5^{a}	2441 ± 1.5^{W}	4477 ± 21^{E}	3961 ± 7.8^{J}	2540 ± 20^{T}
Zn100	$747 \pm 5^{q,r}$	1519 ± 3.2^{m}	2104 ± 4.5^{d}	1981 ± 4^{f}	2355 ± 4^{X}	4409 ± 8.3^{F}	3908 ± 8.3^{K}	2474 ± 9^{V}
Zn120	734 ± 0.5^{r}	1487 ± 6.5^{n}	2038 ± 25^{e}	1877 ± 11^{g}	2131 ± 11.3^{c}	4206 ± 3.2^{G}	3811 ± 9.2^{L}	2254 ± 30^{Y}

Mean values are presented ± standard error (SE). Different letters show significant difference at $p \leq 0.05$. Capital letters indicate the highest values. After completely using upper case letters up to Z, lower case letters are used to show the decrease of mean values. AMF inoculated (AM); non-inoculated (NM); P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; Zn control = 0, Zn1 = 20, Zn2 = 40, Zn3 = 60, Zn4 = 80, Zn5 = 100, and Zn6 = 120 mg Zn kg^{-1}.

Root accumulation of P significantly improved with P addition irrespective of soil Zn status. Statistical analysis showed the two-way interaction between P addition and root P concentration (Table 2). Root P concentration was also maximized by inoculation with AMF in maize plants. AM plants take up more P from the soil than NM plants under all P and Zn application rates. Root P uptake was also affected by Zn. Increasing soil Zn concentration (Table 7) negatively affects root P uptake.

Accumulation of shoot and root Zn followed the same pattern shown in Table 8, so results were considered together. Application of Zn increased the concentration of Zn in both shoots and roots, irrespective of P application. Statistical analysis showed a two-way interaction between the Zn concentration in plants and inoculation by AMF (Table 2). The addition of Zn at higher rates significantly increased the uptake and accumulation of Zn in all maize plants. However, inoculation with AMF seems to impart a protective effect on maize plant Zn concentration. Under low Zn addition rates, AM plants showed a higher concentration of Zn in their roots and shoots than NM plants and at higher Zn addition rates, AMF imparts a protective effect by lowering the accumulation of Zn in plant parts. Zinc concentrations in shoots and roots decreased significantly with P application, as clearly shown in Table 8. Under lower Zn conditions, P addition at higher rates drastically reduced plant Zn uptake, which negatively affected the plant.

Table 8. Shoot and root P of AMF inoculated (AM) and non-inoculated (NM) plants grown under various P (0 to full recommended dose) and Zn (0–120 mg Zn/kg soil) application rates. Mean values are presented ± standard error (SE). Alphabets denote the significant difference among treatments. Small alphabets after capital showed a decrease in the values of attributes.

Zn (mg/kg)	Shoot Zn (mg/kg)							
	$P_{control}$		P1		P2		P3	
	NM	AM	NM	AM	NM	AM	NM	AM
$Zn_{control}$	24.9 ± 1.1 ^{d,e}	30.5 ± 1.5 ^{b,c,d}	21.9 ± 0.4 ^{e,f}	20.3 ± 0.6 ^{e,f,g}	18.1 ± 0.1 ^{f,g}	30.5 ± 1.8 ^{b,c,d}	15.5 ± 0.4 ^{g}	14.2 ± 0.7 ^{g}
Zn20	34.9 ± 1.7 ^{Z,a,b}	42 ± 0.2 ^{X,Y}	31.4 ± 0.5 ^{X,Y}	34.9 ± 0.2 ^{Z,a,b}	28 ± 1.4 ^{c,d}	41.1 ± 1 ^{X,Y}	25.1 ± 2.3 ^{d,e}	38.2 ± 0.5 ^{Y,Z,a}
Zn40	46.1 ± 1 ^{W,X}	49.2 ± 1.7 ^{W}	43.1 ± 0.8 ^{X,Y}	45.7 ± 0.6 ^{W,X}	38.7 ± 3.3 ^{Y,Z,a}	45 ± 0.2 ^{W,X}	33.4 ± 0.5 ^{a,b,c}	40.2 ± 0.6 ^{X,Y,Z}
Zn60	78.4 ± 1.5 ^{S}	62.3 ± 0.8 ^{U}	70.5 ± 2.7 ^{T}	59.16 ± 0.7 ^{U,V}	64.4 ± 4.8 ^{U}	55.4 ± 2.2 ^{V}	60.2 ± 3 ^{U,V}	49.2 ± 1 ^{W}
Zn80	140.7 ± 5.1 ^{L}	126.9 ± 1.1 ^{M,N}	123.7 ± 1.5 ^{N}	115.9 ± 3.6 ^{O}	103.6 ± 2.6 ^{P}	89.4 ± 2.1 ^{Q,R}	92.2 ± 0.9 ^{Q}	84 ± 2.3 ^{R,S}
Zn100	244.1 ± 1 ^{C}	200.8 ± 3.1 ^{F}	211.4 ± 5.6 ^{E}	175.7 ± 5.5 ^{H}	197 ± 7.8 ^{F}	159.3 ± 2.3 ^{I}	182.9 ± 6.3 ^{G}	130.8 ± 2.8 ^{M}
Zn120	320.1 ± 10 ^{A}	243.6 ± 10.7 ^{C}	256 ± 5 ^{B}	197.4 ± 7.7 ^{F}	220.8 ± 10.5 ^{D}	167.6 ± 2.5 ^{J}	198.5 ± 3.7 ^{F}	149.1 ± 3.7 ^{K}
	Root Zn (mg/kg)							
$Zn_{control}$	46.03 ± 4.8 ^{d,e,f}	55.3 ± 1.5 $^{b–f}$	42.8 ± 8.4 ^{e,f}	50.5 ± 3.3 ^{d,e,f}	43.1 ± 7.8 ^{e,f}	53.1 ± 6 $^{c–f}$	49.5 ± 1.9 ^{d,e,f}	41.3 ± 4.6 ^{f}
Zn20	76.8 ± 1.5 $^{a–d}$	83.9 ± 0.9 ^{Z,a}	67.9 ± 4.2 $^{a–e}$	82.4 ± 4.8 ^{Z,a}	69.8 ± 1.7 $^{a–d}$	80.5 ± 5.9 $^{Z–c}$	71.2 ± 1 $^{a–d}$	77.6 ± 8.7 ^{a,b,c}
Zn40	131.3 ± 2.9 ^{X,Y}	140.4 ± 1.4 ^{X}	123.3 ± 4.5 ^{X,Y}	126.9 ± 7 ^{X,Y}	140.6 ± 4.3 ^{X}	120.9 ± 8.5 ^{X,Y}	130.3 ± 3.8 ^{X,Y}	106.2 ± 9.1 ^{Y,Z}
Zn60	620.5 ± 16.8 ^{S}	570.2 ± 37 ^{T}	610.7 ± 12.6 ^{S}	519.5 ± 13.8 ^{U}	527.7 ± 6.2 ^{U}	489.6 ± 14.7 ^{V}	534.4 ± 8 ^{U}	420.9 ± 10.2 ^{W}
Zn80	1044.3 ± 28 ^{J}	721.2 ± 29.7 ^{PQ}	929.3 ± 7.2 ^{L}	702.3 ± 15.4 ^{Q,R}	879 ± 9.6 ^{M}	685.3 ± 6.8 ^{R}	756.5 ± 4.9 ^{O}	611.8 ± 10.4 ^{S}
Zn100	1542.9 ± 56 ^{E}	921.3 ± 26.8 ^{L}	1442 ± 9.3 ^{F}	888.5 ± 11.2 ^{M}	1314.3 ± 12 ^{G}	810.7 ± 10.7 ^{N}	1281 ± 9.6 ^{H}	729.3 ± 23 ^{P}
Zn120	1879.9 ± 22.7 ^{A}	1099.9 ± 30.7 ^{I}	1802.6 ± 20.5 ^{B}	1040.9 ± 31.8 ^{J}	1620.3 ± 4.5 ^{C}	979.6 ± 15.3 ^{K}	1580.3 ± 13.4 ^{D}	932.1 ± 26.7 ^{L}

Mean values are presented ± standard error (SE). Different letters show significant difference at $p \leq 0.05$. Capital letters indicate the highest values. After completely using upper case letters up to Z, lower case letters are used to show the decrease of mean values. AMF inoculated (AM); non-inoculated (NM); P1 = 14.5 kg, P2 = 29 kg, P3 = 58 kg; Zn control = 0, Zn1 = 20, Zn2 = 40, Zn3 = 60, Zn4 = 80, Zn5 = 100, and Zn6 = 120 mg Zn kg^{-1}.

4. Discussion

Optimum P and Zn applications improved maize growth and nutrition with or without AMF inoculation. Results showed that AMF colonization depended on external nutrient concentration, in agreement with Zhang et al. [55,56] and Watts-William et al. [36]. Excess concentration of both nutrients (P and Zn) reduced the AMF colonization rate and root growth, also in agreement with Watts-William and Cavagarno [36] and Watts-William et al. [36]. Reduction in the colonization percentage at high levels of Zn could be due to the toxic effects of Zn on spore germination and hyphae growth, as reported by Smith and Read [34]. There is a negative correlation between Zn and root colonization. High Zn imparts a more pronounced effect on root AMF colonization than on root growth [36]. The soil used in the present study had low Zn availability. Consequently, AMF species had low resistance to toxic Zn levels, unlike AMF isolated from contaminated sites. Irrespective of the mechanisms involved, the effect of high Zn concentration on AMF colonization seems to operate independently of soil P supply.

The growth of maize was significantly reduced under low soil Zn and P and high soil Zn supply, irrespective of AMF inoculation (Table 2). Statistical analysis of growth parameters showed that AMF colonization of roots and P concentration in plant tissues is positively correlated with growth parameters but negatively correlated with Zn tissue concentration. AMF improved maize's growth parameters even at a high level of Zn, which was also reported in previous studies [36,57]. Watts-William et al. [36] reported a greater root-to-shoot ratio at higher soil Zn addition rates, especially in AM plants. That trend showed that the allocation of biomass was significantly affected by Zn addition, which demonstrated that more root growth is a normal tolerance mechanism at toxic Zn rates. Two suggested mechanisms were adopted by plants under toxic Zn conditions. Roots may grow beyond the toxic Zn zone due to the heterogeneous nature of soil [58]. More roots can uptake more P from the rhizosphere, promoting plant growth [36]. Reduced growth of maize at toxic Zn supply reflected the accumulation of the toxic amount of Zn in plant tissues as reported by Watts-William et al. [36]. Addition of P at high soil Zn supply positively affected plant growth by diluting the Zn concentration. The results showed that P application imparted a protective effect on maize growth at toxic soil Zn concentrations. AMF colonization is positively correlated with the plant height and stem girth of maize. Symbiosis was most likely observed at half of the recommended P supply compared with the control, quarter, or full P application. However, in AM plants, the colonization rate decreased with high P supply, which also influenced plant growth parameters, and the results were consistent with various other studies [59]. Improved physical plant growth also increased fresh plant weight, as reported by Zhang et al. [55]. AMF increased the nutrient absorption area of plant roots, which increased plant nutrition and growth parameters. The difference in plant growth between AM and NM plants was consistent with other studies [55,60].

Chlorophyll content was increased to a greater extent in AM plants than in NM plants, with similar results observed by Baslam et al. [61]. High Zn concentrations negatively affected the chlorophyll content, possibly a consequence of oxidative damage, but AM protected the chlorophyll pigments [62]. Application of P promotes the synthesis of chlorophyll; a similar observation was observed with 0–40 mg/kg Zn application, results that were in agreement with Babaeian et al. [63]. Total soluble protein content was increased with the inoculation of AMF as it aids in the synthesis of TSP. Kaur and Kumar [64] observed that AMF inoculation increased the TSP content by 20–30% in mung beans under salinity stress. To a lesser extent, the accumulation of P in plants was significantly influenced by P and Zn fertilization and AMF colonization. Maize can grow in low P soil. However, P accumulation in maize tissues was higher in AM maize than NM maize. As the Zn supply increased in soil, the accumulation of P in maize tissues was decreased. The concentration of P in plants rose in parallel with the AMF root colonization percentage, as reported earlier [27]. The concentration of P did not influence AMF colonization at a high P supply rate but was severely decreased by high soil Zn supply [65]. In terms of AMF symbiosis effect of maize, root colonization of AMF provided maximum benefit to plants by enhancing P accumulation even under deficient soil P and Zn conditions. The results of the present study are consistent with Watts-William and Cavagarno [27], who reported improved P and Zn nutrition in inoculated plants.

The zinc concentration in maize shoots varied from low Zn to moderate and toxic Zn concentrations. Reduced Zn concentrations in maize tissues due to P application increased the growth rate with high soil P application. There is a negative correlation between P and Zn, as shown in Table 7, with results in agreement with Watts-William and Cavagarno [27]. In Zn-deficient soils, the application of P at higher rates lowered the Zn accumulation in maize, so that the results were in line with other studies that reported P-induced Zn deficiency [66,67]. Even at toxic Zn application rates, increased P supply reduced the accumulation of Zn in plant tissues. Few studies have indicated that this reduced accumulation could be related to reduced AMF root colonization percentage, which lowers Zn uptake. However, in higher Zn application rates, P supply did not significantly affect Zn concentration. There was no evidence of the increased P concentration in AM plants due to Zn toxicity [39]. In short, AMF symbiosis showed a beneficial response when P and Zn were deficient in the soil. At a high concentration of Zn, there was a protective effect on plant growth and nutrition, as reported in earlier studies [68,69].

5. Conclusions

It was concluded from this study that P at a rate of 29 kg acre^{-1} and Zn at 20 mg kg^{-1} played a significant positive role in the improvement of the observed maize traits. Maximum root colonization occurred at a level of 29 kg P acre^{-1} and Zn 20 mg kg^{-1} compared to the control. The results also showed that mycorrhizal inoculation enhanced the nutrient uptake and suppressed the antagonistic effects of Zn and P. More studies are suggested at a field level to ascertain that 29 kg P acre^{-1} and 20 mg Zn kg^{-1} soil with mycorrhizal inoculation is the best treatment for the improvement of maize growth.

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