

Effect of Graded Levels of Zn in Combination with or without Microbial Inoculation on Zn Transformation in Soil, Yield and Nutrient Uptake by Maize for Black Soil

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Abstract Field experiment was conducted in black soils in order to study the Zn transformation in soil, yield and nutrient uptake of maize using Arbuscular Mycorrhizal (AM) fungi and Zinc Solubilizing Bacteria (ZSB) in combination with graded levels of ZnSO₄. Treatment consisted of two factors viz., microbial inoculation (M₁: control, M₂: AM fungi, M₃: ZSB and M₄: M₂ + M₃) and graded levels of ZnSO₄ (S₁: 0, S₂: 12.5, S₃: 25, S₄: 37.5, S₅: 50 kg/ha⁻¹ and S₆: 0.5% foliar spray @ 45 and 65 DAS) replicated three times in RBD. The results revealed that the microbial inocula-

tion significantly increased the soil available Zn content at all stages of observation. Among the graded doses of Zn, the highest available Zn content was recorded with the treatment S₅ followed by S₄, S₃ and S₂ over the control. Increased availability of Zn in soil enhanced the maize grain and stover yield indicating response to added Zn under microbial inoculation. The Zn uptake by maize grain and stover also increased significantly for the treatment combinations.

Keywords Maize (*Zea mays* L.), Arbuscular Mycorrhizal (AM) fungi, Zinc Solubilizing Bacteria (ZSB), Graded levels of ZnSO₄, Transformation of Zn.

Introduction

In India, maize is grown in a wide range of environments, extending from extreme semiarid to sub humid and humid regions. It is grown in about 9.09 M ha and utilized for versatile purposes. It is a main source of calories and minerals for most rural populations. Zn is essential for development of immune system and brain function in humans and also plays an important role in enzymatic reactions and metabolic activities in plant system. Zinc deficiency is prevalent worldwide in temperate and tropical climates [1]. Forty seven percent of Indian soils and fifty per cent of Tamilnadu soils are deficient in zinc. Many factors influence supply of Zn to the plants, such as soil texture, soil or-

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Table 1. Initial soil characteristic of the experiment.

Sl. No.	Particulars	Black soil
1.	Taxonomy	<i>Typic Chromustert</i>
2.	Textural class	Clay
3.	pH (1:2.5 soil : water)	8.34
4.	EC (dS m ⁻¹)	0.30
5.	Free CaCO ₃ (g kg ⁻¹)	90.4
6.	Organic carbon (g kg ⁻¹)	5.10
7.	CEC (c mol (p ⁺) kg ⁻¹)	36.2
8.	Alkaline KmnO ₄ -N (kg ha ⁻¹)	221
9.	Olsen-P (kg ha ⁻¹)	20.0
10.	Neutral N NH ₄ Oac-K (kg ha ⁻¹)	245
11.	DTPA Zn (mg kg ⁻¹)	0.80
12.	DTPA Cu (mg kg ⁻¹)	2.52
13.	DTPA Fe (mg kg ⁻¹)	10.2
14.	DTPA Mn (mg kg ⁻¹)	3.45

ganic matter, soil EC, soil reaction, soil calcium carbonate, soil phosphorus, soil temperature and moisture. Customary application of inorganic zinc partially caters the plant need as 96—99% of applied Zn is converted into different insoluble forms depending upon the soil types and physicochemical reactions within 7 days of application. Microbes are potential alternate that could cater plant zinc requirement by solubilizing the complex zinc in soil. Microbes solubilize the metal forms by protons, chelated ligands, and oxidoreductive systems present on the cell surface and membranes. These bacteria also exhibit other traits beneficial to plants, such as production of phytohormones, antibiotics, siderophores,

vitamins, antifungal substances, and hydrogen cyanide, Zinc is immobile in soil and is taken up by plant mainly by diffusion, arbuscular mycorrhizal fungi also helps to increase the availability of Zn in soils as a consequence of rhizospheric acidification and siderophore production besides hyphal transport of nutrients through the external mycelium. Keeping these in view the present study is contemplated to study the Zinc transformation under Zn deficient black soil and its effect on yield and uptake of maize.

Materials and Methods

Experimental soil

The filed experiment was conducted at Cotton Research Station (TNAU), Perambalur. The soil was clay, deep black in color belonging to *Typic Chromustert* (Peelamedu series). The details of soil characteristics are given in Table 1. The initial soil was moderately alkaline, non saline, medium in organic status and low, low and medium in available N, P and K, respectively. With regards to the available micronutrient status of the soil, DTPA-Fe, DTPA-Cu and DTPA-Mn were found to be in sufficient level but the availability of DTPA-Zn was found to be deficient.

Field experiments

Treatment consisted of two factors viz., microbial inoculation (M₁: control, M₂: AM fungi, M₃: ZSB and M₄: M₂+M₃) and graded levels of ZnSO₄ (S₁: 0, S₂:

Table 2. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on DTPA-Zn (mg kg⁻¹) in black soil.

Treatments	Vegetative stage							Tasselling stage						
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean
M ₁	0.80	1.20	1.44	1.55	1.57	0.89	1.24	0.71	1.19	1.38	1.51	1.52	0.75	1.18
M ₂	1.07	1.38	1.56	1.66	1.66	1.07	1.40	0.96	1.30	1.50	1.63	1.64	0.95	1.33
M ₃	1.29	1.47	1.71	1.78	1.82	1.30	1.56	1.24	1.41	1.59	1.71	1.73	1.21	1.48
M ₄	1.48	1.90	2.14	2.26	2.26	1.45	1.92	1.41	1.83	2.06	2.18	2.19	1.30	1.83
Mean	1.16	1.49	1.71	1.81	1.82	1.18	1.53	1.08	1.43	1.63	1.75	1.77	1.05	1.46
		SEd			CD (0.05)				SEd			CD (0.05)		
M		0.02			0.04				0.02			0.03		
S		0.02			0.05				0.02			0.04		
M × S		0.05			0.10				0.04			0.08		

Table 2. Continued.

Treatments	Harvest stage						Mean
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	
M ₁	0.69	1.18	1.30	1.36	1.50	0.54	1.09
M ₂	0.90	1.28	1.45	1.61	1.61	0.95	1.30
M ₃	1.20	1.40	1.55	1.71	1.72	1.16	1.46
M ₄	1.39	1.80	2.06	2.17	2.18	1.35	1.83
Mean	1.04	1.41	1.59	1.71	1.75	1.00	1.42
		SEd		CD (0.05)			
M		0.02		0.04			
S		0.02		0.05			
M × S		0.04		0.10			

12.5, S₃: 25, S₄: 37.5, S₅: 50 kg ha⁻¹ and S₆: 0.5% foliar spray @ 45 and 65 DAS) replicated three times in RBD. Seeds of maize hybrids (NK 6240) were sown on the sides of the ridges by adopting a spacing of 60 × 25 cm along with vermiculite based mycorrhizal inoculum (*Glomus intraradices* TNAU-03-08) 2 g per hill at a depth of 5 cm. Zinc solubilizing bacteria was applied at 2 kg ha⁻¹ after mixing it with 25 kg each of sand and farm yard manure. The recommended fertilizer for maize viz., 250:75:75 kg N, P₂O₅ and K₂O kg ha⁻¹ was followed. The full dose of P and K were applied basally and N was applied at three splits viz., basal (25%), vegetative (50%) and tasselling (25%) stages. Calculated quantities of ZnSO₄ were applied basally as per the treatment schedule. The soil and plant samples were drawn at vegetative (30th), tasselling (60th) and harvest stage (110th) and analyzed for DTPA-Zn and Zn content respectively in soil and

plant samples. The grain and stover yields were recorded treatment wise and Zn uptake were computed.

Results and Discussion

Soil available Zn

The soil DTPA-Zn content was found to be gradually reduced from the vegetative stage to the harvest stage and this reduction might be due to the crop removal and other Zn transformation in soil (Table 2). Among the microbial inoculations, the highest value was recorded in the treatments which received AM fungi + ZSB (M₄) followed by ZSB (M₃) and AM fungi (M₂) over control (M₁). This treatment increased the soil DTPA-Zn content for M₄ (40.4%), M₃ (25.3%) and M₂ (16.2%) over absolute control. The data clearly indicated that the introduced mycorrhizal species *Glomus intraradices* inoculation had consistent favorable effect on availability of Zn in soil. The mycorrhizal colonization facilitates acidification of rhizosphere, solubilization of tightly bound residual form of zinc besides hyphal transport of metallic micronutrients collectively for the availability. These observations are in conformity with the observations of earlier reports [2]. Zinc solubilizing bacterium a *Bacillus* sp. produce organic acids viz., gluconic acids. Gluconic acid as the major anion may be an important agent that helped in the solubilization of insoluble Zn compounds resulting in enhanced availability of Zn. Among the graded doses of Zn, the highest available Zn content was recorded with the treatment S₅ (40.6%)

Table 3. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Maize grain and Stover yield (kg ha⁻¹).

Treatments	Grain yield							Stover yield						
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean
M ₁	5270	6697	7109	7289	7307	6496	6695	9450	13081	13996	14138	14584	12999	13041
M ₂	6607	6767	7325	7498	7576	6790	7094	12011	13661	14123	14972	15182	13046	13833
M ₃	6814	7205	7508	7625	7607	7031	7299	12997	14047	15068	15986	16011	13906	14669
M ₄	73.06	7688	7806	8473	8598	7496	7895	13367	15408	15982	18273	18741	14999	16061
Mean	6499	7090	7437	7721	7772	6953	7246	11956	14049	14692	15842	16130	13738	14401
		SEd		CD (0.05)				SEd		CD (0.05)				
M		46.8		149				114.9		366				
S		36.5		76				246.6		514				
M × S		81.4		199				464.6		1000				

Table 4. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Zinc uptake (g ha^{-1}) by grain

Treatments	Grain uptake							Stover uptake						
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean
M ₁	106	136	150	161	169	132	142	209	290	315	347	350	265	296
M ₂	134	145	163	175	184	138	156	255	328	366	370	389	279	331
M ₃	145	160	175	185	186	152	167	284	329	367	396	399	295	345
M ₄	148	170	183	199	206	159	177	289	341	376	435	441	333	368
Mean	133	153	168	180	186	145	161	259	322	356	387	395	293	335
		SEd		CD (0.05)				SEd		CD (0.05)				
M		4.29		9				5.71		12				
S		5.71		12				6.67		14				
M × S		11.50		24				13.3		28				

followed by S₄ (39.2%), S₃ (34.6%) and S₂ (26.2%) over the control. The increase of soil DTPA-Zn was spectacular up to 25 kg of ZnSO₄ ha⁻¹ in combination with AM fungi (M₂S₃) and Zinc solubilizing bacteria (M₃S₃), beyond that the increase was not so well pronounced. In case of M₄ (AM fungi + Zinc solubilizing bacteria) along ZnSO₄ application, the treatment M₄S₅ registered higher soil DTPA-Zn which was being comparable with M₄S₄.

Maize grain and stover yield

Application of Arbuscular mycorrhizal fungi (M₂), Zinc solubilizing bacteria (M₃) and their combinations (M₄) had significant influence over untreated control. The arbuscular mycorrhizal fungi + Zinc solubilizing bacteria (M₄) recorded the highest grain and stover yield and the increase in grain yields were 15.2% and the stover yields were 18.8% over control (Table 3). Mycorrhizal colonization would have been increased by the inoculation which would have facilitated the acidification of rhizosphere, solubilization of precipitated Zn compounds resulting in the improvement in the soil available Zn status leading to higher amount of nutrient uptake by the crop, translocated from source to sink which had reflected in the yield. This observation was also in line with the findings of Balakrishnan and Subramanian [3].

Zinc solubilizing bacteria in soil has the ability to produce gluconic acid which help in enhancing the supply of nutrient including Zn by dissolving the insoluble nutrients might be the reason for increased

yield for the application of Zinc solubilizing bacteria which also contributed to the yield increase 8.3% for the inoculation of ZSB (M₃). Similar results were earlier reported by Saravanan et al. [4].

It is quite obvious that the response for added Zn in maize crop under Zn deficient soils. Graded levels of ZnSO₄ application increased the maize grain and stover yield correspondingly. It is noteworthy that the crop responded significantly up to 37.5 kg of ZnSO₄ ha⁻¹ as soil application, beyond that the response was not spectacular. The yield increase was 15.8% over control. The reason for the increasing yield might be due to Zn application favored the metalloenzymes systems, regulatory function, auxin production and extended period of photosynthesis which improve the production of carbohydrate and their transportation to the seeds. The result of the present study also in agreement with Kanwal et al. [5] under calcareous soil and Mari et al. [6] under clayey soil. Foliar application of ZnSO₄ had increased the grain yield over control (6.5%). The increased yield might be due to the beneficial effect of Zn in the plant system. Khan et al. [7] stated that the cumulative beneficial effect of Zn on growth and yield attributing characters would finally reflect on grain yield of maize.

Zn uptake

The results had shown that the application of AM fungi + Zinc solubilizing bacteria (M₄) had resulted in significantly higher uptake of Zn than the uninoculated check. The maize grain uptake of Zn was in-

creased to 40.1% for the application of AM fungi + ZSB (M_4). The same trend was also observed for the uptake of Zinc by maize stover also (Table 4). These results corroborate with the findings of Kaur et al. [8] and Kanimozhi et al. [9] who reported the increased nutrients uptake due to the application of microbial inoculation to the crop. Regarding Zn fertilizer leaves, graded levels of Zn application enhanced the nutrient uptake correspondingly. However, the increase was not significant beyond 37.5 kg of $ZnSO_4$ ha⁻¹. The probable reason might be due to the increase in soil nutrient status which favored the enhanced uptake. Similar findings were also reported by Muthumanickam et al. [10].

References

1. Slaton NA, Normon RJ, Wilson JCE (2005) Effect of Zn source and application. *J Ind Soc Soil Sci* 44 : 562—581.
2. Liu AC, Hamel RL, Hamilton BL, Ma Smith DL (2004) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza* 9 : 331—336.
3. Balakrishnan N, Subramanian KS (2012) Mycorrhizal symbiosis and bioavailability of micronutrients in maize grain. *Maydica* 57 : 129—138.
4. Saravanan VS, Subramanian R, Anthoni Raj (2003) Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. *Brazilian J Microbiol* 4 : 121—125.
5. Kanwal S (2009) Zinc requirement of maize cultivars on alkaline calcareous soils. PhD thesis. Dept Soil Sci Inst of Soil & Environm Sci Univ Agric, Faisalabad, Pakistan.
6. Mari GF, Prado RM, Soares AVL, Caione G, Silva CN (2015) Residual effect of zinc application doses and methods on nutrition and productivity of corn. *Am J Pl Sci* 6 : 298—305.
7. Khan HZ, Abdullah M, Amin N, Akbar MF, Saleem Iqbal A (2015) Impact of zinc and manganese application to increase productivity of autumn planted maize (*Zea mays* L.). *Cercetari Agronomice in Moldova* 47 : 65—70.
8. Kaur R, Singh A, Kang JS (2014) Influence of Different Types Mycorrhizal Fungi on Crop Productivity. *Curr Agri Res Jour* 2 : 51—54.
9. Kanimozhi A, Santhy P, Chandra sekaran N, Subramanian KS (2015) Response of maize plants to mycorrhizal colonization at varying level of phosphorus fertilization. In: Proc the Nat Sem on “Soil resilience-2015”. Madurai, pp 146—147.
10. Muthumanickam D, Stalin P, Shukla AK (2015) Enhancing use efficiency and crop productivity of maize through Zn and B Application. In: Proc Nat Sem on “soil resilience-2015”. Madurai, pp 4445.