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Effect of Mycorrhizal Co-Inoculation with Selected Rhizobacteria on Soil Zinc Dynamics

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A B S T R A C T

Co-inoculation of *Arbuscular mycorrhizal* fungi (AMF) and certain species of Plant Growth Promoting Rhizobacteria (PGPR) help to bring the poorly soluble nutrients into the soil solution, thus enhancing nutrient uptake. The present investigation was carried out with objectives to study the effect of co-inoculation of AMF and PGPR (viz. *Burkholderia cepacia* and *Azospirillum brasilense*) on soil Zn fractions and Zn uptake by maize. A field experiment was conducted in Randomized Block Design with 9 treatments, each replicated thrice. The treatments included an absolute control and 100 % recommended fertilizer dose along with different combinations of AMF with PGPR at varied P doses. Laboratory analysis of experimental soil for Zn fractions and plant-available Zn was done at 2 stages *viz.*, at flowering and after harvesting. Results from the experiment obtained at both the stages show highest values for grain Zn content (52.58 mg kg^{-1}) and its uptake (0.390 kg ha^{-1}) under the treatment where AMF@10kg/ha + *Burkholderia cepacia* @20g/kg seed + *Azospirillum brasilense* @20 g/kg seed + 75%P was used. Residual fraction of Zn comprised of more than 80 % of total soil Zn while water soluble-exchangeable Zn accounted for the least (about 1%). The co-inoculation treatments had a significant effect on the water soluble-exchangeable and organically bound Zn, while the oxide bound Zn fractions had higher values in the non-organic treatments *i.e.* Control (13.4 mg kg^{-1}) and 100% RDF (12.9 mg kg^{-1}). The results for co-inoculation effects can be considered as possible strategies towards improving Zn nutrition to plants.

Keywords

Zinc fractions, Maize, Mycorrhiza, PGPR, Sustainability

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Introduction

Maize is the 3rd major crop in India after rice and wheat (Reddy *et al.*, 2013) which provides food, feed, and fodder, and also serves as a source of basic raw material for numerous industrial products. Bihar has emerged as one of the most promising states for maize production in India. The average productivity of *rabi* maize in the state is much higher as compared to the national average. Zinc (Zn) and Phosphorus (P) are the major nutrients limiting plant growth and their bioavailability to nutrient-exhaustive plants like maize, is a major concern. Zn, a transition metal, plays an important role in photosynthesis, membrane integrity, protein synthesis, pollen formation, and immunity system (Alloway, 2008), and thus has an increasing importance in crop production under the present day exploitative agriculture. Although Zn is required by the plant in micro concentration, its bio available fraction in soil is very low. Zn deficiency in India remains the most important nutritional disorder affecting majority of the crop production.

Factors like parent material, pedochemical transformations and anthropogenic interventions contribute in the distribution of Zn and its bioavailability for plant uptake (Chirwa and Yorokun, 2012). Knowledge about the total content of an element in soils is of little importance as it does not provide any substantial information about the chemical behaviour and availability of the nutrients to plants. Crops differ not only in utilization of native and added fertilizer nutrients, but also in the utilization from different chemical pools. Such differences are even more important for micronutrients such as Zn, Cu, Fe and Mn, and crops with contrasting soil root environment. For an efficient nutrient management through fertilizers, a deep understanding of the distribution of Zn fractions is important. Thus, it is important to establish a relationship between nutrient fractions and plant uptake.

The beneficial plant-microbe interaction in the rhizosphere is the primary determinant of plant health and soil fertility. The use of microbial inoculants plays an important role in sustainable agriculture. Arbuscular mycorrhizal fungi (AMF) are known to improve the nutritional status, growth and development of plants, protect plant against root pathogens and offer drought resistance to drought and salinity. AMF colonize roots of host plants and promote plant growth, which is generally attributed to the improved uptake of nutrients with particular emphasis on P nutrition (Smith and Read, 2008). AMF increase the growth of plants and may effectively substitute for the functions of some fertilizers mainly due to improved plant nutrition, particularly in soils of low fertility status. There have been numerous reports (Kothari *et al.,* 1991) that VAM fungi may increase the uptake of many nutrients such as P, N, S, Ca, K, Zn, and Cu. Nevertheless, the effects of Vescicular Arbuscular Mycorrhiza on Zn uptake by plants are contradictory in the literature, especially in P fertilizer trials. For example, Lambert *et al.,* (1979) demonstrated that P fertilization reduced both mycorrhizal colonization and Zn concentration in soybean plants. However, Lu and Miller (1989) showed that both high P fertilization and benomyl treatment reduced VAM colonization but had no effects on Zn uptake by corn both in field and growth chamber conditions.

Soil bacteria which are important for plant growth are termed as plant growth promoting rhizobacteria (PGPR). They can affect plant growth by different direct and indirect mechanisms. PGPR influence direct growth promotion of plants by fixing atmospheric nitrogen, solubilizing insoluble phosphates, secreting hormones such as IAA, GAs, and Kinetics besides ACC deaminase production, which helps in regulation of ethylene. Induced systemic resistance, antibiosis, competition for nutrients, parasitism, production of metabolites (hydrogen cyanide, siderophores) suppressive to deleterious rhizobacteria are some of the mechanism that indirectly benefit plant growth. PGPR is multifunction microbes functioning in sustainable agriculture. PGPR are a diverse group of bacteria that can be found in the rhizosphere on root surfaces as well as in association with roots. These bacteria move around from the bulk soil to the living plant rhizosphere and antagonistically colonize the rhizosphere and roots of plant. In addition to phosphate mobilization they are responsible to play key role in carrying out the bioavailability of soil P, potassium, iron, Zn and silicate to plant roots. This necessitates a system that releases essential quantity of Zn from the unavailable state in which it is retained in the soil to the plants for good growth. Numerous bacteria, especially those associated with the rhizosphere have the ability to transform unavailable form of a metal into available form through solubilization mechanism. The secretion of organic acids appears to be the functional mechanism involved in metal solubilization. Gluconic acid is considered to be the major organic acid involved in the solubilization of insoluble minerals. Organic acids secreted by micro-flora increase soil Zn availability by sequestering cations and by reducing rhizospheric pH.

Application of a single strain of inoculants might lead to efficiency inconsistencies in field conditions. Inorder to overcome this problem different species or strains of beneficial microbes can be included in the same microbial formulation. Co-inoculation helps to increase plant performance, and enhance the efficacy and reliability of constituent inoculants (*Trabelsi* and *Mhamdi*, 2013). Co- inoculation of AMF and PGPR is of particular importance for Zn uptake compared to other micronutrients.

This study was formulated to evaluate the effect of co-inoculation of AMF with selected PGPR (include P solubilizers and Zn solubilizers) on the distribution of different Zn fractions in soil.

Materials and Methods

Experimental site

A field experiment was conducted at the Research Farm of Bihar Agricultural University, Sabour, Bhagalpur (Bihar) in the initial soil sample was collected from the research farm of Bihar Agricultural University, Sabour with the help of a spade from randomly selected places. The soil samples were air dried and sieved through a 2 mm IS sieve and stored in good quality polythene packets.

Experimental details

The experiment was laid out in a Randomized block design (RBD) with three replications. The test crop was Maize (Variety–DKC 9081). Details of treatments are given in Table 1.

Inoculum procurement and application

Inoculum of the AM species *Glomus mosseae* was a commercial product obtained from The Energy Resource Institute (TERI), New Delhi, India. It consisted of fragments of colonized roots and spores of AM fungi in a vermiculite substrate. The spores of mycorrhiza were multiplied with Sudan grass in a net house of Department of Soil Science, BAU. The inocula of AMF and PGPR were applied at the time of sowing before planting the seed at the 3.0 cm below from the seed. The PGPR used were collected from the Biofertilizer laboratory at BAU. These include *Burkholderia cepacia* and *Azospirillum brasilense* identified for possessing nutrient solubilization properties especially phosphorus and zinc.

Experimental soil collection and preparation

The surface soil samples (at 0-15 m depth) were collected from each treatment at flowering stage and after harvest of maize. For estimation of microbiological parameters, freshly collected samples were used, and for chemical analysis, the samples were air-dried, processed and passed through a 2.00 mm aperture sieve to remove roots, clods and debris.

Laboratory analysis of soil and plant samples

Initial soil analysis

Physico-chemical analysis of the initial experimental soil was done for parameters like soil pH, electrical Conductivity (dSm^{-1}), textural class, oxidisable organic carbon (%), mineralizable N (kg ha^{-1}), available P (kg ha^{-1}), available K (kg ha^{-1}) and DTPA-Zn (mg kg^{-1}). The data suggest that the experimental soil had a sandy loam texture, was alkaline in reaction (pH 7.69), non-saline (EC-0.28 dS/m), with low available N (165.16 kg ha^{-1}) status, medium P (18.11 kg ha^{-1}), medium K (144.25 kg ha^{-1}) and medium organic carbon content (0.59%). The soil was deficient in DTPA extractable Zn (0.52 ppm).

pH of the initial soil sample was done by soil: water (1:2.5) and followed by the Beckman Glass Electrode Method (Jackson, 1973).

Electrical conductivity of the initial soil sample was estimated through Conductivity Bridge method as described by *Jackson* (1973). Soil texture of initial soil was done by following the hydrometer methods described by Bouyoucos (1962). Organic carbon content of the soil was estimated by the Walkley and Black (1934) wet oxidation method described by Jackson (1973). Available soil nitrogen was estimated by alkaline permanganate oxidation method as outlined by Subbaiah and Asija (1956). Available phosphorus content of soil samples was estimated by Olsen's method (Jackson, 1973). Available potassium of soil samples was determined in 1:5 ammonium acetate extract of the soil using flame photometer (Jackson, 1973). DTPA method for extraction of available micronutrient in the soil as proposed by Lindsay and Norvell (1978) was used for determination of available soil Zn.

Sequential Zn fractionation

A modified sequential fractionation procedure was used to determine the distribution of soil Zn into various fractions in soil. Water soluble and Exchangeable *(WSEX-Zn)* was estimated by method proposed by Gupta and Chen (1975); the Organically bound *(OB-Zn)* by *Mc* Laren and Crawford (1973); Mn-oxides bound *(MnOB-Zn)* as described by Chao (1972); Amorphous Fe-oxides bound *(AmFeOB-Zn)* and Crystalline Fe-oxides bound *(CrFeOB-Zn)* by that of Shuman (1985).

Finally, the residual fraction was calculated by subtracting all other fractions of Zn from Total soil Zn content.

Total Zn content in soil and plant was determined by diacid digestion method (Nitric acid: Perchloric acid::9: 4) as outlined by Zoroski and Bureau (1977).

Zn uptake by plant

The Zn uptake (mg kg^{-1}) was calculated for each treatment separately using the following formula: Plant uptake (kg ha^{-1}) = [Plant nutrient content (%) x Biological yield (q ha^{-1})].

Grain uptake (kg ha^{-1}) = [Grain nutrient content (%) x Grain yield (q ha^{-1})].

Statistical analysis

The obtained field experiment data were analyzed by using standard analysis of variance (ANOVA) as described by Gomez and Gomez (1984) to determine the effects of various treatments. Critical difference (CD) at 5% level of probability and P values was used to examine differences among treatment means. They were also subjected to analysis of correlation (Gomez and Gomez, 1984) through the requisite statistical computations to predict the cause and effect relationship of various treatments and fractions with the productivity of Maize and nutrient uptake.

Results and Discussion

Effect of the treatments on redistribution of different fractions of Zn (mg kg^{-1}) in soil at flowering stage and harvesting stage

Information regarding the various chemical fractions of Zn in soil is necessary to understand its bioavailability to plants since different fractions contribute differently to the plant available pool. The understanding of the distribution of Zn among various fractions of soils helps to characterise the chemistry of Zn in soils and possibly its availability for plant uptake. The water soluble and exchangeable fraction has been reported to be readily available, while the organically bound, MnO_2 bound and amorphous Fe-oxides bound fractions are potentially available to plants. On the other hand, the crystalline Fe-oxide bound and the residual fractions are known to be unavailable to plants (Mandal and Mandal, 1986; Singh and Abrol, 1986). Sequential fractionation quantifies the element distribution between fractions of different binding strengths, as defined by properties of selected extractants. Reports on the dynamics of these fractions in soils subjected to co-inoculation of AMF with PGPR are scanty. In this experiment an attempt was made to this end.

Ammonium acetate was used to extract the water soluble and exchangeable fraction of Zn in soil. Hydroxylamine hydrochloride is a mild reducing reagent and has been found highly selective in mobilizing Zn from its oxides, or trace metals adsorbed on or occluded in manganese oxides (*Chao*, 1972). Pyrophosphate has been used to extract trace metals that are 'organically bound' or 'held by specific adsorption' on organic sites (Iyengar *et al.,* 1981; Miller *et al.,* 1986). Acid ammonium oxalate after extraction with hydroxylamine hydrochloride has been reported to dissolve amorphous iron oxides or hydroxides from soil (Smith and Mitchell, 1984; Shuman, 1986).

Figures 1 and 2 show that application of different treatments did not lead to any significant change in the Total and Residual Zn content of experimental soil. Values for water soluble-exchangeable and organically bound fractions of Zn were recorded to be significantly higher under the treatment T_6 *i.e.* AMF@10kg/ha + *Azospirillum brasilense* @20g/kg seed + *Burkholderia cepacia* @20 g/kg seed + 75%P (1.27 mg kg^{-1}) and T_5 *i.e.* AMF@10kg/ha + *Azospirillum brasilense* @20g/kg seed + 75%P (6.23 mg kg^{-1}) respectively at flowering stage and the said treatments registered values of 1.01 mg kg^{-1} and 5.61 mg kg^{-1} for water soluble-exchangeable and organic fractions respectively after harvesting. The oxide bound Zn fractions had higher values in the non-organic treatments *i.e.* Control (13.4 mg kg^{-1} at flowering and 12.3 mg kg^{-1} after harvesting) and 100% RDF (12.9 mg kg^{-1} at flowering and 11.4 mg kg^{-1} after harvesting). The content of zinc fractions in the soils was found to be in the following order: Residual Zn> AmFeOB-Zn> OB-Zn> MnOB-Zn> CrFeOB-Zn> WSEX-Zn. Residual fraction of Zn comprised of more than 80 % of total soil Zn while water soluble-exchangeable Zn was accounted to be the least dominant (about 1%) among all the Zn fractions studied. Zn in water soluble-exchangeable form is mobile and readily available for the plant. Samples collected at flowering stage as well as after harvesting recorded a similar pattern for sequential Zn fractions results.

Fig.1 Effect of different treatments on fractions of Zn at flowering stage

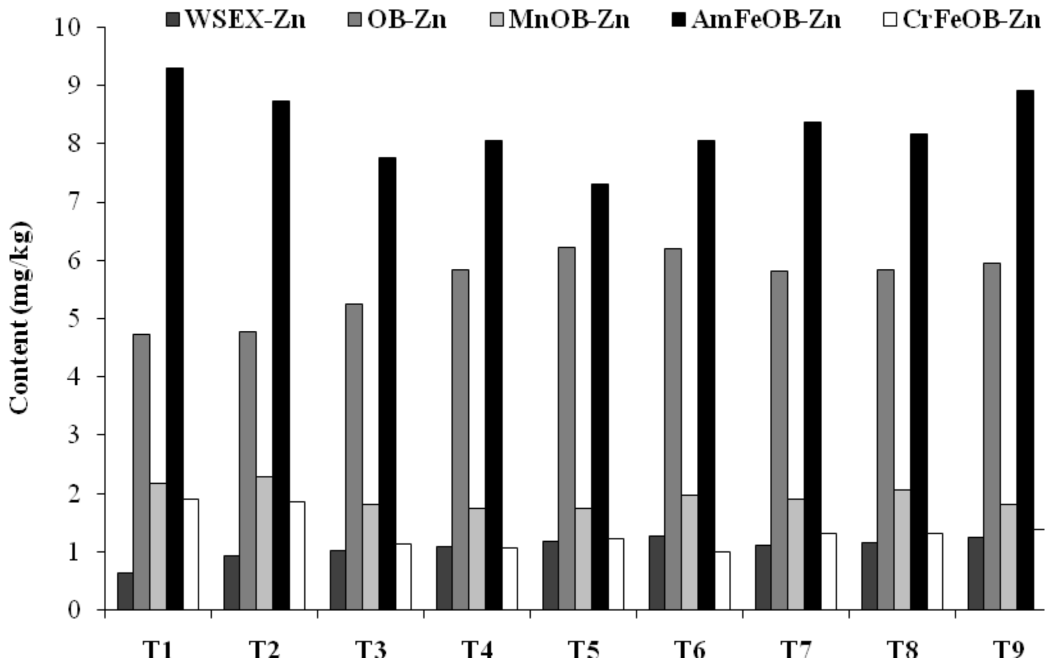

Fig.2 Effect of different treatments on fractions of Zn after harvesting

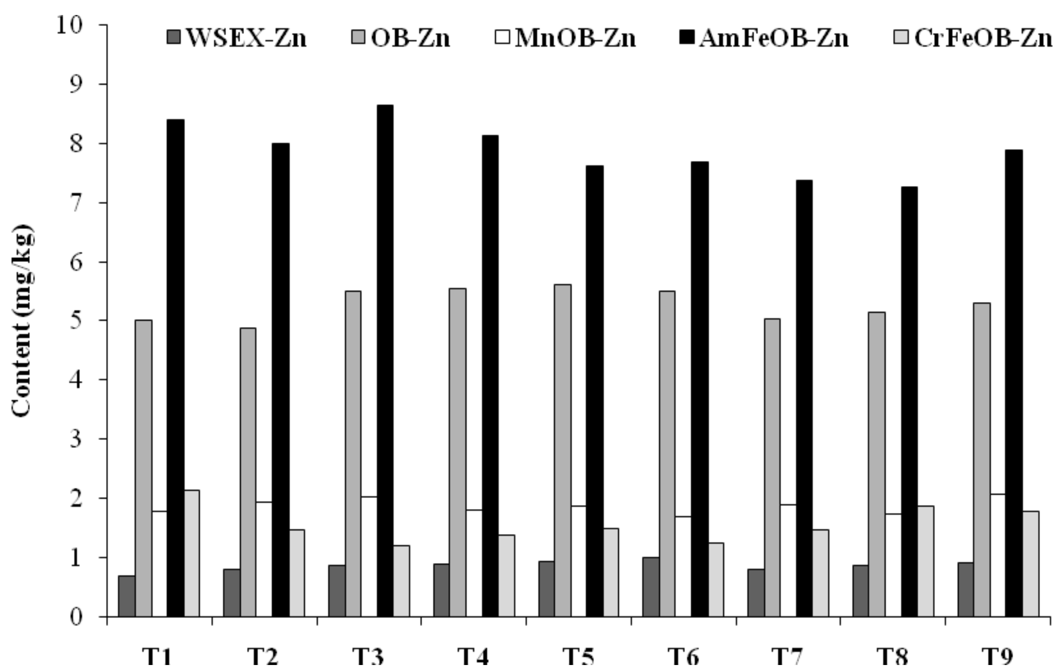

Fig.3 Effect of different treatments on Zn uptake by plant and grain

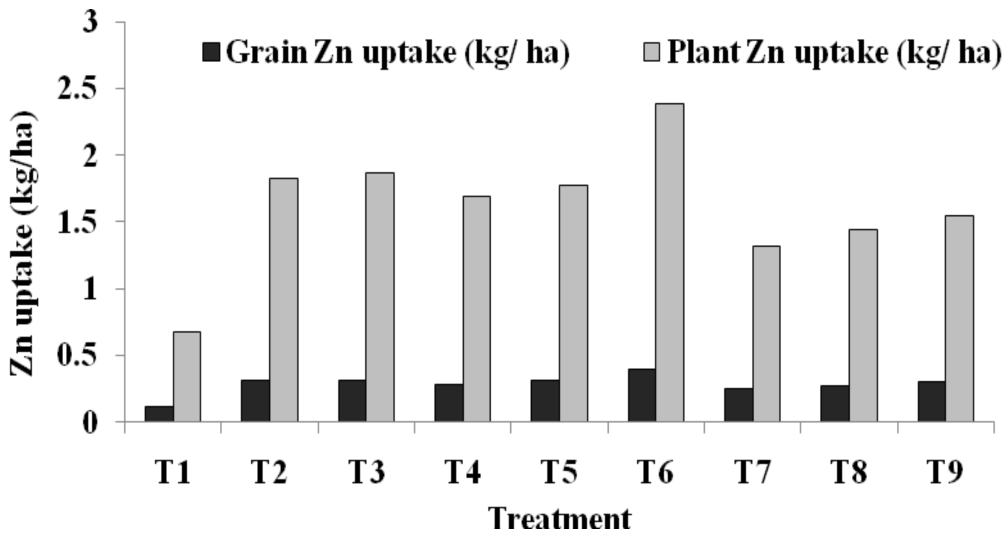

Table.1 Treatment details

Notation	Treatment Details
T_1	Control
T_2	100% Recommended Dose of Fertilizers (RDF)
T_3	AMF @10kg/ha + 75%P
T_4	AMF@10kg/ha + BC @20g/kg seed + 75%P
T_5	AMF@10kg/ha + AB @20g/kg seed + 75%P
T_6	AMF@10kg/ha + AB @20g/kg seed + BC@20 g/kg seed + 75%P
T_7	AMF@10kg/ha + BC @20g/kg seed + 50%P
T_8	AMF@10kg/ha + AB @20g/kg seed + 50%P
T_9	AMF@10kg/ha + AB @20g/kg seed + BC@20g/kg seed + 50%P

RDF: 120:80:60; AMF: *Glomus mosseae;* BC*: Burkholderia cepacia*; AB: *Azospirillum brasilense*

Table.2 Intercorrelations among different Zn fractions in soil

	WSEX Zn	OB Zn	MnOB Zn	AmFe OB Zn	CrFeOB Zn	Residual Zn	Total Zn
WSEX Zn	1.000						
OB Zn	0.882**	1.000					
MnOB Zn	-0.231	-0.400	1.000				
AmFeOBZn	-0.148	-0.194	0.590**	1.000			
CrFeOB Zn	-0.722**	-0.723**	0.323	0.237	1.000		
Residual Zn	0.140	0.160	-0.211	-0.437	-0.362	1.000	
Total Zn	0.325	0.332	0.046	-0.018	-0.360	0.862**	1.000

** Significant at 5% and ** Significant at 1% level of significance*

Table.3 Correlation of different zinc fractions with grain Zn content and uptake

	WSEX Zn	OB Zn	MnOB Zn	AmFeOB Zn	CrFeOB Zn	Residual Zn	Total Zn
Grain Zn	0.701**	0.545*	-0.237	-0.329	-0.522*	0.219	0.241
Zn uptake	0.751**	0.547*	-0.289	-0.501*	-0.810**	0.539*	0.455

Zinc uptake (kg/ha) by grain and plant

Figure 3 shows the Zinc uptake by grain and plant as a whole. The treatment T_6 *(G. mosseae* @10 kg ha^{-1} + *Azospirillum brasilense* @ 20.0 g kg $^{-1}$ seed+ *Burkholderia cepacia* @ 20.0 g kg^{-1} seed +75% P) showed significantly higher uptake of Zinc i.e. 0.39 kg/ha and 2.39 kg/ha by grain and plant respectively as compared to T_1 (control) condition and T_2 (100% RDF).

Correlation studies

Correlation matrices were prepared to get an insight into the intercorrelations among different zinc fractions in soil (Table 2), as well as to find out the important fractions of Zn in terms of their contribution towards plant Zn uptake (Table 3). When the content of different zinc fractions were correlated with Grain Zn content and its uptake (Table 3), it was found that the water soluble and exchangeable fraction was the major contributor towards grain Zn content and its uptake (R^2= 0.701** and 0.751** respectively for content and uptake) followed by organically bound Zn (R^2= 0.545* and 0.547* respectively for content and uptake). On the other hand, the oxide-bound Zn fractions (both, Mn oxide-bound as well as Fe-oxide bound) showed a negative correlation with grain Zn content and its uptake. It was also observed that the residual Zn fraction contributes significantly towards Zn uptake with R^2=0.539*. A highly positive and significant correlation was observed between Water soluble and exchangeable Zn and organically bound Zn (R^2=0.882**).

The data suggest that mycorrhizal co-inoculation orchestrates biochemical changes and Zn release pattern in soils, which might be responsible in facilitating the increased availability of Zn even under deficient conditions.

The results are in accordance with the findings of *Subramanian et al.,* (2009). Co-inoculation of AMF along with both the PGPR used increased the water soluble-exchangeable and organically bound Zn, while led to a comparative decline in the oxide-bound Zn fractions indicating enhancement in plant available Zn by slow transformation of unavailable forms into available forms.

Correlation matrices on the other hand give an insight into the intercorrelations among different zinc fractions as well as to find out the fractions of Zn important in terms of their contribution towards plant Zn uptake. The highly positive and significant correlation between water soluble-exchangeable Zn and organically bound Zn suggests that a depletion of Zn from the water soluble and exchangeable fraction is exclusively replenished by the organically bound fraction.

Also the water soluble and exchangeable Zn is negatively correlated with the oxide-bound fractions which suggests that the oxide-bound Zn has the least possibility to transform into the water soluble or available fractions.

The use of microbial inoculants plays an important role in sustainable agriculture. Arbuscular mycorrhizal fungi have immense potential to improve the nutritional status, growth and development of plants. The present study was formulated to evaluate the effect of co-inoculation of AMF (*G. mosseae*) with selected potentially nutrient solubilizing PGPR (*Burkholderia cepacia* and *Azospirillum brasilense*) on the redistribution of different Zn fractions in soil. AMF enhances the surface area of absorption for both nutrients (*viz.*, Zn and P) solubilized by both *B. cepacia* and *A. brasilense*. This mechanism is also responsible for the higher uptake of both the nutrients by the plant and consequently by the grain which is the economic part of the plant. The various treatments used in the study did not lead to any significant change in the Total and Residual Zn content.

Values for water soluble-exchangeable and organically bound fractions of Zn were recorded to be significantly higher under the co-inoculation treatment as compared to control and RDF while the oxide bound Zn fractions had higher values in the non-organic treatments in both the stages.

The content of zinc fractions in the soils was found to be in the following order: Residual Zn> AmFeOB-Zn> OB-Zn> MnOB-Zn> CrFeOB-Zn> WSEX-Zn. Co-inoculation of AMF along with both the PGPR used increased the organically bound Zn, while led to a comparative decline in the oxide-bound Zn fractions indicating enhancement in plant available Zn by slow transformation of unavailable forms into available forms.

Possessing individual capabilities to enhance the nutrient concentration in the rhizosphere, when the three microbial inoculants were co-inoculated, their efficiency increased which led to the higher values for potential and readily available Zn content in soil. After perusal of the results obtained from the experiment, it may be concluded that although the effects have to be revalidated over a longer period of time, they can be considered as positive signs and possible sustainable strategies towards improved Zn nutrition to plants.

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