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RESEARCH PAPER

Effect of Zn fertilizer in combinations with arbuscular mycorrhizal fungi and zinc solubilising bacteria on micronutrients transformations and uptake in maize grown on calcareous soils

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Abstract A field experiment was conducted in black soils in order to study the micronutrients *viz.,* iron (Fe), Manganese (Mn) and copper (Cu) transformation in soil under maize cultivation and their uptake of by maize using Arbuscular Mycorrhizal (AM) fungi and Zinc Solubilizing Bacteria (ZSB) in combination with graded levels of $ZnSO_4$. Treatment consisted of two factors *viz.,* microbial inoculation (M_1: control, M_2: AM fungi, M_3: ZSB and M_4: M_2+M_3) and graded levels of $ZnSO_4$ (S_1: 0, S_2: 12.5, S_3: 25, S_4: 37.5, S_5: 50 Kg ha^{-1} and S_6: 0.5% foliar spray @ 45 and 65 days after sowing) replicated three times in randomized block design (RBD). The results revealed that the microbial inoculation significantly increased the soil available micronutrients Fe, Mn and Cu content at vegetative, flowering and harvesting stages of maize crop. Among the graded doses of Zn, the highest available micronutrients content was recorded with the treatment S_5 followed by S_4, S_3 and S_2 over the control. The application of M_4S_5 resulted in higher DTPA extractable micronutrients Fe, Mn, and Cu compared to the other treatment combinations. Application of AM fungi + ZSB (M_4) registered the highest uptake of micronutrients by grain. Graded levels of Zn application enhanced the nutrient uptake correspondingly. However, beyond 25 kg of $ZnSO_4$ ha^{-1}, the increase in Fe, Mn and Cu was not statistically significant.

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Introduction

Maize (*Zea mays* L.) is the third most important cereal crop after wheat and rice worldwide, as well as in India. It is cultivated in both tropical and temperate regions. Maize is often called as "Queen of cereals" and "miracle crop" because of its higher productive potential compared to other cereal crops. Maize is vital cereal feed for livestock and a critical staple food in various forms (Suganya, 2015). In India, it is cultivated on 9.2 m ha with the productivity of 3.0 t ha^{-1} (DACNET, 2020). On average, maize grain is composed of 60% carbohydrate, 10% protein, 4.5% oil, 3.5% fibre and 2% minerals, making it an important source of calories and minerals (Suganya *et al.*, 2020).

Micronutrients, required in small quantity, are essential for the production of hormones, enzymes and other essential substances for plant growth (Ayyar *et al.*, 2019). Elements such as iron, manganese, and copper play crucial roles in maize physiology for instance, iron is involved in redox reactions and forms part of structural components like porphyrin, while manganese activates various enzymes, and copper is vital for photosynthetic and respiratory electron transport (Nurul *et al.,* 2014). Deficiencies or toxicities of these micronutrients can adversely affect plant yield and overall health. Given that calcareous soils are often deficient in micronutrients due to factors such as pH, texture, and organic matter content, alternative strategies such as microbial inoculation to enhance micronutrient solubilization are of significant research interest (Harender, 2019). Micronutrient is immobile in soil and is taken up by plant mainly by diffusion, arbuscular mycorrhizal fungi also helps to increase the availability of micronutrients in soils as a consequence of rhizospheric acidification and siderosphore production besides hyphal transport of nutrients through the external mycelium (Ayyar and Appavoo, 2017; Suganya and Saravanan, 2016). This study, therefore, investigates the effect of zinc fertilizer in combination with AM fungi and ZSB on micronutrient transformation and uptake in maize cultivated on calcareous black soils.

Materials and methods

Experimental site and soil characteristics

The field experiment was conducted at the Cotton Research Station (TNAU), Perambalur. The experimental soil was clayey, deep black in colour belonging to *Typic Chromustert* (Peelamedu series). The detailed characteristics are given in Table 1. The initial soil conditions were moderately alkaline and non – saline, with a medium level of organic matter. Nutrient analysis revealed low to medium availability of nitrogen (N), phosphorus (P), and potassium (K), while the DTPA – extractable micronutrient levels indicated the zinc deficient relative to iron, copper, and manganese.

Treatment design and crop management

The experiment was arranged in a randomized block design (RBD) with three replications. The Treatments consisted of two factors *viz.,* microbial inoculation (M_1: control, M_2: AM fungi, M_3: ZSB and M_4: M_2+M_3) and graded levels of $ZnSO_4$ (0, 12.5 25, 37.5, 50 Kg ha^{-1} and 0.5% foliar spray @ 45 and 65 DAS). Seeds of maize hybrids (NK 6240) were sown on the sides of the ridges at a spacing of 60 x 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, P_2O_5 and K_2O 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 $ZnSO_4$ were applied basally as per the treatment schedule.

Table 1. Initial soil characteristic of the experiment

S.No.	Particulars	Black soil
1.	Taxonomy	*Typic Chromustert*
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_4Oac- 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

Sampling and analytical methods

Composite surface (0-15 cm) soil samples were collected from the site before the experiment and analysed for their physical, physio – chemical and chemical properties. Soil samples were collected at three key growth stages: vegetative (30 days after sowing), tasselling (60 days after sowing) and harvest (110 days after sowing). The samples were air dried and passed through 2 mm sieve prior to analysis. The pH and EC were determined in 1:2.5 (soil:water) suspension using digital pH meter. Organic carbon (OC) content of soil was determined by chromic acid wet oxidation method (Walkley and Black, 1934); available N content in soil was determined by alkaline permanganate method (Subbiah and Asija, 1956); available P content in soil was estimated using olsen's extraction method (Olsen *et al.,* 1945); available K in soil was analysed using neutral normal ammonium acetate method as outlined by Jackson (1973); exchangeable calcium and magnesium in soil was determined by versenate titration – neutral normal ammonium acetate extract (Jackson, 1973) and available micronutrient (Zn, Cu, Fe and Mn) in soil was estimated by Atomic Absorption Spectrophotometer (AAS) after extracting soil samples with DTPA (Diethelene Triamine Penta Acetic Acid) extractant (Linday and Norwell, 1978).

Plant samples, including both grain and stover, were shade –dried for 72 hours, oven – dried, ground, and then analyzed for micronutrient content using Triacid extraction method followed by atomic absorption spectrophotometer (Jackson,1973). Grain and stover yields were recorded for each treatment, and zinc uptake was calculated based on nutrient concentration and dry matter production. Data were statistically analysed using ANOVA package method of Gomez and Gomez (1984) and least significant difference (LSD) at 5% probability level was computed to compare the treatments.

Result and discussion

Micronutrients Transformation in soil

The soil micronutrients *viz.,* Fe, Mn and Cu contents were 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 micronutrient transformations in soil (Suganya *et al.,* 2020).

The soil DTPA-Fe content of the black soil during vegetative stage ranged from 15.2 to 18.8 mg kg^{-1}. The highest soil DTPA- Fe was noticed under M_4 (17.8 mg kg^{-1}) followed M_3 (17.4 mg kg^{-1}), M_2 (17.1 mg kg^{-1}) and M_1 (16.8 mg kg^{-1}). The graded levels of Zn application had significantly increased the soil DTPA-Fe. Higher dose of $ZnSO_4$ @ 50 kg ha^{-1} (S_5) recorded higher soil DTPA-Fe 18.2 mg kg^{-1} was on par with S_4 (18.1 mg kg^{-1}) followed S_3 (17.8 mg kg^{-1}) and S_2 (17.1 mg kg^{-1}). The spray treatment S_6 (16.2 mg kg^{-1}) and control S_1 (16.2 mg kg^{-1}) had similar effect on soil DTPA- Fe. The interaction effect of M X S on DTPA-Fe was found to be significant. The trend of results during the tasselling and post harvest stage of crop on soil DTPA-Fe was similar to that of vegetative stage.

For manganese, during the vegetative stage, treatments involving AM fungi (M2) and the combination of AM fungi and ZSB (M4) significantly increased the soil DTPA-Mn compared to the control. Highest soil DTPA-Mn was noticed for M_4 (11.01 mg kg^{-1}) followed by M_3 (10.91 mg kg^{-1}) and M_2 (10.86 mg kg^{-1}) while M_1 (10.79 mg kg^{-1}) had lowest soil DTPA- Mn. With regards to graded doses of $ZnSO_4$ soil application, the treatment S_5 registered highest soil DTPA-Mn (11.00 mg kg^{-1}) which was on par with S_4 (10.98 mg kg^{-1}) and S_3 (10.95 mg kg^{-1}) followed by S_2 (10.87 mg kg^{-1}). The spray treatment S_6 (10.77 mg kg^{-1}) and control S_1 (10.77 mg kg^{-1}) had similar effect on soil DTPA-Mn. The interaction effect of M X S on soil DTPA-Mn was not significant. Similar trend was observed at harvest stage.

Table 2. Graded levels of Zn with arbuscular mycorrhizal fungi and zinc solubilizing bacteria on DTPA- Fe (mg kg^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest 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	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	15.2	16.6	17.6	17.7	17.9	15.6	16.8	15.1	16.2	17.2	17.4	17.6	15.5	16.5	15.0	16.1	17.1	17.2	17.5	15.3	16.4
M_2	16.0	16.9	17.7	17.8	18.0	16.1	17.1	15.9	16.8	17.4	17.6	17.9	16.1	17.0	15.7	16.5	17.3	17.5	17.8	16.0	16.8
M_3	16.6	17.3	17.9	18.2	18.2	16.3	17.4	16.3	17.1	17.7	18.1	18.2	16.5	17.3	16.2	16.9	17.5	18.0	18.0	16.2	17.1
M_4	17.1	17.5	18.1	18.6	18.8	16.8	17.8	17.0	17.1	17.9	18.2	18.3	17.1	17.6	16.3	16.9	17.5	18.1	18.0	16.8	17.3
Mean	16.2	17.1	17.8	18.1	18.2	16.2	17.3	16.1	16.8	17.6	17.8	18.0	16.3	17.1	15.8	16.6	17.4	17.7	17.9	16.1	16.9
		SEd			CD (0.05)				SEd			CD (0.05)				SEd			CD (0.05)		
M		0.05			0.1				0.04			0.1				0.04			0.1		
S		0.10			0.2				0.14			0.3				0.11			0.2		
M X S		0.12			0.2				0.10			0.2				0.10			0.2		

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – M_2 + M_3
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

Table 3. Graded levels of Zn with arbuscular mycorrhizal fungi and zinc solubilizing bacteria on DTPA - Mn (mg kg^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest 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	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	10.59	10.83	10.88	10.92	10.92	10.59	10.79	10.58	10.83	10.86	10.90	10.91	10.58	10.78	10.53	10.76	10.80	10.87	10.89	10.55	10.73
M_2	10.74	10.85	10.92	10.94	10.95	10.75	10.86	10.73	10.83	10.90	10.91	10.93	10.58	10.81	10.70	10.76	10.87	10.89	10.91	10.69	10.80
M_3	10.85	10.87	10.94	10.97	10.98	10.86	10.91	10.83	10.84	10.89	10.94	10.95	10.82	10.88	10.81	10.82	10.89	10.91	10.93	10.81	10.86
M_4	10.90	10.95	11.05	11.10	11.16	10.91	11.01	10.92	10.94	10.96	11.07	11.09	10.91	10.98	10.87	10.92	10.93	10.96	10.99	10.88	10.92
Mean	10.77	10.87	10.95	10.98	11.00	10.77	10.89	10.76	10.86	10.90	10.95	10.97	10.72	10.86	10.73	10.81	10.87	10.91	10.93	10.73	10.83
		SEd		CD (0.05)					SEd		CD (0.05)					SEd		CD (0.05)			
M		0.08		0.16					0.09		0.16					0.08		0.13			
S		0.10		0.21					0.11		0.24					0.08		0.13			
M X S		0.21		NS					0.24		0.41					0.15		NS			

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – $M_2 + M_3$
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

Table 4. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on DTPA - Cu (mg kg^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest 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	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	2.52	2.55	2.56	2.57	2.58	2.52	2.55	2.50	2.54	2.54	2.54	2.53	2.50	2.52	2.49	2.52	2.53	2.54	2.55	2.50	2.52
M_2	2.56	2.58	2.60	2.63	2.64	2.54	2.59	2.53	2.57	2.56	2.60	2.61	2.53	2.57	2.51	2.56	2.56	2.58	2.60	2.53	2.56
M_3	2.56	2.58	2.62	2.63	2.64	2.55	2.60	2.56	2.58	2.60	2.62	2.63	2.56	2.59	2.55	2.56	2.58	2.59	2.60	2.54	2.57
M_4	2.58	2.60	2.63	2.64	2.65	2.57	2.61	2.57	2.60	2.61	2.61	2.62	2.57	2.60	2.57	2.58	2.60	2.60	2.60	2.56	2.58
Mean	2.55	2.58	2.60	2.62	2.63	2.54	2.59	2.54	2.57	2.57	2.59	2.60	2.54	2.57	2.53	2.56	2.57	2.58	2.58	2.53	2.56
		SEd		CD (0.05)					SEd		CD (0.05)					SEd		CD (0.05)			
M		0.02		0.05					0.01		0.01					0.003		0.01			
S		0.03		0.07					0.01		0.01					0.004		0.01			
M X S		0.06		0.14					0.01		NS					0.008		NS			

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – $M_2 + M_3$
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

Table 5. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Iron uptake (g ha^{-1}) by grain and stover

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	351	456	504	535	529	451	471	1292	2053	2501	2688	2653	1855	2173
M_2	446	488	518	551	553	482	506	1678	2052	2457	2632	2616	2004	2240
M_3	454	473	523	536	549	492	504	1853	2321	2536	2773	2751	2127	2393
M_4	489	536	554	605	615	513	552	1858	2586	2652	3173	3195	2485	2658
Mean	435	488	524	557	561	484	508	1670	2253	2536	2816	2803	2118	2366
		SEd			CD (0.05)				SEd			CD (0.05)		
M		4.44			9				47.4					
S		5.44			11				58.05					
M X S		10.87			22				116.09					

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – M_2 + M_3
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

Table 6. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Manganese uptake (g ha^{-1}) by grain and stover

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	36	43	44	45	45	41	42	279	307	317	322	330	282	306
M_2	41	43	46	48	51	41	45	295	323	329	352	356	311	328
M_3	44	47	50	53	53	45	49	328	340	361	380	382	331	354
M_4	47	52	54	60	60	50	54	341	379	382	449	463	359	395
Mean	42	46	49	51	53	44	47	311	337	347	376	383	321	346
		SEd			CD (0.05)				SEd			CD (0.05)		
M		1.90			4				10.8			23		
S		2.38			5				13.3			27		
M X S		3.81			8				26.5			54		

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – M_2 + M_3
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

Table 7. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Copper uptake (g ha^{-1}) by grain and stover

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	26	34	37	39	40	33	35	101	120	122	126	133	105	116
M_2	33	35	41	42	44	33	38	120	125	131	141	147	124	131
M_3	33	36	39	44	46	35	39	133	138	144	153	161	135	144
M_4	35	39	44	49	51	37	42	137	145	154	162	166	146	152
Mean	31	36	40	44	45	34	38	123	129	138	146	152	128	136
		SEd			CD (0.05)				SEd			CD (0.05)		
M		1.38			3				2.86			6		
S		1.71			4				3.33			7		
M X S		3.48			7				3.81			8		

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – M_2 + M_3
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

Regarding copper, the effect of microbial inoculation was significant; treatments with AM fungi (M2), ZSB (M3), and their combination (M4) all increased soil DTPA-Cu. M_4 had highest soil DTPA-Cu 2.61 mg kg^{-1} which was on par with M_3 (2.60 mg kg^{-1}) followed by M_2 (2.59 mg kg^{-1}) which had similar effect as that of M_1 (2.55 mg kg^{-1}). Increased levels of $ZnSO_4$ application increased the soil DTPA-Cu significantly. Highest DTPA-Cu was recorded for S_5 (2.63 mg kg^{-1}) which was on par with S_4 (2.62 mg kg^{-1}), S_3 (2.60 mg kg^{-1}) and S_2 (2.58 mg kg^{-1}) indicating that the soil application of Zn had no influence on soil DTPA-Cu. However, the soil application had higher soil DTPA-Cu than the $ZnSO_4$ spray S_6 (2.54 mg kg^{-1}) and control S_1 (2.55 mg kg^{-1}) both which was on par. Although the interaction between microbial inoculation and $ZnSO_4$ was significant for DTPA – Cu at certain stages, its effect was less pronounced during the tasselling and harvest stages.

The available micronutrient *viz.,* Fe, Mn and Cu increased in the microbial inoculation combination with graded doses of $ZnSO_4$ application in black soil (Table 2, 3 and 4). The soil available micronutrients *viz.,* Fe, Mn and Cu were found to be increased for the application of M_4 (AM fungi + ZSB) followed by M_3 (ZSB) and M_2 (AM fungi) during vegetative, tasselling and harvesting stages of crop growth periods of maize. Mycorrhizal symbiosis improves the biological activities of soil that would have helped to release wide range of organic acid and siderophores which assists in the availability of micronutrient (Ayyar and Appavoo, 2017). This is in confirmation with Kanimozhi *et al.* (2015) who reported a significant increase in the soil available micronutrient content in the soil due to the AM fungi inoculation. The increase availability of soil available micronutrients might be due to the solubilization by ZSB. Zinc solubilizing bacteria produced organic acid like gluconic acid it is solubilized the applied fertilizers and native resource in the soil (Suganya *et al.*, 2020). Consequentially, Zinc solubilizing bacteria increased the availability of micronutrients in black soil (calcareous soil). Irrespective of microbial inoculations, the highest soil available micronutrients *viz.,* Fe, Mn and Cu were registered for S_5 followed by S_4, S_3 and S_2 at vegetative, tasselling and harvesting stage of maize crop. It is quite expected that the graded levels of Zn application was reflected in soil available micronutrient at all the stages of observation. Acid forming fertilizer applications in calcareous soil

Table 8. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on total Fe uptake (g ha^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest 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	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	91	123	171	225	228	92	155	612	912	1330	1764	1765	698	1180	1641	2509	3005	3222	3182	2306	2644
M_2	104	146	203	231	234	125	174	804	1140	1594	1870	1875	999	1380	2124	2540	2974	3183	3169	2485	2746
M_3	128	187	251	306	311	155	223	1021	1376	1791	2167	2159	1209	1619	2306	2793	3056	3309	3299	2619	2897
M_4	149	230	328	375	370	194	274	1241	1798	2194	2538	2545	1589	1984	2347	3121	3206	3778	3810	2998	3210
Mean	118	172	238	284	286	141	207	920	1306	1725	2085	2086	1124	1541	2104	2741	3060	3373	3365	2602	2874
		SEd		CD (0.05)					SEd		CD (0.05)					SEd			CD (0.05)		
M		18.1		37					30.0		62					56.7			116		
S		22.2		49					36.2		75					69.5			147		
M X S		44.5		NS					72.3		NS					138.9			283		

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – M_2 + M_3
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

Table 9. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on total Mn uptake (g ha^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest 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	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	40	46	56	56	59	43	50	110	106	114	137	151	105	120	314	350	361	366	374	322	348
M_2	44	46	51	57	62	45	51	112	118	121	132	153	119	126	336	365	375	400	407	353	373
M_3	45	52	55	55	63	49	53	109	124	122	127	142	115	123	372	387	412	433	435	376	402
M_4	53	55	60	62	72	57	60	129	162	193	198	220	133	172	389	430	436	509	524	408	450
Mean	45	49	55	58	64	48	53	115	127	137	148	166	118	135	353	382	396	427	435	365	393
		SEd			CD (0.05)				SEd			CD (0.05)				SEd			CD (0.05)		
M		1.96			4				4.75			10				11.09			23		
S		2.40			5				5.82			12				13.62			29		
M X S		4.79			NS				11.63			NS				27.24			57		

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – M_2 + M_3
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

Table 10. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on total Cu uptake (g ha^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest 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	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	8.2	8.6	10.4	11.9	13.3	8.7	10.1	50	65	74	91	105	52	73	127	143	159	165	173	138	151
M_2	7.3	9.6	12.2	12.2	13.2	7.9	10.4	56	69	79	97	113	64	79	153	160	171	184	191	157	169
M_3	8.6	10.8	13.5	13.4	16.5	10.5	12.2	66	86	86	102	114	70	87	166	173	183	197	207	170	183
M_4	10.2	12.2	15.7	16.1	19.9	11.9	14.3	88	113	113	118	129	91	108	171	184	198	211	217	182	194
Mean	8.6	10.3	12.9	13.4	15.7	9.7	11.8	65	83	88	102	115	69	87	154	165	178	189	197	162	174
		SEd			CD (0.05)				SEd			CD (0.05)				SEd			CD (0.05)		
M		0.56			1.2				3.56			7				17.0			36		
S		0.69			1.4				4.36			9				21.0			NS		
M X S		1.38			NS				8.72			NS				42.1			NS		

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – M_2 + M_3
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

Table 11. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Maize grain yield (kg ha^{-1})

Treatments	Black soil						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	5270	6697	7109	7289	7307	6496	6695
M_2	6607	6767	7325	7498	7576	6790	7094
M_3	6814	7205	7508	7625	7607	7031	7299
M_4	7306	7688	7806	8473	8598	7496	7895
Mean	6499	7090	7437	7721	7772	6953	7246
		SEd			CD (0.05)		
M		46.8			149		
S		36.5			76		
M X S		81.4			199		

M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – M_2 + M_3
S_1 - Control; S_2 – $ZnSO_4$ @ 12.5 kg ha^{-1}; S_3 - $ZnSO_4$ @ 25 kg ha^{-1}; S_4 - $ZnSO_4$ @ 37.5 kg ha^{-1};
S_5 - $ZnSO_4$ @ 37.5 kg ha^{-1}; S_6 – 0.5% $ZnSO_4$ Foliar spray @ 45 and 65 DAS

reduce the soil pH, which in turn increase the availability of micronutrients in calcareous soil.

Micronutrients uptake

Micronutrient uptake by maize depends on both the availability of nutrients in the soil and the plants dry matter accumulation. The results had shown that the application of AM fungi + Zinc solubilizing bacteria (M_4) had resulted in significantly higher uptake of micronutrients *viz.,* Fe, Mn and Cu than the uninoculated check (Table 5, 6 and 7). The application of M_4 (AM fungi + ZSB) was able to produce high yield (Table 11) which may be the reason for higher uptake of nutrients by the crop. The uptake of Fe by the grain was increased to the tune of 36.4% for black. The increase in Mn uptake by grain was increased 33.3% while the increase in Cu uptake by grain 38.1% over control. The same trend was also observed for the uptake of micronutrients by maize stover also. Similarly the trends of results were observed in total uptake of micronutrients *viz.,* Fe, Mn and Cu for maize crop (Table 8, 9 and 10). These results corroborate with the findings of Kaur *et al*. (2014) and Kanimozhi *et al.* (2015) who reported the increased nutrients uptake due to the application of microbial inoculation to the crop. Regarding Zn fertilizer levels, 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.* (2015) and Suganya *et al*. (2021).

Conclusion

This study aimed to provide to understanding the uptake of Fe, Mn and Cu. The results revealed that the microbial inoculation significantly increased the soil available micronutrients Fe, Mn and Cu content at vegetative, flowering and harvesting stages of maize crop. Among the graded doses of Zn, the highest available micronutrients content was recorded with the treatment S_5 followed by S_4, S_3 and S_2 over the control. The application of M_4S_5 resulted in higher DTPA extractable micronutrients Fe, Mn, and Cu compared to the other treatment combinations. Application of AM fungi + ZSB (M_4) registered the highest uptake of micronutrients by grain. Graded levels of Zn application enhanced the nutrient uptake correspondingly. However, beyond 25 kg of $ZnSO_4$ ha^{-1}, the increase in Fe, Mn and Cu was not statistically significant. Application of Zinc solubilizing bacteria and Arbuscular mycorrhizal fungi enhance the availability of Fe, Mn and Cu. The plant growth parameters and yield parameter improved, and which favored to enhanced the uptake of Fe, Mn and Cu.

References

Ayyar, S. & Appavoo, S. (2017). 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. *Environment & Ecology*, **35**(1): 172-176.

Ayyar, S., Appavoo, S., Basker, M., Pandiyarajan, P. & Kavimani, R. (2019). Effect of zinc and microbial inoculation on soil enzyme activities for maize (*Zea mays* L.) in black soil. *International Journal of Current Microbiology and Applied Sciences*, **8**(8): 1804-1814.

DACNET. (2020). India maize scenario report 2020-21. Department of agriculture and cooperation, Indian Institute of Maize Research.

Gomez, K. A. & Gomez, A. A. (1984). Statistical Procedures for Agricultural Research, Pub: John Wiley and Sons, New Delhi. p 680.

Harender, Kapil, M., Naveen, R., Kavinder, Raman, S. & Shishram J. (2019). Role of micronutrients in maize production under haryana ecological conditions. *Advances in Agriculture and Biodiversity*, 177-179.

Jackson, M. L. (1973). Soil chemical analysis. Prentice Hall of India Private Ltd., New Delhi, pp. 56-70.

Kanimozhi, A., Santhy, P., Chandra Sekaran, N. & Subramanian, K. S. (2015). Response of maize plants to mycorrhizal colonization at varying level of phosphorus fertilization. *In:* Proceedings of the National Seminar on "soil resilience-2015" held at Madurai, pp. 146-147.

Kaur, R., Singh, A. & Kang. J. S. (2014). Influence of different types mycorrhizal fungi on crop productivity. *Current Agriculture Research Journal*, **2**(1): 51-54.

Lindsay, W. L. & Norvell, W. A. (1978). Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal*, **42**: 421-428.

Muthumanickam, D., Stalin, P. & Shukla, A. K. (2015). Enhancing use efficiency and crop productivity of maize through Zn and B application. In: Proceedings of the National Seminar on "soil resilience-2015" held at Madurai, pp. 44-15.

Nikhil, K. & Salakinkop, S. R. (2018). Agronomic Biofortification of Maize with Zinc and Iron Micronutrients. *Modern Concepts & Developments in Agronomy*, 1(5): 87-90.

Nurul, S., Sarwar, J., Mohammad, M. K., Nashriyah, M., Mohd, K., Mohd, N. & Mohd, H. R. (2014). Application of copper increased corn yield through enhancing physiological functions. *Australian Journal of Basic and Applied Sciences*, **8**(16): 282-286.

Olsen, S. R., Cole, C. V., Watenabe, F. S. & Dean, L. A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S.D.A. Circ. 939, U.S. Govt. Printing Office, Washington, DC.

Prasanna, B. M., Vasal, S. K., Kassahun, B. & Singh, N. N. (2001). Quality protein maize. *Current Science*, **81**: 48-53.

Subbiah, B. V. and Asija, G. C. (1956). A rapid procedure for estimation of available nutrients in soils. *Current Science*, **25**: 259-260.

Suganya, A., Saravanan, A. & Manivannan, N. (2020). Role of zinc nutrition for increasing zinc availability, uptake, yield, and quality of maize (*Zea mays* L.) Grains: An Overview, *Communications in Soil Science and Plant Analysis,* **51**(15): 2001-2021, doi: https://doi.org/10.1080/00103624.2020.1820030.

Suganya, A., Saravanan, A., Baskar, M., Pandiyarajan, P. & Kavimani, R. (2021). Agronomic biofortification of maize (*Zea mays* L.) with zinc by using of graded levels of zinc in combination with zinc solubilizing bacteria and Arbuscular mycorrhizal fungi. *Journal of Plant Nutrition,* **44**(7): 988-994, https://doi.org/10.1080/01904167.2020.1845383.

Suganya, A., Saravanan, A., Basker, M., Pandiyarajan, P. & Kavimani, R. (2020). Effect of Arbuscular Mycorrhizal (AM) Fungi and Zinc Solubilizing Bacteria in combination with graded levels of $ZnSO_4$ on micronutrient transformation and their uptake of maize. *Journal of Xidian University,* **14**(6): 2862-2877.

Walkley, A. & Black, C. A. (1934). An examination of the Deglgareff method for determining soil organic matter and proposed modification of chromic acid titration method. *Soil Science*, **37**: 29-38.