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Biochemical Changes of Mycorrhiza Inoculated and Uninoculated Soils under Differential Zn and P Fertilization

Chandrasekaran Bharathi[1], Natarajan Balakrishnan[2*] and Kizhaeral S. Subramanian[2]

[1]Department of Soil Science and Agricultural Chemistry, [2]Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

**Corresponding author*

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A B S T R A C T

Greenhouse experiment was conducted to assess the soil biochemical change patterns in soils of arbuscular mycorrhizal fungus (AMF)-inoculated and uninoculated maize plants fertilized with varying levels of P and Zn. Soil samples were collected for mycorrhizal spores, microbial communities, available micronutrients and phosphorus (P) contents besides organic and biomass carbon (BMC), soil enzymes and glomalin. Major portion of Fe and Zn fractionations was found to occur in the residual form. AM symbiosis significantly modulated the microbial communities in the soil regardless of low or high P concentration. The results showed that mycorrhizae had pronounced influence on increasing bacterial population, while less effect was found in the case of fungi and actinomycetes activity in the soil. The positive interaction between P and Zn in mycorrhizae treated soil resulted in enhanced growth especially root and nutrient uptake. Soil enzymes, viz. dehydrogenase and acid phosphatase activities in M+ soils, were significantly higher than M− soil consistently. Overall, the data suggest that mycorrhizal symbiosis enhanced the availability of P and Zn as a result of preferential nutrient uptake and biochemical changes that may alleviate micronutrient deficiencies in soil.

Introduction

Indian agricultural soils are 60 % zinc deficient causing reduction in crop productivity to the tune of 30-40% (Singh *et al.*, 2005). Zinc use efficiency by crops is hardly exceeds 1% as the major portion gets fixed in the soil. In addition, soils of arid and semiarid regions of India are very low in organic status as a result of faster decomposition of organic matter that aggravates deficiency of Zn in soils and mobility of phosphorus in the soil is very low because of its strong adsorption towards clay mineral Fe and Al oxides. Arbuscular mycorrhizal fungi (AMF) are obligate endosymbionts, colonize with more than 80 % of terrestrial plant species (Allen, 1991) and live on carbohydrates obtained from root cells. They are key components of the soil biota and account for about 5-50% of agricultural soils microbial biomass (Olsson *et al.*, 1999) which facilitates in sustaining the fertility status through favorable biochemical changes. Soil

microbial biomass, the living part of soil organic matter characterizes the microbiological status and quality of the soil. AMF hyphae as they are the main components of soil biomass (Hamel *et al.,* 1991) distribute C compounds and energy in soil. Since AMF are closely associated with plant roots, most of the biomass retained within top 0-20 cm of the soil. AMF fungal inoculation increases soil biomass carbon content (Alguacil, 2005) with time (Kim *et al.,* 1998) as a result of increased biomass production. The C allocated to AM and thus their contribution to soil C is of particular importance in tropics because of the low nutrient levels in highly weathered tropical soils).

The increased biomass carbon due to AM symbiosis, promotes soil microbial population and their activities (Tarafdar and Marschner, 1994) by altering root exudation of carbohydrates (Wamberg *et al.,* 2003) and are expected to influence rhizosphere population as well (Hayman, 1983). In turn, biologically active substances such as amino acids and hormones produced by soil microorganism stimulate the growth of AMF. The carbonaceous product produced by AM fungal hyphae in soil is glomalin, a recalcitrant glycoprotein containing 30-40% C. It may comprise as much as 2% of soil by weight which makes a large contribution to active soil organic C pools (Rillig *et al.,* 2003). Concentration of glomalin ranges from 2-15 mg g^{-1} of soil in temperate climate and 3.94 mg cm^3 in tropical rain forest accounting for approximately 3.2% of total soil C in the 0-10 cm soil layer (Lovelock *et al.,* 2004). Pools of organic carbon such as glomalin produced by AMF may even exceed soil microbial biomass by a factor of 10-20 (Rillig *et al.,* 2001). Mycorrhizal symbiosis enhances soil enzymatic activities viz., acid phosphatase and dehydrogenase, which favours the availability of P and Zn. Acid phospahtase aids in increased uptake of P from the soil (Leadir *et al.,* 1998) by the mechanisms such as hydrolysis of soil organic P (Tarafdar and Claassen, 1988) after the hydrolysis of C-O-P bond by phosphatase enzyme (Tarafdar, 2008) and more utilization of P in primary metabolism. Moreover, the phosphtase activity was higher in mycorrhizal treated plants particularly with the supply of organic P (Tarafdar and Marchner, 1994). Dehydrogenase enzyme activity serves as a marker of microbiological redox system by measuring microbial oxidative activities in soil (Garcia *et al.,* 1997) and its activity was more in rhizosphere than non rhizosphere soil. It is evident that Zn is important for the activation of several enzymes. However, dehydrogenase activity was decreased by 95% due to Zn addition in metal contaminated soil (Kelly and Tate 1998). Recently Subramanian *et al.,* (2008) reported that mycorrhizal symbiosis improved both the availability of P and Zn as a consequence of synergistic interaction between these two nutrients.

We hypothesized that mycorrhizal symbiosis orchestrates biochemical changes such as biomass carbon, glomalin concentration and soil enzyme activities that collectively contribute for the improved availability of Zn in deficient soils. Further the response to mycorrhizal inoculation may vary with the degree of P fertilization. The synergistic interaction between Zn and P may also assist in increased availability of Zn in soils.

Materials and Methods

Experimental soil characteristics

A greenhouse experiment was conducted on a red sandy loam soil belonging to Alfisol (Typic Haplustalf). The experimental soil was neutral in pH (7.25), free from salinity (EC 0.14 dSm^{-1}) and extremely low in organic carbon status (0.22%). Regarding macronutrients, soil had low available N (102

mg kg^{-1}) and P (2.60 mg kg^{-1}) and high in available K (199 mg kg^{-1}). The DTPA extractable (available) Zn was 0.6 ppm, which is considered as severely Zn deficient soil. The experimental soil had indigenous viable AMF spore population (< 10 spores $100g^{-1}$ soil) and the soil was sterilized at121^{o}C, and pressure 15 lbs for 20 minutes three times in order to eliminate the interference of native mycorrhizal fungi.

Greenhouse experiment

The greenhouse experiment was maintained at 24-28^{o}C, light intensity (800 -1000 µmols provided by natural light), relative humidity (60-65%) and 12-h photoperiod. The treatments consisted of two levels of P (15 and 30 mg kg^{-1}) and three levels of Zn (0, 1.25 and 2.5 mg kg^{-1}) in the presence or absence of AM inoculation. There were 12 treatments each was replicated seven times in a factorial randomized block design (FRBD). Three replications were kept for sampling at 55 days after sowing (DAS) and the remaining four replications at 75 DAS. In a 10 kg capacity pot, 10 kg soil was filled and over laid with AM inoculum carrying *Rhizoglomus intraradices* @10 g pot^{-1} as a thin layer. AM was inoculated 5 cm below the seeds prior to sowing (applied uniformly as a thin layer). Vermiculite based Am fungal inoculum (*Glomus intraradices* TNAU-03-06) used in this study was provided by the Department of Microbiology of this university. This strain was cultured in maize plants and propagules comprised of infected root bits and spores were blended in sterile vermiculite. For nonmycorrhizal treatments inoculum without mycorrhizal spres was applied. Pre-germinated maize hybrid seeds (COHM-5) were sown on the thin layer of AM inoculum overlaid on 1 kg of soil. Germination percentage was nearly 95% on the seventh day of sowing and the seedlings were thinned leaving one plant per pot throughout the experiment. Half the dose of N and full dose of K were applied in the form of urea and muriate of potash, respectively, as basal at the time of sowing. Full basal dose of P was applied as per treatment in the form of single superphosphate. In addition to the macronutrients, three levels of Zn as $ZnSO_4$ was applied as per treatment. Soil samples collected at 55 and 75 DAS were used for the analysis of enzyme activities, organic carbon, biomass carbon, glomalin, Olsen's P, DTPA extractable Zn and microbial population.

Soil assay

Enumeration of mycorrhizal spores in soil

The indigenous mycorrhizal population in an experimental soil was determined using wet sieving and decantation technique. 100 g of soil sample was stirred for 1 hour with 1 litre water and the supernatant solution was passed through 45, 106 and 180 µm sieves staked one over the other. The washings collected in each sieve was transferred into grid line petriplates and observed under stereo zoom microscope for viable spores at 10X (Gardmann and Nicolson, 1963).

Enumeration of microbial communities in rhizosphere soil

One gram of soil added to 100 ml of distilled water and 1 ml of the suspension was used for serial dilution up to 10^{-7}. The dilutions of 10^{-6}, 10^{-4} and 10^{-2} were used for bacteria, fungi and actinomycetes, respectively. Transferred 1 ml of appropriate dilution to petridishes and mixed with 15 ml of melted and cooled media (luck worm condition) shaked clockwise and anticlockwise direction and allowed for complete solidification and incubated for 2-7 days in inverted position. The media used for bacteria was nutrient agar medium, fungi were rose bengal agar medium and for actinomycetes was Kenknights agar medium.

Bacterial colonies were observed after 2 days, for fungi 5-7 days and for actinomycetes 7 days (Allen, 1953).

Soil biochemical analyses

Biomass carbon

Soil microbial biomass carbon was determined through chloroform fumigation and K_2SO_4 extraction (conversion coefficient K is 0.45). Culturing in closed containers and alkali absorption were employed to obtain soil basal respiration (Jenkinson and Poulson, 1976).

Organic carbon

Accurately 0.5g of **s**oil was weighed and passed through 0.2 mm sieve added 10 ml of 1N $K_2Cr_2O_7$ and 10 ml of concentrated H_2SO_4 allowed for digestion for 30 minutes.

After the expiry of time, 10 ml ortho phosphoric acid, 200 ml distilled water were added and titrated against 0.5 N ferrous ammonium sulphate using diphenylamine indicator. Blank was run without soil sample and from the amount of $K_2Cr_2O_7$ used for oxidizing organic matter, the organic carbon content in soil was calculated (Walkley and Black, 1934).

Glomalin

The easily extractable glomalin (EEG) fraction was extracted with 20 mM citrate, pH 7.0 at 121ºC for 30 min (Wright and Updahyaya, 1998). The supernatant was removed by centrifugation at 5000 rpm for 20 min. Extraction was continued till the supernatant was devoid of red brown colour. The supernatant was taken in the test tube and 5 ml of alkaline copper tartarate and 0.5 ml of folin reagent were added. Thirty minutes after colour development, OD was measured at 660 nm using spectrophotometer.

Dehydrogenase

Twenty grams of moistened inoculated or uninoculated soil samples were added with 0.2 g $CaCO_3$ and 2 ml of 1% triphenyl tetrazoilum chloride and incubated for 24 hours at 30º C. At the end of incubation period, soil samples were extracted with 25 ml methanol. The microbial activity produces H^+ ions, which reduces triphenyl tetrazolissum chloride into triphenyl tetrazoilum formazan, which is red in colour. Dehydrogenase activity, the index of microbial activity was determined by measuring the intensity of red colour at 485 nm (Tate and Terry, 1980).

Acid phosphatase

One-gram soil was mixed with 10 ml 0.2 M sodium acetate buffer and 0.2 ml 10 mM ρ-nitrophenol phosphate and kept in water bath for 30 minutes. The reaction was terminated by the addition of 2 ml 200 mM Na_2CO_3. The mixture was mixed thoroughly, filtered and determined acid phosphatase activity as µ moles ρ-nitrophenol produced per gram per minute at 37ºC using spectrophotometer at 420 nm (Tabatabai, 1982).

Soil nutrient analyses

Olsen's phosphorus

Five grams of soil sample was mixed with 50 ml 0.5 M $NaHCO_3$ (pH 8.5) and pinch of Darco G 60. The mixture was shacked in mechanical shaker for 30 minutes and filtered through Whatman No. 40 filter paper. Five ml of the filtrate was pipette out into a 25 ml volumetric flask, added with 4 ml of reagent (1.056 g ascorbic acid in 200 ml of reagent containing ammonium molybdate, antimony potassium tartarate and sulphuric acid) and made up to 25 ml. The intensity of blue color developed was measured at 660 nm using spectrophotometer.

DTPA extractable micronutrients

Ten grams of soil sample was shaken with 20 ml DTPA extractant (13.1 ml triethanolamine, 1.967 g (Diethylene Triamine Penta Acetic acid) DTPA and 1.47 g $CaCl_2$ mixed together, made up to 1 litre and pH adjusted to 7.3) for 2 hrs and filtered through Whatman No. 42 filter paper and fed into Atomic Absorption Spectrophotometer (Varian Spectra AA 220), Australia.

Statistical analysis

A two-way analysis of variance (ANOVA) was done for all data and comparisons among means were made using LSD (least square difference) test, calculated at $p \leq 0.05$.Statistical procedures were carried out with the software package IRRI stat (IRRI, Manila, Philippines).

Results and Discussion

Microbial population

Soil treated with AM fungus had significantly higher number of bacteria, fungi and actinomycetes populations than uninoculated control at 75 DAS (Table 1). In contrast application of P and Zn had no such influence on bacteria and actinomycetes population while the significant response was observed in the case of fungal population.

Biochemical properties

Soil enzymes

Acid phosphatase and dehydrogenase activities of soil inoculated with AM fungus increased significantly ($P \leq 0.01$) at 55 and 75 DAS in comparison to respective uninoculated soil (Table 2). However the magnitude of increase in acid phosphatase and dehydrogenase activities in rhizosphere soil were found to be observed more at 75 DAS rather than 55DAS. The activity of acid phosphatase was increased with P levels in both the inoculated and uninoculated mycorrhizal soil at 55 and 75 DAS, regardless of zinc levels. Incremental levels of zinc linearly increased the dehydrogenase activity in both the stages of M+ and M- soil. Such reaction was not seen in the activity of soil acid phosphatase except at the stage of 75 DAS.

Biomass carbon

Biomass carbon of content of inoculated (M+) soil was significantly ($P \leq 0.05$) higher than uninoculated (M-) soil regardless of P and Zn levels with the percent increase of 32% and 15% respectively (Table 3). The biomass carbon content in the AM treated soil increased significantly ($P \leq 0.01$) in correspondence with increasing levels of P at 55 DAS. However the magnitude of increase was more at 55 than 75 DAS. Application of incremental levels of zinc progressively increased the biomass carbon content under inoculated condition at 55 DAS over 75 DAS.

Glomalin

The soil treated with AM fungus had a considerable role on increasing the concentration of glomalin (Table 3). The inoculated soil had significantly ($P \leq 0.01$) higher glomalin content by 30% and 25% at 55 and 75 DAS respectively, over uninoculated soil. The addition of P in both the inoculated and uninoculated soil significantly increased the glomalin content irrespective of stages. At 75 DAS, graded levels of zinc addition progressively increased ($P \leq 0.01$) the glomalin content in both AM fungus treated and untreated soil. Whereas the glomalin content was not significantly influenced by Zn addition at 55DAS.

Soil fertility status

Organic carbon

Organic carbon content in mycorrhizal soil was significantly ($P \leq 0.01$) higher than untreated soil at 55 and 75 DAS regardless of P and Zn levels (Table 4). The increases in organic carbon content of both inoculated and uninoculated soils at two P levels were 14% and 12% at 55 DAS and 16% and 11% at 75 DAS, respectively. The graded levels of Zn had no effect on organic carbon content in both stages.

Available P and Zn

Soil treated with *Glomus intraradices* had higher available P and Zn than uninoculated soil regardless of varying levels of P or Zn application (Table 4 and 5). The treatment with AM fungus was very effective for increasing the concentration of available P and Zn significantly ($P \leq 0.01$) in the rhizosphere soil of *Zea mays* by about 22% and 26% at the time of 55 DAS and 30% and 42% at 75 DAS respectively, when compared to non-mycorrhizal plants. Increasing levels of Zn gradually increased the available P status of both inoculated and uninoculated soils however the values were consistently higher for inoculated soils. The available (DTPA-extractable) Zn increased significantly ($P \leq 0.01$) with mycorrhizal inoculation under varying levels of P or Zn. The percent increase in DTPA- Zn at 55 DAS was 41 and 25 % by P15 and P30, respectively. Conversely, P30 had higher percentage of increase at 75 DAS. Similarly uninoculated soil had higher Zn in P15 than P30 at 75 DAS

Available micronutrients

In both stages mycorrhizal inoculation increased the DTPA- Fe, Mn and Cu concentration in soil above the critical limit fixed for experimental soil (Table 5 and 6) over uninoculated control regardless of P and Zn levels. However the difference between inoculated and uninoculated soil was more at 55DAS. Similarly application of P had positive impact on increasing the DTPA- Mn and Cu while Fe content found to be decreased. The incremental levels of Zn addition showed gradual increase in DTPA-Fe, Mn and Cu content in 55and 75 DAS

The zinc availability in the soil is highly restricted due to fixation of major portion of available form of Zn caused by chemical reactions. Mycorrhizal symbiosis appears to facilitate release of Zn from unavailable forms which in turn tend to enhance the availability of Zn. In this study, arbuscular mycorrhizal (AM) fungus inoculation in maize improved organic status, dehydrogenase and phosphatase activities of soils that collectively contributed for the availability of P and Zn and may assist in alleviating Zn deficiency in crop plants.

AM symbiosis significantly modulated the microbial communities in the soil regardless of low or high P concentration. The results showed that mycorrhizae had pronounced influence on increasing bacterial population, while less effect was found in the case of fungi and actinomycetes activity in the soil. This can be explained by altering root exudation through the changes made in root physiology. Numerous studies have shown conclusively that AM is having synergistic interaction with other beneficial soil microorganism such as N fixers and P solubilizers (Caravaca *et al.,* 2003) while AM fungi decrease the activity of some of the microorganism (Ames *et al.,* 1984). AM fungi are the key component of soil micro biota and obviously interacted with other microorganism in the rhizosphere. The interactive effect of AM fungi and phosphate solubilizing bacteria were evaluated by Toro *et al.,* (1997) reported

that AM fungi increased the size of the phosphate solubilizing bacteria population while bacteria behaved as mycorrhiza helper. The effect of AM fungi on wider soil biota including nematode, fungal biomass as indicated by ergosterol, microbial biomass carbon, phospholipid fatty acid profiles were less pronounced (Cavagnaro *et al.,* 2006).

Analysis of the activity of soil enzymes provides information on biochemical processes proceeding in the soil. Mycorrhizal inoculation increased acid phosphatase activity in all the experimental treatments. Acid phosphates in the rhizosphere play an important role for acquisition of P by roots (Kabir *et al.,* 1998) and through the hydrolysis of organic P (Tarafdar and Claassen 1988; Helal and Saverbeck, 1991). Acid phosphatase released due to a direct fungal secretion or an induced secretion by plant roots as pointed by Joner *et al.,* (2000); Tarafdar and Marschner, (1994) and its activity was higher in the close vicinity (0.2 - 0.8 mm) of maize roots (Kandeler *et al.,* 2002); 2.0-3.1 mm in cumbu (Tarafdar, 2008).

Dehydrogenase activity of AM fungus inoculated soil was consistently higher under varying levels of P and Zn and it is considered as a measure of soil microbial activity (Garcia *et al.,* 1997). Therefore due to the central role that soil microorganisms play in the degradation of organic matter and the cycling of nutrient in soil ecosystems, a decrease in dehydrogenase activity could have a significant effect on soil ecosystem. The similar result of increased dehydrogenase activity due to the addition of AM fungi was also reported by Caravaca *et al.,* (2003) in *Rhamnus lyciodes* seedling. In the present study addition of P and Zn also enhanced dehydrogenase activity, which indicates the importance of these nutrients on enzyme activity. However Kelly *et al.,* (1999) reported a reduction of dehydrogenase activity due to the addition of Zn above the toxic level.

Soil biomass carbon is the active component of soil organic matter. The changes of microbial biomass carbon reflect the process of microorganism propagation and degradation utilizing soil carbon. In this study, mycorrhizal inoculation in soil had intensive microbial population besides higher dehydrogenase activities. On decomposition of microbial tissues, the residues serve as source of carbon for heterotrophic microorganism, which may have contributed for the accumulation of biomass carbon. This was supported by Caravaca *et al.,* (2003) who reported that biomass carbon content of rhizosphere soil was increased by 240% with respect to control. Over short period changes in microbial biomass carbon can be a sensitive index of changes in the organic matter content of soil.

Glomalin, a iron containing glycoprotein produce by AM fungi as a component of hyphal and spore wall (Rillig *et al.,* 2001) considered as a major sequester of C and potentially important active soil. Our study also revealed the increased glomalin concentration in mycorrhizal treated soil than untreated soil due to increased biological activity as indicted by increased dehydrogenase activity and biomass carbon. The amount of C in glomalin represented 4-5% of total C which might have contributed to the increased soil C under AM inoculated soil. Radio carbon dating defined glomain has residence time of 6 - 42 years in soil, which is much longer than the residence time reported for hyphae, this could influence soil C storage indirectly by stable soil aggregates (Rillig *et al.,* 2002). Rillig *et al.,* (2003) report that glomalin concentration was consistently and highly positively correlated with soil C. Our results also suggest that glomalin acts as C sink in tropical condition and it act as adsorptive site of Zn thus made it available to plants.

Table.1 Mean for bacteria, fungi and actinomycetes population in soils at 75 (n = 4) days after sowing (DAS) under different levels of P (15 and 30 mg kg^{-1}) and Zn (0, 1.25 and 2.5 mg kg^{-1}) with (M+) or without (M-) mycorrhizal inoculation. Values in parentheses indicate the standard error and the levels of significance for ANOVA *P ≤ 0.05; ** P ≤ 0.01; NS, not significant

Treatments (kg ha^{-1})	75 DAS					
	Bacteria		Fungi		Actinomycetes	
	M+	M-	M+	M-	M+	M-
P15 Zn 0	30.0 (1.528)	18.3 (1.453)	17.3 (1.202)	13.3 (0.882)	7.7 (0.662)	2.0 (0.577)
Zn1.25	32.3 (2.028)	24.0 (2.082)	19.7 (0.882)	14.7 (1.453)	6.7 (0.662)	3.0 (0.577)
Zn2.5	29.3 (2.333)	20.7 (3.844)	23.0 (1.528)	14.3 (2.186)	6.7 (0.331)	2.3 (0.882)
P30 Zn 0	35.7 (3.283)	21.7 (1.202)	20.3 (1.453)	9.7 (1.453)	7.0 (1.000)	2.7 (0.667)
Zn1.25	30.7 (1.764)	20.3 (1.453)	16.3 (0.882)	8.7 (0.882)	6.7 (0.882)	3.3 (0.667)
Zn2.5	30.0 (3.464)	22.3 (2.186)	20.0 (1.732)	10.7 (0.667)	7.3 (1.202)	2.0 (0.577)
Anova: M (Mycorrhiza), P (Phosphorus), Zn (Zinc)						
M	*		**		**	
P	NS		*		NS	
Zn	NS		*		NS	
M x P	NS		*		NS	
P x Zn	NS		*		NS	
M x Zn	NS		NS		NS	
M x P x Zn	NS		NS		NS	

Table.2 Mean for dehydrogenase and acid phosphatase activities in soil at 55 (n = 3) and 75 (n = 4) days after sowing (DAS) under different levels of P (15 and 30 mg kg^{-1}) and Zn (0, 1.25 and 2.5 mg kg^{-1}) with (M+) or without (M-) mycorrhizal inoculation. Values in parentheses indicate the standard error and the levels of significance for ANOVA *P ≤ 0.05; ** P ≤ 0.01; NS, not significant

Treatments (kg ha^{-1})	Dehydrogenase (Δ in OD at 485 nm)						Acid Phosphatase (µg ofPNP/g/min)					
	55DAS			75 DAS			55DAS			75 DAS		
	M+	M-	**Mean**	M+	M-	**Mean**	M+	M-	**Mean**	M+	M-	**Mean**
P15 Zn 0	0.417 (0.011)	0.323 (0.012)	**0.37**	0.658 (0.380)	0.443 (0.256)	**0.55**	1.921 (0.063)	1.518 (0.071)	**1.72**	3.266 (1.887)	2.360 (1.377)	**2.81**
Zn1.25	0.441 (0.009)	0.341 (0.004)	**0.39**	0.674 (0.389)	0.544 (0.315)	**0.61**	2.291 (0.083)	1.496 (0.057)	**1.89**	3.392 (1.959)	2.447 (1.414)	**2.92**
Zn2.5	0.462 (0.008)	0.375 (0.004)	**0.41**	0.727 (0.420)	0.577 (0.334)	**0.65**	1.912 (0.054)	1.095 (0.078)	**1.50**	3.403 (1.965)	2.558 (1.478)	**2.98**
Mean	**0.44**	**0.35**		**0.68**	**0.52**		**2.04**	**1.37**		**3.35**	**2.46**	
P30 Zn 0	0.642 (0.009)	0.425 (0.006)	**0.53**	0.735 (0.425)	0.637 (0.368)	**0.69**	2.009 (0.053)	1.300 (0.043)	**1.65**	3.332 (0.916)	2.693 (1.555)	**3.01**
Zn1.25	0.712 (0.016)	0.445 (0.007)	**0.59**	0.752 (0.434)	0.653 (0.378)	**0.70**	2.872 (0.226)	2.326 (0.050)	**2.59**	3.419 (0.975)	2.743 (1.584)	**3.08**
Zn2.5	0.749 (0.010)	0.542 (0.009)	**0.65**	0.769 (0.444)	0.682 (0.395)	**0.73**	2.802 (0.086)	2.275 (0.226)	**2.54**	3.552 (2.052)	2.799 (1.616)	**3.17**
Mean	**0.70**	**0.47**		**0.75**	**0.66**		**2.56**	**1.97**		**3.43**	**2.75**	
Anova: M (Mycorrhiza), P (Phosphorus), Zn (Zinc)												
M	**			**			**			**		
P	**			**			**			**		
Zn	**			**			**			**		
M x P	**			**			NS			**		
P x Zn	**			**			**			NS		
M x Zn	*			**			NS			NS		
M x P x Zn	NS			**			NS			*		

Table.3 Mean for biomass carbon and glomalin content in soil at 55 (n = 3) and 75 (n = 4) days after sowing (DAS) under different levels of P (15 and 30 mg kg^{-1}) and Zn (0, 1.25 and 2.5 mg kg^{-1}) with (M+) or without (M-) mycorrhizal inoculation. Values in parentheses indicate the standard error and the levels of significance for ANOVA *P ≤ 0.05; ** P ≤ 0.01; NS, not significant

Treatments (kg ha-1)	Biomass carbon (mg kg^{-1})						Glomalin (mg g^{-1})					
	55 DAS			75 DAS			55 DAS			75 DAS		
	M+	M-	**Mean**	M+	M-	**Mean**	M+	M-	**Mean**	M+	M-	**Mean**
P15 Zn0	36.00 (9.000)	27.00 (0.000)	**31.500**	54.00 (0.000)	47.00 (0.0013)	**50.50**	0.40 (0.060)	0.31 (0.076)	**0.36**	0.56 (0.345)	0.42 (0.246)	**0.51**
Zn1.25	45.00 (9.000)	27.00 (0.000)	**36.000**	54.00 (0.0011)	54.00 (0.0011)	**54.00**	0.43 (0.040)	0.32 (0.065)	**0.38**	0.70 (0.362)	0.49 (0.288)	**0.56**
Zn2.5	54.00 (15.58)	36.00 (9.000)	**45.000**	61.00 (0.0007)	47.00 (0.0007)	**54.00**	0.52 (0.070)	0.40 (0.090)	**0.49**	0.75 (0.385)	0.57 (0.331)	**0.62**
Mean	**45.00**	**30.00**		**56.33**	**49.33**		**0.45**	**0.34**		**0.63**	**0.49**	
P30 Zn0	45.00 (9.000)	27.00 (0.000)	**36.000**	68.00 (0.0008)	47.00 (0.0007)	**57.50**	0.61 (0.070)	0.43 (0.060)	**0.52**	0.81 (0.471)	0.61 (0.357)	**0.71**
Zn1.25	54.00 (15.58)	36.00 (9.000)	**45.000**	61.00 (0.0007)	54.00 (0.0011)	**57.50**	0.65 (0.040)	0.45 (0.051)	**0.55**	0.89 (0.517)	0.63 (0.366)	**0.76**
Zn2.5	72.00 (9.000)	36.00 (9.000)	**54.000**	74.00 (0.0007)	47.00 (0.0013)	**60.50**	0.69 (0.026)	0.46 (0.040)	**0.58**	0.96 (0.561)	0.72 (0.417)	**0.84**
Mean	**57.00**	**33.00**		**67.67**	**49.33**		**0.65**	**0.43**		**0.89**	**0.65**	
ANOVA: M (Mycorrhiza), P (Phosphorus), Zn (Zinc)												
M	*			*			**			**		
P	**			NS			**			**		
Zn	*			NS			NS			**		
M x P	NS			NS			NS			NS		
P x Zn	NS			NS			NS			NS		
M x Zn	NS			NS			NS			NS		
M x P x Zn	NS			NS			NS			NS		

Table.4 Mean for organic carbon and available P content in soil at 55 (n = 3) and 75 (n = 4) days after sowing (DAS) under different levels of P (15 and 30 mg kg^{-1}) and Zn (0, 1.25 and 2.5 mg kg^{-1}) with (M+) or without (M-) mycorrhizal inoculation. Values in parentheses indicate the standard error and the levels of significance for ANOVA *P ≤ 0.05; ** P ≤ 0.01; NS, not significant

Treatments (kg ha^{-1})	Organic carbon(%)						Soil P (mg kg^{-1})					
	55 DAS			75 DAS			55 DAS			75 DAS		
	M+	M-	Mean	M+	M-	Mean	M+	M-	Mean	M+	M-	Mean
P15 Zn 0	0.23 (0.020)	0.21 (0.017)	**0.22**	0.30 (0.017)	0.23 (0.015)	**0.26**	9.91 (0.97)	7.30 (1.04)	**8.66**	7.10 (0.18)	5.63 (0.29)	**6.40**
Zn1.25	0.23 (0.010)	0.21 (0.000)	**0.22**	0.31 (0.008)	0.26 (0.009)	**0.28**	10.90 (1.11)	8.23 (1.09)	**9.54**	8.72 (0.16)	6.14 (0.17)	**7.42**
Zn2.5	0.26 (0.010)	0.22 (0.026)	**0.24**	0.31 (0.008)	0.23 (0.008)	**0.27**	12.42 (0.71)	9.76 (1.28)	**11.13**	9.90 (0.18)	7.60 (0.21)	**8.80**
Mean	**0.24**	**0.21**		**0.31**	**0.24**		**11.10**	**8.40**		**8.62**	**6.42**	
P30 Zn 0	0.27 (0.032)	0.25 (0.020)	**0.26**	0.31 (0.008)	0.26 (0.009)	**0.28**	15.42 (1.57)	12.02 (1.53)	**13.70**	12.10 (0.13)	10.00 (0.25)	**11.33**
Zn1.25	0.28 (0.013)	0.23 (0.026)	**0.26**	0.33 (0.021)	0.27 (0.024)	**0.30**	16.80 (1.36)	13.00 (1.11)	**14.92**	13.57 (0.22)	10.31 (0.41)	**11.90**
Zn2.5	0.28 (0.026)	0.25 (0.019)	**0.27**	0.35 (0.016)	0.28 (0.031)	**0.31**	17.93 (1.18)	14.82 (1.86)	**16.40**	15.55 (0.23)	9.72 (0.33)	**12.74**
Mean	**0.33**	**0.27**		**0.33**	**0.27**		**16.74**	**13.34**		**13.70**	**10.20**	
ANOVA: M (Mycorrhiza), P (Phosphorus), Zn (Zinc)												
M	**			**			**			**		
P	**			**			**			**		
Zn	NS			NS			**			**		
M x P	NS			NS			NS			**		
P x Zn	NS			NS			NS			**		
M x Zn	NS			NS			NS			**		
M x P x Zn	NS			NS			NS			**		

Table.5 Mean for DTPA-Fe and Mn content in soil at 55 (n = 3) and 75 (n = 4) days after sowing (DAS) under different levels of P (15 and 30 mg kg^{-1}) and Zn (0, 1.25 and 2.5 mg kg^{-1}) with (M+) or without (M-) mycorrhizal inoculation. Values in parentheses indicate the standard error and the levels of significance for ANOVA *P ≤ 0.05; ** P ≤ 0.01; NS, not significant

Treatments (kg ha^{-1})	DTPA-Fe						DTPA-Mn					
	55DAS			75 DAS			55DAS			75 DAS		
	M+	M-	**Mean**	M+	M-	**Mean**	M+	M-	**Mean**	M+	M-	**Mean**
P15 Zn 0	8.11 (0.116)	6.20 (0.067)	**7.15**	8.32 (0.331)	7.04 (0.399)	**7.68**	11.12 (0.320)	8.16 (0.754)	**9.64**	11.56 (0.295)	7.14 (0.703)	**9.35**
Zn1.25	8.70 (0.097)	6.63 (0.036)	**7.67**	8.78 (0.241)	7.30 (0.484)	**8.04**	11.46 (0.659)	8.76 (0.583)	**10.11**	12.72 (0.271)	7.87 (0.650)	**10.30**
Zn2.5	9.22 (0.125)	7.45 (0.137)	**8.34**	9.34 (0.265)	7.34 (0.573)	**8.34**	12.14 (0.249)	9.14 (1.007)	**10.64**	13.14 (0.427)	8.59 (0.460)	**10.87**
Mean	**8.68**	**6.76**		**8.81**	**7.23**		**11.57**	**8.69**		**12.47**	**7.87**	
P30 Zn 0	7.54 (0.050)	5.63 (0.127)	**6.59**	7.81 (0.320)	6.72 (0.561)	**7.27**	12.79 (1.003)	9.67 (0.777)	**11.23**	13.57 (0.286)	8.21 (0.651)	**10.89**
Zn1.25	8.01 (0.113)	6.06 (0.076)	**7.03**	8.14 (0.260)	6.81 (0.427)	**7.48**	13.12 (1.147)	10.05 (0.981)	**11.59**	13.88 (0.307)	7.56 (0.612)	**10.72**
Zn2.5	8.49 (0.066)	6.33 (0.073)	**7.41**	8.52 (0.254)	6.95 (0.497)	**7.74**	13.45 (0.621)	10.22 (0.662)	**11.84**	14.02 (0.347)	7.80 (0.575)	**10.91**
Mean	**8.01**	**6.01**		**8.16**	**6.83**		**13.12**	**9.98**		**13.82**	**7.86**	
Anova: M (Mycorrhiza), P (Phosphorus), Zn (Zinc)												
M	**			**			**			**		
P	**			*			**			*		
Zn	**			NS			NS			NS		
M x P	NS			NS			NS			*		
P x Zn	*			NS			NS			NS		
M x Zn	NS			NS			NS			NS		
M x P x Zn	NS			NS			NS			NS		

Table.6 Mean for DTPA Zn and Cu in soils at 55 (n = 3) and 75 (n = 4) days after sowing (DAS) under different levels of P (15 and 30 mg kg^{-1}) and Zn (0, 1.25 and 2.5 mg kg^{-1}) with (M+) or without (M-) mycorrhizal inoculation. Values in parentheses indicate the standard error and the levels of significance for ANOVA *P ≤ 0.05; ** P ≤ 0.01; NS, not significant

Treatments (kg ha^{-1})	DTPA-Zn						DTPA-Cu					
	55DAS			75 DAS			55DAS			75 DAS		
	M+	M-	**Mean**	M+	M-	**Mean**	M+	M-	**Mean**	M+	M-	**Mean**
P15 Zn 0	3.02 (0.21)	2.50 (0.41)	**2.80**	3.74 (0.25)	3.70 (0.15)	**3.72**	0.513 (0.137)	0.313 (0.022)	**0.413**	0.314 (0.029)	0.221 (0.017)	**0.268**
Zn1.25	6.40 (0.45)	3.82 (0.36)	**5.10**	6.76 (0.99)	4.34 (0.12)	**5.63**	0.535 (0.028)	0.327 (0.020)	**0.431**	0.511 (0.014)	0.238 (0.059)	**0.375**
Zn2.5	8.17 (0.35)	4.18 (0.56)	**6.17**	8.90 (0.11)	7.80 (0.08)	**8.30**	0.649 (0.018)	0.342 (0.020)	**0.496**	0.545 (0.018)	0.242 (0.099)	**0.394**
Mean	**5.80**	**3.50**		**6.54**	**5.28**		**0.566**	**0.327**		**0.457**	**0.234**	
P30 Zn 0	3.82 (0.35)	4.25 (0.62)	**4.13**	4.10 (0.09)	2.40 (0.11)	**3.20**	0.614 (0.020)	0.344 (0.017)	**0.479**	0.394 (0.159)	0.278 (0.018)	**0.336**
Zn1.25	9.70 (0.53)	6.60 (0.59)	**8.24**	9.92 (0.15)	3.52 (0.10)	**6.71**	0.654 (0.022)	0.372 (0.020)	**0.513**	0.524 (0.184)	0.303 (0.040)	**0.414**
Zn2.5	11.42 (0.49)	7.92 (0.56)	**9.60**	10.76 (0.39)	3.91 (0.08)	**7.46**	0.675 (0.026)	0.375 (0.016)	**0.525**	0.574 (0.200)	0.325 (0.068)	**0.450**
Mean	**8.36**	**6.38**		**8.29**	**3.20**		**0.648**	**0.364**		**0.497**	**0.302**	
Anova: M (Mycorrhiza), P (Phosphorus), Zn (Zinc)												
M	**			**			**			**		
P	**			NS			*			**		
Zn	**			**			NS			**		
M x P	NS			**			NS			NS		
P x Zn	**			**			NS			NS		
M x Zn	**			**			NS			NS		
M x P x Zn	NS			**			NS			NS		

Soil organic carbon pools an important component of terrestrial ecosystems. In the present study AM fungi inoculation improved soil organic carbon status through the growth and turnover of extraradical hyphae besides exudates from hyphae as shown by Marschner *et al.,* (1997). Percentage of colonized root was significantly correlated to labile C fraction in the rhizosphere soil. Mechanisms influencing soil organic carbon storage depend mainly on net primary production and the distribution of photosynthates between above and below ground structure. Graham (2000) reported that AM could drain 4-20 % of carbon from host plant which indirectly influence C storage in soils. Which in turn influenced the microbial activity in soil. Rillig *et al.,* (2003) reported that organic carbon is positively correlated with glomalin. In the present study also the increased glomalin concentration, biomass carbon in mycorrhizae treated soil might have contributed the presence of high organic carbon in the soil as indicated by Rillig *et al.,* 2002. As mycorrhizal symbiosis utilizes at least 10% of the host plant photosynthetic C (Fitter, 1988) and the transferred C enriches microbial activities in the rhizosphere, which may have contributed for the enhancement of active C pool in the soil.

The primary effect of AM symbiosis is to increase the supply of mineral nutrient to the plant particularly those whose ionic forms have a poor mobility rate such as P, Zn (Barea, 1991) which resulted in enhanced growth. We found that positive interaction between P and Zn in mycorrhizae treated soil resulted in enhanced growth especially root and nutrient uptake. This was supported by Caragnaro *et al.,* (2006). AM can deliver up to 80% of plant P and 25% of plant Zn (Marschner and Dell 1994). In general, only P in the soil solution and labile pool can be readily taken up by roots and AM fungal hyphae. Efficient nutrient acquisition by AM fungi is generally attributed to the external hyphal growth beyond the nutrient depletion zone surrounding the root and also due to an increase in the no. of uptake sites per unit area of roots (Kim *et al.,* 1988) so that external hyphae are able to explore a large volume of soil. AM increased P uptake by dissolving complex soil phosphate due to release of organic substance by the roots (Bolan, 1991) especially organic acids *viz*., citric acid, lactic acid, formic acid and malic acid. Organic acid can carry varying negative charges, thereby allowing the complextation of metal cations in solution and the displacement of anions from the soil matrix. Organic anions function as a organic ligands, which can increase P in solution by replacing P sorbed at metal hydroxide surfaces through ligand–exchange reactions, dissolving metal oxide surfaces that sorb P and complexing metals in solution and thus preventing precipitation of metal phosphates. Further AM infection increased P availability more from organic P compared to inorganic P. In our study mycorrhizal inoculation modified the soil biochemistry by increasing organic carbon, biomass carbon and soil enzyme activities which had a positive role on Zn release from soil. The enhance growth of mycorrhizal hyphae had many adsorptive site for Zn and also organic substance produced by AM fungi acts as chelating agent and complexed with metallic micronutrient such as Zn and made it available. Rupa *et al.,* (2003) concluded that p addition up to 40 mg kg^{-1} in soil increased plant available zinc in soil whereas at higher P levels, inhibits zinc translocation. In our study we used P only up to 30 mg kg^{-1}, hence it might have shown synergistic interaction between P and zinc.

In both stages AM inoculation increased DTPA- Fe, Mn, Cu in soil above the critical level over uninoculated control regardless of P and Zn level. This might be due to enhanced growth of external hyphae which

acts as extension to plant roots (Turk *et al.*, 2006). At low P levels in soils of soyabean mycorrhiza substantially increases the availability of Cu and Zn content (Lambert and Weidebsaul, 1991).

To conclude, Arbuscular mycorrhizal inoculation could improve soil biochemical, enzymatic and organic carbon status, which altogether improved Zn availability due to synergistic interaction between Zn and P. Over all this study reveals that AM fungal inoculation is one of the major biochemical component in the soil needed to be considered to nutrient especially micronutrient deficiency.

Abbreviations: AMF- arbuscular mycorrhizal fungus, BMC- biomass carbon, P-Phosphorous, Zn-Zinc, Mn-Manganese, Fe-Iron.

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