EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON MAIZE (*ZEA MAYS* L.) UNDER ZINC DEFICIENT AND TOXIC FIELD CONDITIONS

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(Received 7th Oct 2020; accepted 21st Dec 2020)

Abstract. Arbuscular mycorrhizal fungi (AMF) have potential to cope with nutrient stress environment when the soil is zinc (Zn) deficient or toxic. Current study was conducted under field conditions to investigate the potential of mycorrhizal inoculation on maize to mitigate Zn nutrient stress condition. The treatments were organized according to randomized complete block design under factorial arrangements with three replicates. During this study soil nutrient status and maize nutrient uptake were observed. Soil analysis and determination of different parameters such as chlorophyll contents, total soluble protein including yield parameters was conducted to compare the change in nutrient status with mycorrhizal inoculation. Results showed that mycorrhizal inoculation (M+) reversed the stress effect of Zn stress and promoted maize growth. Inoculation increased Zn uptake by 98% in Zn deficient conditions while reduced the uptake of Zn by 39% in Zn toxic conditions. Plants height increased by up to 16% with fungal inoculation as compared to non-inoculated maize plants. The increased cob length ultimately resulted in higher grain yield with an increase of 15% and 8% under medium Zn toxicity and severe toxicity respectively with mycorrhizal inoculation. Moreover, inoculated maize also showed significant improvement in maize plant root colonization, chlorophyll contents and total soluble protein.
Keywords: *alkaline soil, growth attributes, physiological growth, yield traits, nutrients*

Introduction

Maize (*Zea mays* L.) is one of the most abundantly cultivated cereal and fodder crop globally. It is an important cereal crop as it provides essential foodstuff to many people all around the world (Khan et al., 2012). The composition of maize grains comprises of starch, protein, fiber, oil, sugar and ash as described in the literature (Chaudhary, 1993). Limited availability of zinc (Zn) in alkaline calcareous soil is one of the major constraints that hamper maize crop productivity (Joy et al., 2017). Deficiency of Zn reduces the plant growth due to less auxin production (Brown et al., 1993). According to an estimation of WHO, about 31% of the world's population have deficiency of Zn (World Health Organization, 2005). As Zn is an essential micronutrient for animals and plants, it plays important roles in various enzymatic activities involved in protein synthesis, lipid metabolism, carbohydrate and nucleic acid synthesis (Hussain et al., 2011; Ali et al., 2013; Broadley et al., 2007).

Due to its structural, catalytic and co-catalytic function, Zn plays a significant role in development, reproduction and signaling (Broadley et al., 2007; Cavagnaro, 2008; Roohani et al., 2013) of plants. Zinc takes part in about 300 enzymatic functions, and it is important for plant functioning and growth (Christie et al., 2004; Hacisalihoglu and Kochian, 2000). The studies showed that alkaline calcareous soils of arid and semi-arid regions comprising of 30% of the soils in the world have deficiency of Zn (Kochian,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1903_21512169

2000) because of low mobility of Zn (Cakmak et al., 1999; Broadley et al., 2007; Alloway, 2009). In soils with limited Zn mobility, it becomes very important to understand the mechanisms of Zn acquisition by plants from the soil (Impa and Johnson-Beebout, 2012). Soil Zn with less than 1 mg/kg concentration in soil is considered as deficient, 1-60 mg/kg is considered as optimum, 60-120 mg/kg is toxic and above 120 mg/kg, it is highly toxic (Alloway, 2009). Requirement of Zn for optimum growth of plants varies among different plant species and crop variety. Maize optimum Zn requirement for its proper growth is 4.7 mg/kg, and for maximizing the maize yield, it should be more than 7 mg/kg (Liu et al., 2017). Contrarily microbial strains confer positive influence by enhancing root colonization which assist in essential nutrient uptake and mitigates any kind of soil nutrient or abiotic stresses (Shahzad et al., 2017). Arbuscular Mycorrhizal Fungi (AMF) develop a symbiotic association with most of the terrestrial plants and increase uptake of mineral nutrients (Wahid et al., 2016). The AMF are reported to increase Zn uptake in plants (Rue et al., 1975; Chen et al., 2003; Kafkas and Ortas, 2009; Ortas, 2012). Through this mechanism, AMF can help to cope up Zn deficiency in animals and humans (Cavagnaro, 2008).

Meanwhile, concentration of heavy metals in soils, including Zn is increasing at a faster rate from the past few decades (Zarcinas et al., 2004). At high level of Zn in soil, stress is induced in plants, which causes stunted root and shoot growth, death of leaf tips, curling of young leaves, leaf chlorosis, reduced photosynthetic rate, etc. (Rout and Das, 2003; Shi et al., 2015). Environmental Zn pollution is usually caused by anthropogenic activities like mining, electroplating, smelting and improper waste disposal (Bacon and Dinev, 2005; Bi et al., 2006). High or toxic amount of Zn availability in soil can have negative effect on plant growth, germination of seeds (Wang et al., 2009), development of roots (Lingua et al., 2008), loss of membrane structure (Stoyanova and Doncheva, 2002) ultimately, leading to the death of cells (Chang et al., 2005).

Under such conditions of high Zn levels, AMF can play a significant role in improving crop growth and development (He and Nara, 2007; Cavagnaro, 2008). Arbuscular mycorrhizal fungi are symbiotic fungi occurring worldwide, they belong to phylum Glomeromycota (Schubler et al., 2001) and form symbiotic association with the majority of terrestrial plants. The prominent function of AMF is its efficiency in absorbing less mobile nutrients, such as P and Zn (Bolan, 1991; Burkert and Robson, 1994; Marschner and Dell, 1994; Jansa et al., 2003). The role of AMF in absorbing P from the soil is well studied; however, their role for uptake of micronutrients is not well established under field relevant conditions. The study aimed to investigate the effects of arbuscular mycorrhizal fungi on the growth, yield, chlorophyll contents and total soluble protein of maize under toxic or deficit zinc concentrations in a field experiment.

Materials and methods

The research experiment of the proposed study was conducted in Multan, Punjab, Pakistan at latitude 29°55N and longitude 71°31E (site selected due to Zn deficiency). Experiment was carried out under field condition at optimized selected toxic and deficient levels of Zn with and without AMF inoculation. The study comprised of the following treatments ($Zn_{0.45}$ zinc deficiency 0.45 mg kg^{-1}, Zn_{60} medium toxicity 60 mg kg^{-1} and Zn_{120} zinc toxicity 120 mg kg^{-1}) with and without mycorrhizal inoculations. Field soil Zn levels were maintained by $ZnSO_4.7H_2O$ salt addition in the surface soil layer. The treatments were arranged according to randomized complete block design (RCBD) under factorial

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DOI: http://dx.doi.org/10.15666/aeer/1903_21512169

arrangements with three repeats. The net plot size was 6 m^2 with 65 cm row to row and 15.5 cm plant to plant distance. The hybrid maize cultivar (YH-1898) was sown on 15th July. Mix consortia of mycorrhizal inoculum having glomus species (inoculum purchased from Bustan urban Gardening Essential, Toronto, Canada having 158 propagule/gram) were used as seed priming. In AMF controlled pots (M-), Topsin M (Thiophanate Methyl 70% WP) was applied at 50 mg/kg soil, for rendering AMF root colonization. Standard agronomic practices of irrigation with tube well water and recommended dose of nitrogen (N) in three split doses, optimum level of half dose of (P) recommended and potassium (K) fertilizer were applied N:P:K@ 92:29:37 kg $acre^{-1}$, respectively. Other agronomic practices like weed and pest control were also applied. At the time of maturity, full plot was harvested, and root and leaf samples were collected randomly from whole plot and the growth, and physiological parameters were recorded.

Mycorrhizal colonization

Roots were harvested for AMF root colonization assessment by gridline intersect method (Giovannetti and Mosse, 1980), cleared in 10% KOH solution and tryphan blue stain was used for staining (Phillips and Hayman, 1970).

Physiological parameters

Chlorophyll a, b was determined by following the procedure of Arnon (1949). The intensity of green color extract of fresh plant leaves in acetone was measured by a spectrophotometer at 645 and 663 nm wavelength and chlorophyll a, b was calculated by the formula proposed by Arnon (1949).

$$\text{Chlorophyll '}a\text{' (mg g}^{-1}) = 100 \times [(0.0127 \times A663 - A645 \times 0.00269)] / 0.5$$

$$\text{Chlorophyll '}b\text{' (mg g}^{-1}) = 100 \times [(0.0229 \times A645 - 0.00468 \times A663)] / 0.5$$

Nutrient concentration

All of the fresh plant leaves and roots were removed and rinsed with water and oven dried at 72 °C for 24 h. Plant leaves and roots fresh weight and oven dried weight was recorded by an analytical/precision electrical balance. Plant Zn was determined by using standard procedure of atomic absorption spectrophotometer by Lindsay and Norvell (1978) method. Phosphorus in plants was analyzed by following the protocol of malachite green method (Ohno and Zibiliski, 1991). Soil Zn was determined by extractable DTPA-Zn as prescribed by Lindsay and Norvell (1978) and available P was extracted by sodium bicarbonate solution (Olsen and Sommers, 1982) method and further quantified by malachite green method (Ohno and Zibiliski, 1991). Soil N analysis was performed by Kjeldahl method (Bremner, 1960) and K was determined by extracting K from soil in ammonium acetate solution by flame photometer instrument (Shuman and Duncan, 1990).

Total soluble protein (mg g^{-1})

Total soluble protein was measured by using the procedure of Bradford (1976). The plant material (200 μL) was extracted from leaves. After this extracted material was added

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into 780 µL deionized water and 20 µL of coomassie blue dye, and absorbance of this prepared mixture was read at 595 nm in a spectrophotometer.

Statistical analysis

The data collected was analyzed statistically using analysis of variance (ANOVA) with arrangement of two factorial randomized complete block design (Steel et al., 1997). Mean values were compared for significance by conducting least significance difference test ($P \leq 0.05$). Principal component analysis (PCA) and correlation matrix was performed by using XLSTAT-2014.

Results

Data regarding mycorrhizal colonization was measured from the roots of both inoculated and un-inoculated maize plants. Significant ($P \leq 0.05$) proportion increase in mycorrhizal colonization was observed under all zinc deficient and toxic soil conditions (*Fig. 1*).

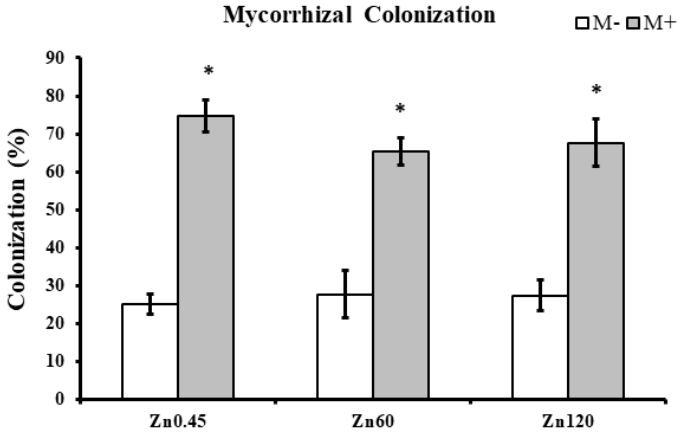

Figure 1. *Effect of arbuscular mycorrhizal fungi inoculation on mycorrhizal colonization by maize under zinc deficient and toxic soil conditions $Zn_{0.45}$ zinc deficiency (0.45 mg Kg^{-1}), Zn_{60} medium zinc toxicity (60 mg Kg^{-1}), Zn_{120} severe zinc toxicity (120 mg Kg^{-1}). Inoculated with AMF (M + grey), un-inoculated (M- white). Vertical bars represent standard error and asterisk (*) shows significant difference ($P \leq 0.05$) among treatments*

Soil nutrient status of the Zn deficient and toxic soil under investigation in the current study was noted. It was observed that the mycorrhizal inoculation enhanced the nutrient status of the soil irrespective of macro nutrients (N and P) as well as micronutrient (Zn) (*Fig. 2*). However, the behavior for Zn contents was different with the subjected Zn environment. Soil Zn content was reported to be increased with the inoculated maize plants under Zn deficiency (0.45 mg Kg^{-1}), however contrasting results were observed under both medium and severe toxic Zn growing medium. Maize inoculated plants showed ameliorative effect against Zn toxicity. Highly significant ($P \leq 0.05$) twofold decrease (11.80 mg Kg^{-1}) was noted with inoculation in soil Zn contents under severe Zn toxicity (*Fig. 2c*).

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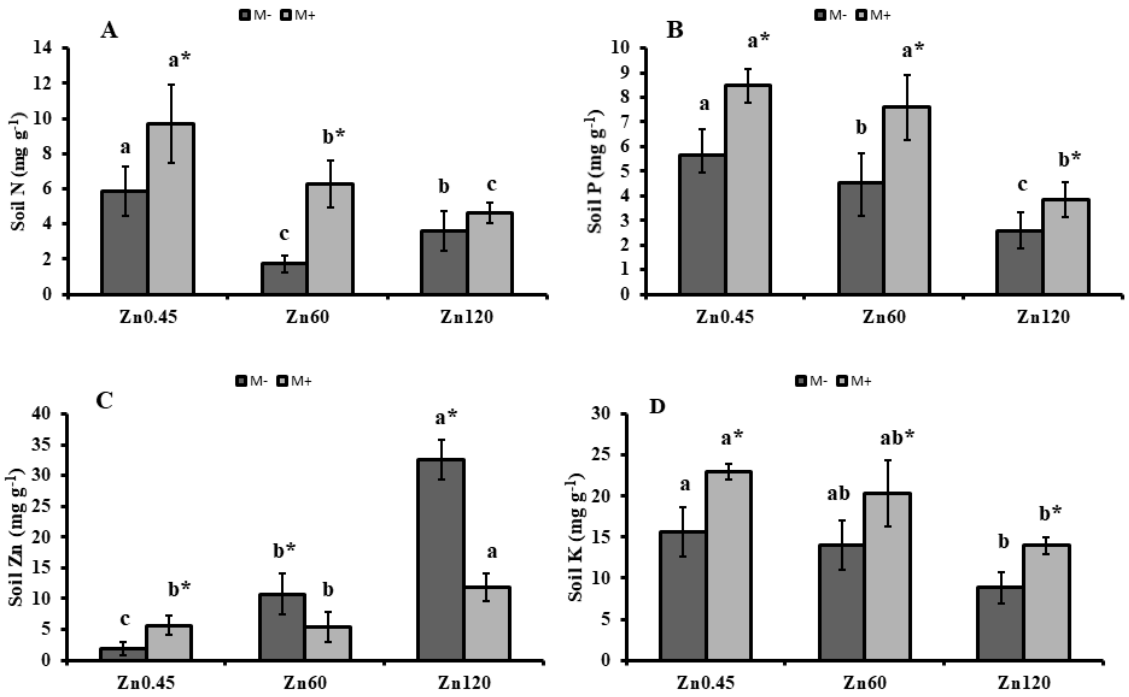

Figure 2. *Influence of arbuscular mycorrhizal inoculation maize plants on soil nutrient status under zinc deficient and toxic soil environments. (a) soil nitrogen contents (b) soil phosphorus contents (c) soil zinc contents (d) soil potassium contents. $Zn_{0.45}$ zinc deficiency (0.45 mg Kg^{-1}), Zn_{60} medium zinc toxicity (60 mg Kg^{-1}), Zn_{120} severe zinc toxicity (120 mg Kg^{-1}). among treatments. Inoculated with AMF (M + grey), un-inoculated (M- white). Vertical bars represent standard error and asterisk (*) shows significant difference ($P \leq 0.05$) among treatments. Alphabets sharing same letter shows non-significant ($P \geq 0.05$) difference whereas, different letter shows significant ($P \leq 0.05$) difference among different treatments*

Plant root is the only organ that is in direct contact with the soil environmental adversities and it is also the base of plant for nutrient uptake to ensure sturdy plant growth. Maize nutrient uptake and its accumulation in roots showed contrary results with inoculated and un-inoculated maize plants. Zinc contents in the root of inoculated maize plant were significantly ($P \leq 0.05$) higher while in the upper portion (shoots) it was lower. However un-inoculated maize roots showed lower Zn contents with a decrease of 96% (0.04 mg g^{-1}) and P content decrease was of 90% (1.90 mg g^{-1}) under severe Zn toxicity (120 mg Kg^{-1}), whereas it created significant nutrient toxic conditions with higher uptake of Zn in shoots of maize plants (*Table 1*).

Table 1. *Effect of arbuscular mycorrhizal fungi inoculation on nutrient accumulation in roots and its uptake by maize under zinc deficient and toxic soil conditions*

	Zn in root (mg g^{-1})		Zn in shoot (mg g^{-1})		P in root (mg g^{-1})		P in Shoot (mg g^{-1})	
	M-	M+	M-	M+	M-	M+	M-	M+
$Zn_{0.45}$	0.04±0.00b	0.10±0.00a	5.21±0.01a	3.18±0.02c	1.90±0.01e	3.32±0.01b	4.14±0.01a	1.51±0.01e
Zn_{60}	0.04±0.01b	0.09±0.01a	4.59±0.01b	2.94±0.03d	2.37±0.02c	5.47±0.01a	3.27±0.01b	1.77±0.01d
Zn_{120}	0.02±0.00c	0.04±0.00b	1.40±0.03f	2.78±0.03e	0.12±0.01f	1.97±0.02d	0.32±0.01f	2.13±0.00c

Mean values ± standard error. Lettering represents significance; different letters shows significant difference ($P \leq 0.05$). $Zn_{0.45}$ zinc deficiency (0.45 mg Kg^{-1}), Zn_{60} medium zinc toxicity (60 mg Kg^{-1}), Zn_{120} severe zinc toxicity (120 mg Kg^{-1})

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Maize plants that were subjected to Zn deficient (0.45 mg kg^{-1}) and severe toxic (120 mg kg^{-1}) soil conditions exhibited lower chlorophyll a and b contents. Maize showed the same trend in decrease of chlorophyll contents in the case of both mycorrhizal inoculation and un-inoculation. Significant ($P \leq 0.05$) difference was noted in chl a and b contents of maize with AMF inoculated plants under both Zn deficient and toxic soil regimes (*Table 1*). Maize plants inoculated with AMF showed higher total soluble protein.

Significant differences were noted with all the inoculated plants under varying Zn levels. The $Zn_{0.45}$ with mycorrhizal inoculation depicted the highest TSP with an increase of 46% (26.49 mg g^{-1}) as compared with the same medium toxicity of Zn but having non-mycorrhizal inoculation (*Table 2*).

Table 2. *Effect of arbuscular mycorrhizal fungi inoculation on chlorophyll and total soluble protein of maize under zinc deficient and toxic soil conditions*

	Chl a (mg g^{-1})		Chl b (mg g^{-1})		TSP (mg g^{-1})	
	M-	M+	M-	M+	M-	M+
$Zn_{0.45}$	1.32±0.29c	1.71±0.49a	0.26±0.03e	0.82±0.01a	18.16±1.80b	26.49±1.67a
Zn_{60}	1.19±0.80d	1.62±0.26a	0.38±0.01d	0.59±0.02b	13.42±2.54c	18.95±2.48b
Zn_{120}	1.29±0.20cd	1.47±0.36b	0.36±0.02d	0.51±0.06c	11.36±1.01c	15.93±0.98b

Mean values ± standard error. Lettering represents significance; different letters shows significant difference ($P \leq 0.05$). $Zn_{0.45}$ zinc deficiency (0.45 mg Kg^{-1}), Zn_{60} medium zinc toxicity (60 mg Kg^{-1}), Zn_{120} severe zinc toxicity (120 mg Kg^{-1}). Chl a and b (chlorophyll a and b contents of maize), whereas TSP represents total soluble protein

Results showed that mycorrhizal inoculation improved maize growth attributes (plant height, stem girth, no of leaves and plant biomass) under zinc deficient and zinc toxic conditions as compared to maize plants grown in un-inoculated treatments. Significant ($P \leq 0.05$) increase of 16% was observed in plant height with fungal inoculation. The stem girth improved with an increase of 8% (8.45 cm) under zinc toxicity. The plants no of leaves were also decreased with zinc toxicity, on the other hand mycorrhizal inoculation resulted in higher number of leaves (*Fig. 3*).

Results regarding yield attributes of maize are presented in *Figure 4*. This data showed that mycorrhizal inoculation was effective in improving the yield attributes (cob length and weight, 1000 grain weight, harvest index, biological yield and grain yield) of maize in zinc toxic and deficient conditions. Increase in cob weight, 1000 grain weight, harvest index, biological yield and grain yield of maize was observed in zinc problematic conditions under inoculation compared to treatment without inoculation. The increase in cob length was 30% (17.03 cm) under slight zinc toxic soil conditions. The increased cob length resultantly conferred higher grain yield with an increase of 17% under $Zn_{0.45}$ zinc toxicity without inoculation to 6.06 Kg ha^{-1} and 8% under severe toxicity 3.93 Kg ha^{-1} to 4.26 Kg ha^{-1} with mycorrhizal inoculation.

Principal component analysis

The interrelationship among the variables under Zn deficient and toxic soil were evaluated by biplot principal component analysis (PCA) as shown in *Figure 5*. It showed that the first two components explained 90.42% variance (contributed by PC1 75.78%, and PC2 14.64%) under Zn deficient and toxic soil SS conditions. PCA biplot showed the grouping of the mycorrhizal and non-mycorrhizal treatments on their response to the

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tested morphological and physiological traits. The mycorrhizal inoculation was highly responsive to influence the tested variables. The Zn1M (mycorrhizal inoculation under Zn deficiency) and Zn2M (mycorrhizal inoculation under medium Zn toxicity), showed higher response for the variables, i.e., cob weight, grain yield, harvest index, plant height, number of leaves, stem girth, cob length, total soluble protein, potassium in soil, P accumulation in roots, Zn in root, nitrogen in soil. The Zn3M (mycorrhizal inoculation under high zinc toxicity) showed average response. The Zn1NM (non-mycorrhizal under zinc deficiency) showed higher accumulation of P in shoot. The Zn2NM and Zn3NM were non-responsive to influence the traits of maize.

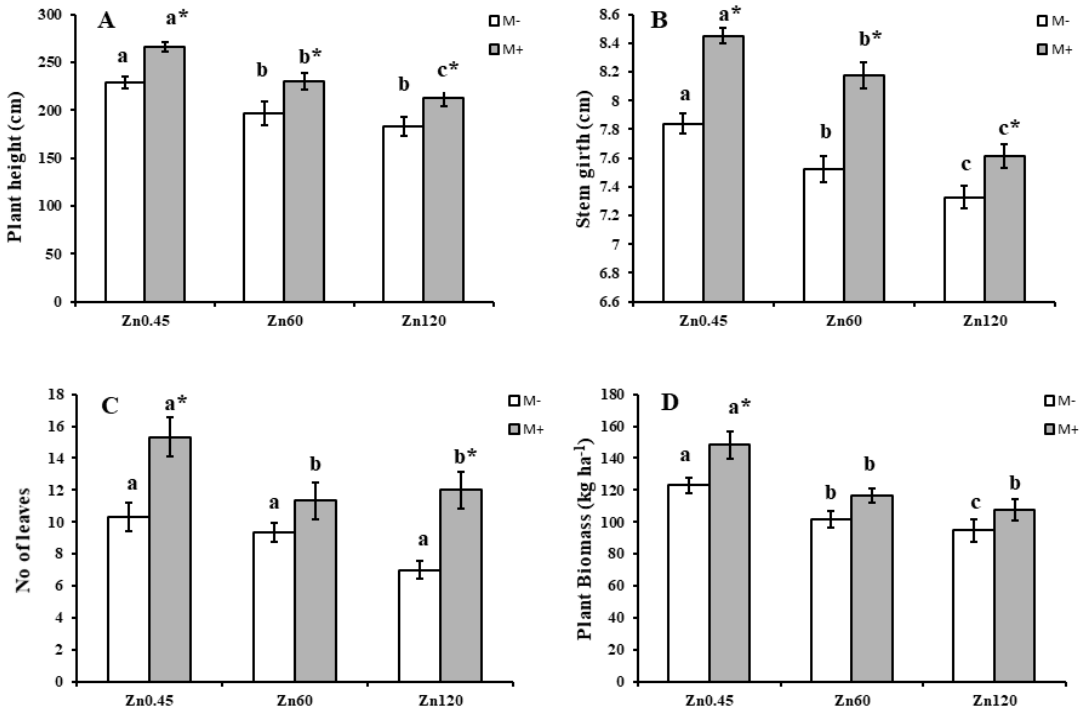

Figure 3. *Influence of arbuscular mycorrhizal inoculation on maize growth characteristics under zinc deficient and toxic soil environments. (a) plant height (cm) (b) stem girth (c) no of leaves (d) plant biomass. Zn0.45 zinc deficiency (0.45 mg Kg^{-1}), Zn60 medium zinc toxicity (60 mg Kg^{-1}), Zn120 severe zinc toxicity (120 mg Kg^{-1}). Inoculated with AMF (M + grey), un-inoculated (M- white). Vertical bars represent standard error and asterisk (*) shows significant difference ($P \leq 0.05$) among treatments. Alphabets sharing same letter shows non-significant ($P \geq 0.05$) difference whereas, different letters show significant ($P \leq 0.05$) difference among different treatments*

Correlation matrix

Results of Pearson correlation coefficient (r) among the morphological and physiological traits of maize under Zn deficient and toxic soil conditions were summarized in *Table 1*. Plant height had a strong positive correlation soil N, soil P, soil K and Zn in root, and negative correlation with Zn in soil. Similarly, grain and yield had positive correlation with soil N, soil P and total soluble protein, whereas both exhibited negative correlation with soil Zn. The soil P had a positive correlation with soil N and K, chlorophyll a, and total soluble protein, and a negative correlation with soil Zn, while strongly correlated with Zn in root (*Table 3*).

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Table 3. *Pearson's correlation between different traits under Zn deficient and toxic soil conditions in maize (n = 6)*

	PH	SG	PB	NL	CL	CW	GW	HI	BY	GY	SN	SP	SZ	SK	ZR	ZS	PR	PS	CHLA	CHLB
PH																				
SG	0.962**																			
PB	0.981**	0.923**																		
NL	0.926**	0.861*	0.885**																	
CL	0.947**	0.922**	0.904**	0.964**																
CW	0.906**	0.921**	0.843*	0.869*	0.836*															
GW	0.973**	0.877*	0.963**	0.957**	0.933**	0.859*														
HI	0.948**	0.840*	0.976**	0.850*	0.857*	0.762*	0.963**													
BY	0.984**	0.907**	0.992**	0.912**	0.925**	0.833*	0.984**	0.986**												
GY	0.925**	0.885**	0.963**	0.801*	0.791*	0.854*	0.891**	0.914**	0.927**											
SN	0.932**	0.901**	0.929**	0.823*	0.923**	0.713	0.890**	0.916**	0.940**	0.811*										
SP	0.916**	0.981**	0.877*	0.796*	0.842*	0.943**	0.812*	0.771*	0.842*	0.887**	0.808*									
SZ	-0.734*	-0.697	-0.668	-0.675	-0.577	-0.894**	-0.725	-0.649	-0.672	-0.718	-0.474	-0.749*								
SK	0.937**	0.981**	0.884**	0.879*	0.900**	0.971**	0.862*	0.784*	0.866*	0.872*	0.812*	0.985**	-.0770*							
ZR	0.866*	0.960**	0.805*	0.818*	0.885**	0.888**	0.760*	0.673	0.779*	0.775*	0.796*	0.957**	-0.601	0.967**						
ZS	0.254	0.17	0.274	0.16	-0.012	0.45	0.287	0.3	0.247	0.447	-0.043	0.284	-0.751*	0.262	0.038					
PR	0.61	0.763*	0.482	0.57	0.607	0.825*	0.477	0.338	0.461	0.495	0.456	0.824*	-0.686	0.829*	0.852*	0.19				
PS	0.128	0.026	0.125	0.065	-0.118	0.335	0.186	0.185	0.123	0.269	-0.158	0.127	-0.704	0.121	-0.113	0.970**	0.114			
CHLA	0.828*	0.871*	0.752*	0.831*	0.938**	0.716	0.766*	0.685	0.777*	0.6	0.882*	0.782*	-0.403	0.827*	0.886**	-0.273	0.667	-0.354		
CHLB	0.735*	0.780*	0.695	0.834*	0.889**	0.658	0.702	0.584	0.696	0.596	0.742*	0.71	-0.271	0.777*	0.861*	-0.306	0.572	-0.437	0.896**	
TSP	0.993**	0.960**	0.987**	0.929**	0.957**	0.880*	0.965**	0.943**	0.984**	0.932**	0.940**	0.910**	-0.668	0.931**	0.877*	0.191	0.58	0.049	0.839*	0.786*

** significant at $p \leq 0.01$; * significant at $p \leq 0.05$; PH: Plant height, SG: Stem girth, PB: Plant biomass, NL: No of leaves, CL: Cob length, CW: cob weight, GW: 1000 grain weight, HI: harvest index, BY: biological yield, GY: grain yield, ZR: Zn in root, ZS: Zn in shoot, PR: Phosphorus in root, PS: phosphorus in shoot, PS: phosphorus in soil, ZS: zinc in soil, SN: nitrogen in soil, SZ: zinc in soil, ZS: zinc in shoot, ZR: zinc in root, PR: phosphorus in root, SK: potassium in soil, TSP, total soluble protein, CHLA: chlorophyll a, CHLB: chlorophyll b

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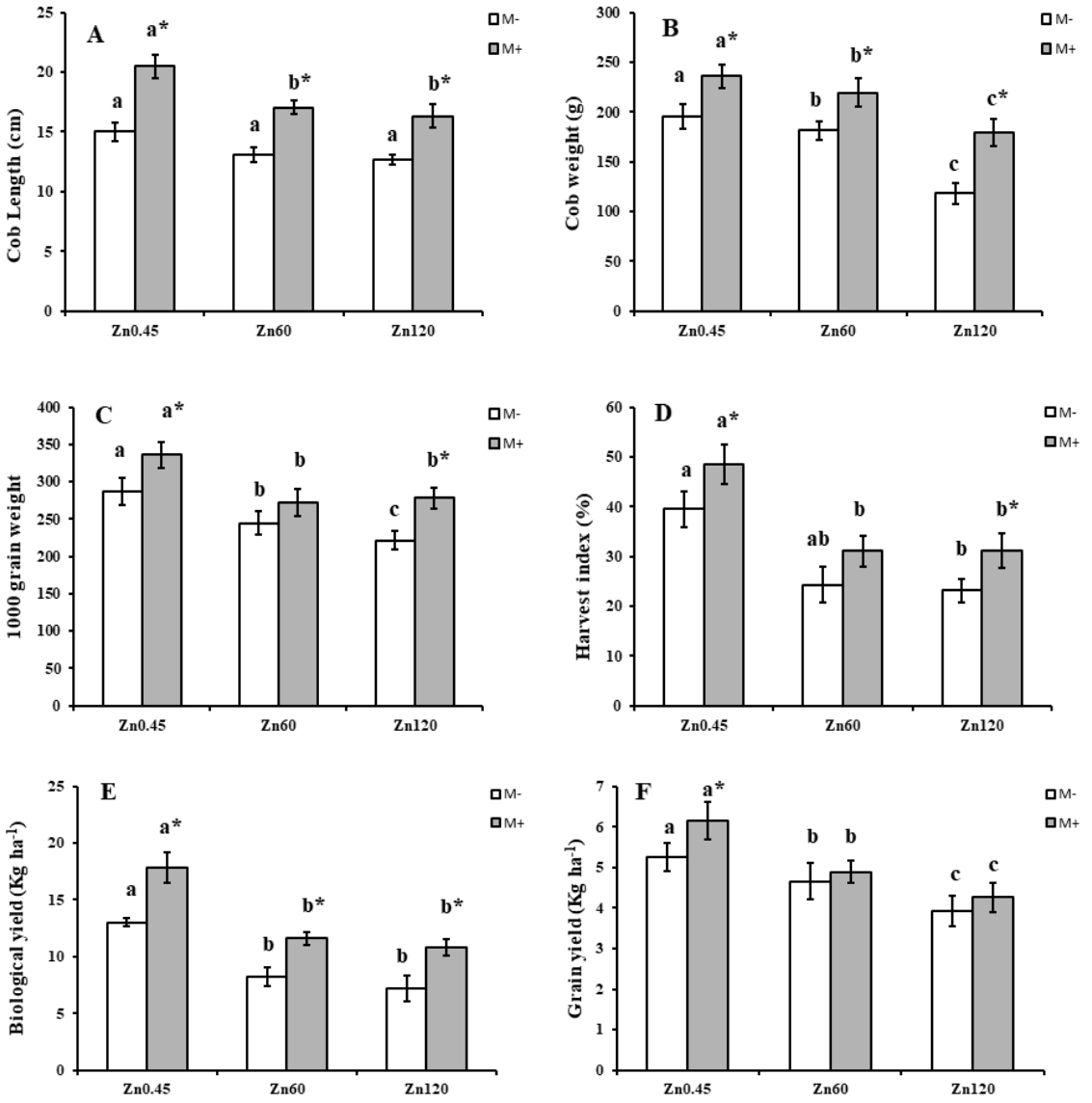

Figure 4. *Influence of arbuscular mycorrhizal inoculation maize yield attributes under zinc deficient and toxic soil conditions. (a) cob length (cm) (b) cob weight (c) 1000 grain weight (d) harvest index (e) biological yield (f) grain yield. $Zn_{0.45}$ zinc deficiency (0.45 mg Kg^{-1}), Zn_{60} medium zinc toxicity (60 mg Kg^{-1}), Zn_{120} severe zinc toxicity (120 mg Kg^{-1}). Inoculated with AMF (M + grey), un-inoculated (M- white). Vertical bars represent standard error and asterisk (*) shows significant difference ($P \leq 0.05$) among treatments. Alphabets sharing same letter shows non-significant ($P \geq 0.05$) difference whereas, different letters show significant ($P \leq 0.05$) difference among different treatments*

Discussion

Zinc deficiency is a common problem for many cereal crops. The deficiency of Zn in crops also provide deficient food among their consumers over the extended period. In this way, much of the world's human population cannot fulfil their daily Zn requirement. This can lead to serious issues regarding human health (Brown and Wuehler, 2000). To avoid nutritional deficiencies, it is important to understand, how plants uptake and utilize Zn from the soil. While the concern for increasing the

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DOI: http://dx.doi.org/10.15666/aeer/1903_21512169

concentration of Zn in staple crops is widely recognized (Brown and Wuehler, 2000; Burns et al., 2010). Microbial inoculants have received attention to fortify the micronutrients. If this is to change, we must develop a sound understanding how plants and AMF acquire Zn from soil. Current study evaluated the effect of mycorrhizal inoculation for plants under deficient and toxic field conditions under optimum P fertilizer application rates. In this study, mycorrhizal inoculation improved the zinc uptake in zinc deficient condition, while reduced the zinc uptake in zinc toxic concentration. That might be due to myccorhizae prevented zinc deficiency by promoting the plant zinc and phosphorons acquisition and under toxic soil Zn conditions immoblize Zn, AMF reduced the soil Zn availability as reported in present study (Chen et al., 2003; Kafkas and Ortas, 2009; Ortas, 2012). Protecting the plants against excessive zinc concentration could also be attributed to mycorrhizal application as mycorrhizae colonize the plant roots lower its uptake and tissue zinc concentration (Chen et al., 2003; Christie et al., 2004). Improvement in phosphorous uptake in stress conditions by mycorrhizal inoculation might be due to that mycorrhizal inoculation solubilized the unavailable phosphorous in stress conditions and promoted the uptake of P in plants (Javot et al., 2007). Improved P nutrition of mycorrhizal maize plants is due to the hyphal P uptake beyond the P depletion zone resulting in absorption of P from the soil solution, which otherwise cannot be replenished, as its mobility is poor in soil (Karandashov and Bucher, 2005). The results of this study are consistent with the earlier report of improved P uptake in mycorrhizal maize plants (Battini et al., 2017). Our results regarding phosphorus solubilization were further strengthened by the report of Wahid et al. (2016) in maize plants. Results reported in this study showed that mycorrhizal inoculation improved the Chlorophyll (a and b) contents under zinc stress conditions. This might be due to mycorrhizae increased the uptake of essential nutrients especially N. Increase in N content in plants promote the chlorophyll content that ultimately assist in improved photosynthetic rate. Results regarding improved chlorophyll contents in response to stress conditions are in agreement with earlier study reported by Sheng et al. (2008). It was also noticed that mycorrhizal inoculation reversed the toxic effect of zinc deficiency and toxicity on maize growth and yield, and improved maize growth such as higher plant height, stem girth, plant biomass, number of leaves and yield attributes which includes cob weight, cob length, 1000 grain weight, harvest index, biological yield and grain yield under zinc toxic and zinc deficient conditions. Improvement in maize growth and yield characteristics under zinc deficient conditions by mycorrhizal inoculation is due to the beneficial role of mycorrhizal inoculation which solubilized unavailable zinc, phosphorous and other essential nutrients and promoted their uptake in maize plants resulting in improved maize growth and yield in zinc deficient conditions (Smith and Read, 2010; Nadeem et al., 2014). The study conducted by Amanullah et al. (2011) also corroborated the results of this study with higher yield of maize with mycorrhizal inoculation in maize plants. While improvement in maize growth and yield attributes under zinc toxic conditions by fungal application might also be due to that inoculation reduced the uptake, accumulation, and translocation of zinc in plant tissues (Smith and Read, 2010). Moreover, it is also due to that mycorrhizal application protects the plants against excessive zinc concentration as mycorrhizae colonize the plant roots, lower uptake and tissue zinc concentration (Chen et al., 2003; Christie et al., 2004).

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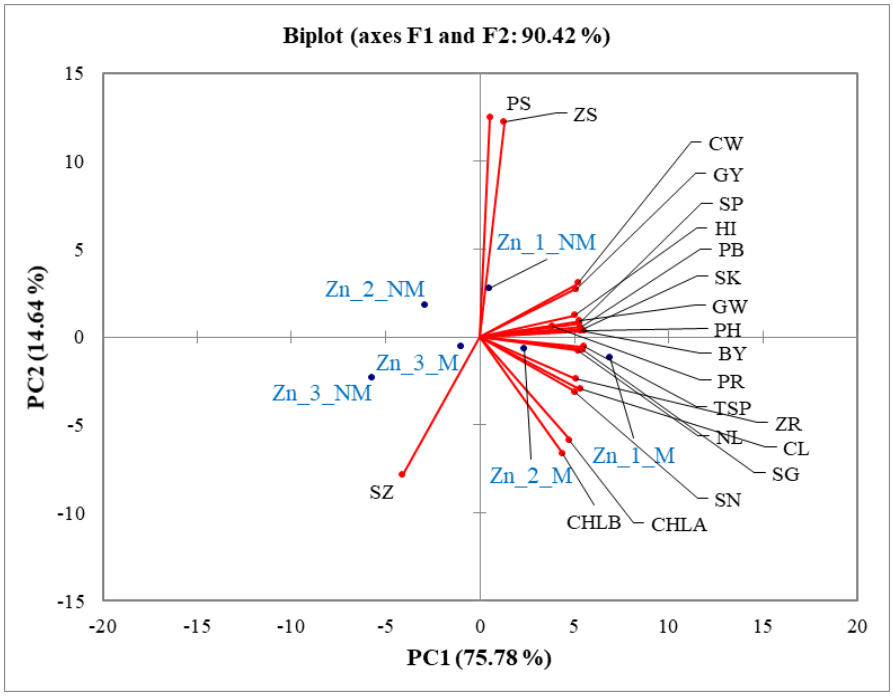

Figure 5. *PCA biplot for morpho-physiological variables of maize grown under zinc deficient and zinc toxic soil conditions with mycorrhizal (M) and non-mycorrhizal (NM) inoculations. PCA biplot is a combination of score plot of zinc treatments with M and NM (represented in blue text) and loading plot of variables (represented by red vectors; black text). Zn1: zinc deficiency (0.45 mg Kg^{-1}), Zn2: Zn60 medium zinc toxicity (60 mg Kg^{-1}), Zn3: Zn120 severe zinc toxicity (120 mg Kg^{-1}), PH: Plant height, SG: Stem girth, PB: Plant biomass, NL: No of leaves, CL: Cob length, CW: cob weight, GW: 1000 grain weight, HI: harvest index, BY: biological yield, GY: grain yield, ZR: Zn in root, ZS: Zn in shoot, PR: Phosphorus in root, PS: phosphorus in shoot, SP: phosphorus in soil, ZS: zinc in soil, SN: nitrogen in soil, SZ: zinc in soil, ZS: zinc in shoot, ZR: zinc in root, PR: phosphorus in root, SK: potassium in soil, TSP, total soluble protein, CHLA: chlorophyll a, CHLB: chlorophyll b*

Conclusion

It was concluded from the study that mycorrhizal inoculation reversed the stress effect of Zn deficiency and promoted maize growth, nutrient uptake, and yield. Inoculation of AMF imparted dual beneficial effect on maize as it increased Zn uptake in Zn deficient conditions while reduced the uptake of Zn in toxic conditions. Moreover, it can be suggested by observing the beneficial role of AMF, that it can be used to mitigate Zn deficiency and toxicity for healthy growth of maize. Further experiments should be conducted for determining the molecular mechanism behind this. Moreover, experiments should be performed on different crops, soils with varying texture, soil pH, nutrient status and moisture contents, as all of these factors strongly influenced Zn availability and translocation.

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APPENDIX

Analysis of variance table for biological yield

Source	DF	SS	MS	F	P
Myco	1	69.896	69.8956		
Zinc	2	144.904	72.4522	65.26	0.0000
Error	14	15.544	1.1103		
Total	17	230.344			

Grand mean 11.448 CV 9.20

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Analysis of variance table for cob length

Source	DF	SS	MS	F	P
Myco	1	85.238	85.2383		
Zinc	2	36.787	18.3933	9.95	0.0020
Error	14	25.877	1.8484		
Total	17	147.902			

Grand mean 15.768 CV 8.62

Analysis of variance table for cob weight

Source	DF	SS	MS	F	P
Myco	1	6063.6	6063.6		
Zinc	2	20506.3	10253.1	69.54	0.0000
Error	14	2064.3	147.5		
Total	17	28634.1			

Grand mean 193.34 CV 6.28

Analysis of variance table for chlorophyll a

Source	DF	SS	MS	F	P
Myco	1	0.49336	0.49336		
Zinc	2	0.05710	0.02855	4.48	0.0313
Error	14	0.08914	0.00637		
Total	17	0.63960			

Grand mean 1.4333 CV 5.57

Analysis of variance table for chlorophyll b

Source	DF	SS	MS	F	P
Myco	1	0.45442	0.45442		
Zinc	2	0.03484	0.01742	1.54	0.2478
Error	14	0.15798	0.01128		
Total	17	0.64724			

Grand mean 0.4944 CV 21.48

Analysis of variance table for grain weight

Source	DF	SS	MS	F	P
Myco	1	9038.4	9038.40		
Zinc	2	13254.1	6627.07	72.21	0.0000
Error	14	1284.8	91.77		
Total	17	23577.3			

Grand mean 273.25 CV 3.51

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Analysis of variance table for grain yield

Source	DF	SS	MS	F	P
Myco	1	0.92934	0.92934		
Zinc	2	7.40468	3.70234	153.47	0.0000
Error	14	0.33774	0.02412		
Total	17	8.67176			

Grand mean 4.8428 CV 3.21

Analysis of variance table for harvest index

Source	DF	SS	MS	F	P
Myco	1	216.94	216.944		
Zinc	2	911.59	455.796	15.52	0.0003
Error	14	411.13	29.366		
Total	17	1539.67			

Grand mean 32.402 CV 16.72

Analysis of variance table for number of leaves

Source	DF	SS	MS	F	P
Myco	1	72.000	72.0000		
Zinc	2	36.111	18.0556	6.71	0.0090
Error	14	37.667	2.6905		
Total	17	145.778			

Grand mean 10.889 CV 15.06

Analysis of variance table for plant biomass

Source	DF	SS	MS	F	P
Myco	1	562.24	562.242		
Zinc	2	1883.36	941.682	65.37	0.0000
Error	14	201.68	14.406		
Total	17	2647.28			

Grand mean 112.00 CV 3.39

Analysis of variance table for plant height

Source	DF	SS	MS	F	P
Myco	1	3618.5	3618.45		
Zinc	2	10581.7	5290.87	118.57	0.0000
Error	14	624.7	44.62		
Total	17	14824.9			

Grand mean 222.26 CV 3.01

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Analysis of variance table for phosphorus in roots

Source	DF	SS	MS	F	P
Myco	1	20.2248	20.2248		
Zinc	2	24.8323	12.4162	76.17	0.0000
Error	14	2.2819	0.1630		
Total	17	47.3390			

Grand mean 2.5256 CV 15.99

Analysis of variance table for phosphorus in shoot

Source	DF	SS	MS	F	P
Myco	1	2.6912	2.69120		
Zinc	2	8.6987	4.34937	3.80	0.0479
Error	14	16.0036	1.14312		
Total	17	27.3936			

Grand mean 2.1889 CV 48.85

Analysis of variance table for mycorrhizal root colonisation

Source	DF	SS	MS	F	P
Myco	1	8149.39	8149.39		
Zinc	2	35.11	17.56	1.20	0.3297
Error	14	204.44	14.60		
Total	17	8388.94			

Grand mean 47.944 CV 7.97

Analysis of variance table for shoot girth

Source	DF	SS	MS	F	P
Myco	1	1.20125	1.20125		
Zinc	2	1.37301	0.68651	74.60	0.0000
Error	14	0.12883	0.00920		
Total	17	2.70309			

Grand mean 7.8206 CV 1.23

Analysis of variance table for soil potassium

Source	DF	SS	MS	F	P
Myco	1	177.033	177.033		
Zinc	2	200.406	100.203	16.71	0.0002
Error	14	83.952	5.997		
Total	17	461.391			

Grand mean 15.975 CV 15.33

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Analysis of variance table for soil nitrogen

Source	DF	SS	MS	F	P
Myco	1	44.305	44.3054		
Zinc	2	55.922	27.9612	12.51	0.0008
Error	14	31.297	2.2355		
Total	17	131.525			

Grand mean 5.3033 CV 28.19

Analysis of variance table for soil phosphorus

Source	DF	SS	MS	F	P
Myco	1	25.2998	25.2998		
Zinc	2	47.6024	23.8012	22.95	0.0000
Error	14	14.5174	1.0370		
Total	17	87.4196			

Grand mean 5.4533 CV 18.67

Analysis of variance table for soil zinc

Source	DF	SS	MS	F	P
Myco	1	251.78	251.777		
Zinc	2	1115.27	557.636	16.60	0.0002
Error	14	470.35	33.596		
Total	17	1837.39			

Grand mean 11.357 CV 51.04

Analysis of variance table for total soluble protein

Source	DF	SS	MS	F	P
Myco	1	209.169	209.169		
Zinc	2	198.507	99.253	51.94	0.0000
Error	14	26.754	1.911		
Total	17	434.430			

Grand mean 17.714 CV 7.80

Analysis of variance table for zinc in root

Source	DF	SS	MS	F	P
Myco	1	0.00720	0.00720		
Zinc	2	0.00668	0.00334	18.21	0.0001
Error	14	0.00257	0.00018		
Total	17	0.01644			

Grand mean 0.0556 CV 24.37

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Analysis of variance table for zinc shoot

Source	DF	SS	MS	F	P
Myco	1	2.6758	2.67576		
Zinc	2	14.8597	7.42987	9.92	0.0021
Error	14	10.4899	0.74928		
Total	17	28.0254			

Grand mean 3.3511 CV 25.83

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