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Impact of levels of zinc and various zinc solubilizing microorganisms on changes in zinc and other soil nutrients in spinach

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Abstract

Field experiment was conducted during *kharif* season of 2018 at Research farm, Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani to assess the response of spinach crop to various zinc solubilizers and graded levels of zinc on changes in zinc and other soil nutrients. Experiment consists of sixteen treatment in which four zinc solubilizers (*Pseudomonas striata*, *Bacillus megaterium*, *Trichoderma viride* and Control) and four graded levels of ZnSO₄ (0 kg ZnSO₄ ha⁻¹, 10 kg ZnSO₄ ha⁻¹, 20 kg ZnSO₄ ha⁻¹ and 30 kg ZnSO₄ ha⁻¹) were used in factorial randomized block design. Seed treatment of spinach was done with zinc solubilizing microbial cultures and application of zinc in graded doses at the time of sowing with recommended dose of fertilizers. Significantly highest values of available N, P₂O₅, K₂O and S were noted in treatment receiving *Pseudomonas striata* + 30 kg ZnSO₄ ha⁻¹ interaction. DTPA Zn, Fe, Mn and Cu were noted maximum in treatment receiving *Pseudomonas striata* + 30 kg ZnSO₄ ha⁻¹.

Keywords: Spinach, zinc solubilizers, zinc levels

Introduction

Spinach (*Spinacea oleracea* L.) is one of the perennial leafy vegetable and grown throughout the world. Spinach has an excellent nutritional values and health benefits. Farmers can grow this crop in greenhouse, polyhouse and hygroscopic system as well. It can be grown for daily use in pots, containers, backyards also in field. Spinach belongs to family of '*Chenopodiaceae*' and genus of '*Spinacea*'. Raw spinach has 91% water, 4% carbohydrates, 3% protein, and contains negligible fat. In a 100 g serving providing only 23 calories, spinach has a high nutritional value, especially when fresh, frozen, steamed, or quickly boiled.

Zinc plays very important role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions and synthesis of cytochrome. Plant enzymes activated by Zn are involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, regulation of auxin synthesis and pollen formation. The regulation and maintenance of the gene expression required for the tolerance of environmental stresses in plants are Zn dependent. Its deficiency results in the development of abnormalities in plants which become visible as deficiency symptoms such as stunted growth, chlorosis and smaller leaves, spikelet sterility. Zinc deficiency can also adversely affect the quality of harvested products, plants susceptibility to injury by high light or temperature intensity and to infection by fungal diseases can also increase. Zinc seems to affect the capacity for water uptake and transport in plants and also reduce the adverse effects of short periods of heat and salt stress (Hafeez *et al.* 2013) [9].

Availability of zinc to plants can be increases by zinc-solubilizing microorganisms through solubilize zinc from inorganic and organic pools of total soil zinc. Both in vitro and in vivo fungi have been extensively studied for solubilization of insoluble zinc compounds (Gadd. 2007) [17]. However, only some bacterial species of the genera *Bacillus*, *Acinetobacter*, *Gluconacetobacter*, and *Pseudomonas* have been reported (Simine, *et al.* 1998) [18]. For zinc solubilization microorganisms play a key role. Some species of *rhizobacteria* are capable of solubilizing zinc in accessible form in soils. Mineral zinc solubilization by microbes which enhances crop growth and yield. Zinc solubilizing bacteria are capable of solubilizing ZnO,

ZnCO₃ and zinc phosphate through production and excretion of organic acids. Several genera of rhizobacteria belonging to *Pseudomonas spp.* and *Bacillus spp.* are reported to solubilize zinc. 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 (Goteti *et al.* 2013) [8]. Microorganisms are capable for zinc solubilization viz., *B. subtilis*, *Thiobacillus thiooxidans* and *Saccharomyces sp.* These microorganisms can be used as bio-fertilizers for solubilization of fixed micronutrients like zinc. The results have shown that a *Bacillus sp.* (Zn solubilizing bacteria) can be used as bio-fertilizer for zinc or in soils where native zinc is higher or in conjunction with insoluble cheaper zinc compounds like zinc oxide (ZnO), zinc carbonate (ZnCO₃) and zinc sulphide (ZnS) instead of costly zinc sulphate (Mahdi *et al.* 2010) [14].

Materials and Method

The field experiment was carried out on spinach crop (Var. *All green*) in *kharif* season during year 2018 on *Typic haplusters* at Research Farm, Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. After completion of preparatory tillage operations, the experiment was laid out in Factorial Randomized Block Design comprising sixteen (16) treatments replicated three (3) times. In which four zinc solubilizers (*Pseudomonas striata*, *Bacillus megaterium*, *Trichoderma viride* and Control) and four graded levels of ZnSO₄ (0 kg

ZnSO₄ ha⁻¹, 10 kg ZnSO₄ ha⁻¹, 20 kg ZnSO₄ ha⁻¹ and 30 kg ZnSO₄ ha⁻¹). Seed treatment was done immediately before sowing with liquid zinc solubilizing culture @ 100 ml 10 kg⁻¹ seed. The crop was raised following recommended agronomic practices. The recommended dose of chemical fertilizers was applied at the time of sowing. Intercultural operations like thinning, weeding, spraying of insecticides, fertilizer application and schedule of irrigation for spinach crop was carefully followed. The data obtained was statistically analyzed and appropriately interpreted as per the methods described in "Statistical Methods for Agricultural Workers" by Panse and Sukhatme.

Results and Discussion

Nitrogen

Perusal of data presented in Table 1 indicate that available N in soil was significantly increased with inoculation of different zinc solubilizing microbial inoculants in spinach. Zinc solubilizers influenced the available nitrogen which ranged between 166.07 to 186.80 kg ha⁻¹ showing significantly higher available nitrogen in *Pseudomonas striata* treated plots followed by *Bacillus megaterium* and *Trichoderma viride*. Whereas, significantly lower available nitrogen per plot were noted in uninoculated control. Similarly, graded levels of zinc in the form of zinc sulphate also increase the available nitrogen with each incremental dose up to 30 kg ZnSO₄ ha⁻¹. The available nitrogen as influenced by Zn application ranged from 155.78 to 188.83 kg ha⁻¹.

Table 1: Effect of zinc solubilizing cultures and zinc levels on nutrient availability in spinach

Treatments	Available N (Kg ha ⁻¹)	Available P ₂ O ₅ (Kg ha ⁻¹)	Available K ₂ O (Kg ha ⁻¹)	Available S (mg kg ⁻¹)
Zinc solubilizers (S)				
S0: Control	166.07	15.61	584.38	10.11
S1: <i>Pseudomonas striata</i>	186.80	18.46	634.86	11.04
S2: <i>Trichoderma viride</i>	171.09	16.51	605.07	10.61
S3: <i>Bacillus megaterium</i>	174.27	17.13	623.74	10.69
S.Em.±	2.41	0.22	6.63	0.08
C.D. at 5 %	6.97	0.63	19.16	0.25
Levels of ZnSO₄ (Zn)				
Zn0: ZnSO ₄ 0 kg ha ⁻¹	155.78	14.89	579.45	9.69
Zn1: ZnSO ₄ 10 kg ha ⁻¹	171.45	17.34	600.48	10.57
Zn2: ZnSO ₄ 20 kg ha ⁻¹	182.16	18.26	626.33	10.74
Zn3: ZnSO ₄ 30 kg ha ⁻¹	188.83	18.53	641.79	10.90
S.Em.±	2.41	0.22	6.63	0.08
C.D. at 5 %	6.97	0.63	19.16	0.25
Interaction (SxZn)				
S.Em.±	4.83	0.44	13.27	0.17
C.D. at 5 %	13.94	1.27	38.33	0.50

Interaction effect of zinc solubilizing cultures and zinc levels on available N in Table 1a. Synergistic effect of each factor was recorded showing significantly highest available nitrogen in *Pseudomonas striata* X ZnSO₄ 30 kg ha⁻¹ (205.11 kg ha⁻¹) it was found at par with *Bacillus megaterium* X ZnSO₄ 30 kg ha⁻¹. However the lower values of available nitrogen (140.74 kg ha⁻¹) were recorded in without microbial inoculant and zinc application.

The increase in available nitrogen might also be attributed to the greater mobilization of soil microbes which converts organically bound N to inorganic form. The zinc solubilizing organism (*Bacillus spp.*) proved to have a favourable effect on the availability of N, P and K, thereby indicating vital role of these organisms in the transformation reaction of major nutrients in the soil. Experimental results are in corroborate with the findings of Kumar *et al.* (2004) [12], Rizvi *et al.* (2013) [16] and Sable *et al.* (2016) [17].

Table 1a: Interaction effect of zinc solubilizers and levels of zinc on nutrient availability in soil

Treatments	Zn0:ZnSO ₄ 0 kg ha ⁻¹	Zn1:ZnSO ₄ 10 kg ha ⁻¹	Zn2:ZnSO ₄ 20 kg ha ⁻¹	Zn3:ZnSO ₄ 30 kg ha ⁻¹	Mean
Available N (kg ha⁻¹)					
S0: Control	140.74	163.30	178.73	181.49	166.06
S1: <i>Pseudomonas striata</i>	156.95	185.58	199.58	205.11	186.80
S2: <i>Trichoderma viride</i>	159.65	168.18	173.79	182.76	171.09
S3: <i>Bacillus megaterium</i>	165.79	168.76	176.55	185.76	174.26
Mean	155.78	171.45	182.16	188.82	
Interaction	S	Zn	SXZn		
SEm+	2.41	2.41	4.83		
CD at 5%	6.97	6.97	13.94		
Available P₂O₅ (kg ha⁻¹)					
S0: Control	12.91	16.33	17.79	17.80	15.61
S1: <i>Pseudomonas striata</i>	16.54	18.78	19.17	19.30	18.46
S2: <i>Trichoderma viride</i>	15.24	17.03	18.29	18.71	16.51
S3: <i>Bacillus megaterium</i>	15.25	17.22	17.77	18.28	17.13
Mean	14.89	17.34	16.78	18.53	
Interaction	S	Zn	SXZn		
SEm+	0.22	0.22	0.44		
CD at 5%	0.63	0.63	1.27		
Available K₂O (kg ha⁻¹)					
S0: Control	517.43	552.51	624.33	643.25	584.38
S1: <i>Pseudomonas striata</i>	602.83	627.18	652.24	657.18	634.85
S2: <i>Trichoderma viride</i>	591.98	604.09	609.00	615.20	605.06
S3: <i>Bacillus megaterium</i>	605.55	618.15	619.74	651.52	623.73
Mean	579.44	600.48	626.32	641.89	
Interaction	S	Zn	SXZn		
SEm+	6.63	6.63	13.27		
CD at 5%	19.16	19.16	38.33		
Available S (mg kg⁻¹)					
S0: Control	8.30	10.26	10.84	11.05	10.11
S1: <i>Pseudomonas striata</i>	10.31	10.96	11.12	11.15	11.04
S2: <i>Trichoderma viride</i>	9.89	10.43	10.69	11.33	10.61
S3: <i>Bacillus megaterium</i>	10.28	10.58	10.77	11.32	10.69
Mean	9.69	10.57	10.74	10.90	
Interaction	S	Zn	SXZn		
SEm+	0.08	0.08	0.17		
CD at 5%	0.25	0.25	0.50		

Phosphorus

Available P₂O₅ in soil was influenced significantly due to various treatments of zinc solubilizing microbial inoculants in spinach are presented in Table 1. Zinc solubilizers influenced the available phosphorus which ranged from 15.61 to 18.46 kg ha⁻¹ showing significantly higher available phosphorus in *Pseudomonas striata* (18.46 kg ha⁻¹) treated plots followed by *Bacillus megaterium* (17.13 kg ha⁻¹) and *Trichoderma viride* (16.51 kg ha⁻¹). Whereas significantly lower available phosphorus per plot were noted in uninoculated control. Similarly, graded levels of zinc in the form of zinc sulphate also increase the available phosphorus with each incremental dose up to 30 kg ZnSO₄ ha⁻¹. The available phosphorus as influenced by zinc application ranged from 14 to 18 kg ha⁻¹. Interaction effect of zinc mobilizing cultures and zinc levels on available phosphorus from soil shown in Table 1a. Significantly highest available phosphorus in *Pseudomonas striata* X ZnSO₄ 30 kg ha⁻¹ (19.30 kg ha⁻¹) it was found at par with *Trichoderma viride* X ZnSO₄ 30 kg ha⁻¹ (18.71 kg ha⁻¹). However the lower value of available phosphorus (12.91 kg ha⁻¹) was recorded in uninoculated control.

Both plant roots and soil microorganisms alter and are affected by soil chemical and physical properties in the rhizosphere. Among the factor affecting soil P availability root exudates, such as organic acids, H⁺ ions, sugar and phosphatase facilitates the solubilization and desorption of mineral P. Alterations in composition and quantity root

exudates through the introduction of new genetic traits may therefore directly affect processes such as mineral P or fixed P solubilization or indirectly affect the availability of P through changes in the activity of rhizosphere microorganisms. Thus, in our case it can be presumed that P availability was enhanced because of changes in rhizospheric conditions under microbial influence.

Our findings are also confirmed with the results of Egamberdiyeva *et al.* (2004) [6] who observed the positive influence of P-solubilizers inoculation on soil available P and observed that phosphate solubilizers increased the available P in the soil. Further, Jayant Raman, (2012) [11] also reported that *Pseudomonas striata* can solubilize the extra phosphorus. Similarly Bodkhe *et al.* (2014a) [5] observed that the 100% RDF and dual inoculation treated plots had highest increase in available phosphorus which might be due to PSB secretes different organic acids like citric and mallic acid which act on insoluble phosphate (PO₄) to convert them into soluble phosphate (H₂PO₄) near the root of the plant and hence availability of phosphorus increased. These beneficial bacteria enhances plant growth by improving soil nutrient status, secreting plant growth regulators as reported by Rizvi *et al.* (2013) [16], Afzal *et al.* (2013) [2] and Kumar *et al.* (2014) [13].

Potassium

In Table 1 shows that availability of potassium is significantly influenced by various treatments of zinc solubilizing microbial inoculants in spinach in which significantly highest

value for potassium availability in *Pseudomonas striata* treated plots which is (634.86 kg ha⁻¹) followed by *Bacillus megaterium* (623.74 kg ha⁻¹) and *Trichoderma viride* (605.07 kg ha⁻¹). Whereas, significantly lower available phosphorus per plot were noted in uninoculated control. Similarly, graded levels of zinc in the form of zinc sulphate also increase the available potassium with each incremental dose up to 30 kg ZnSO₄ ha⁻¹. The available potassium as influenced by zinc application ranged from 579.45 to 641.79 kg ha⁻¹.

Interaction effect of zinc solubilizing microbial cultures and zinc levels on available potassium from soil shown in Table 1a. Significantly highest available potassium in *Pseudomonas striata* X ZnSO₄ 30 kg ha⁻¹ (657.18 kg ha⁻¹) it was found at par with *Bacillus megaterium* X ZnSO₄ 30 kg ha⁻¹ (651.52 kg ha⁻¹). However the lower value of available potassium (517.43 kg ha⁻¹) was recorded in uninoculated control.

Microorganisms are able to mobilize metals by formation organic or inorganic acids (eg. citric and mallic acid) by oxidation and reduction reaction and by the secretion of complexing agents. Contents of organic acids released in the rhizosphere could be responsible for increased K solubilization. The marked effect of FYM + ZSB on turmeric rhizome yield might have been attributed to the direct role played by zinc solubilizer on enhancing the availability of not only zinc but also potassium (Kumar *et al.* 2014) [13].

Sulphur

The data presented in Table 1 shows significant increase in available sulphur in soil after harvest of spinach with zinc solubilizers as compare to control. Significantly highest value of available sulphur is in *Pseudomonas striata* treated plot which is 11.04 mg kg⁻¹ followed by *Bacillus megaterium* 10.69 mg kg⁻¹ treated plot is at par with *Trichoderma viride* 10.61 mg kg⁻¹. Whereas, significantly lower available sulphur per plot were noted in uninoculated control. Similarly, graded levels of zinc in the form of zinc sulphate also increase the available sulphur with each incremental dose up to 30 kg ZnSO₄ kg ha⁻¹. The value of available sulphur for 30 kg ZnSO₄ha⁻¹ is 10.90 mg kg⁻¹.

Interaction effect of zinc solubilizing microbial cultures and zinc levels on available sulphur from soil shown in Table 1a. Significantly highest available sulphur in *Trichoderma viride* X ZnSO₄ 30 kg ha⁻¹ (11.33 mg ha⁻¹) it was found at par with *Bacillus megaterium* X ZnSO₄ 30 kg ha⁻¹ (11.32 mg ha⁻¹).

However the lower value of available sulphur (8.30 mg ha⁻¹) was recorded in uninoculated control.

Due to application of P solubilizers, there was greater mobilization of insoluble phosphorus along with enhanced transport of soil nutrients within the plant system. Increasing S availability due to application of P solubilizers could be due to greater root proliferation and increasing activity of sulphur solubilizing bacteria. It may also attribute to the contribution of sulphur from single super phosphate which was used as a source of phosphorus. These results are in agreement with the findings of Trivedi *et al.* (1997) [19] and Pasricha and sarkar (2002).

DTPA extractable zinc

The content of DTPA Zn in soil showed significant increase under all microbial inoculated treatments compared to control treatment. The data pertaining to periodical changes in DTPA Zn in soil is narrated in Table 2 and depicted in Fig.1. Zinc mobilizing cultures influenced the DTPA zinc which ranged from 0.38 to 0.64 mg kg⁻¹ showing significantly higher DTPA zinc in *Pseudomonas striata* treated plots follows by *Bacillus megaterium* and *Trichoderma viride*. Whereas, significantly lower DTPA zinc per plot were noted in uninoculated control. Similarly, increase in zinc content in soil with application of graded levels of zinc in the form of zinc sulphate also increase the DTPA zinc with each incremental dose up to 30 kg ZnSO₄ kg ha⁻¹.

Interaction effect of zinc solubilizers and zinc levels on DTPA Zn in Table 2a. Showing significantly the highest build-up of soil DTPA Zn was noticed under in *Pseudomonas striata* X ZnSO₄ 30 kg ha⁻¹ (0.815 mg kg⁻¹) followed by *Bacillus megaterium* X ZnSO₄ 30 kg ha⁻¹ (0.718 mg kg⁻¹) it was found at par with *Trichoderma viride* X ZnSO₄ 30 kg ha⁻¹ (0.683mg kg⁻¹). However the lower values of DTPA zinc (0.296 mg kg⁻¹) was recorded in uninoculated control plots.

Mechanisms employed by different solubilizers in enhancing Zn availability by solubilization and chelation of minerals can increase plant metabolism leading to the enhancement of plant physiological activity and the development of the root system with production of some organic acids in the rhizosphere such as gluconic, citric and/or fumeric acids by solubilizers which decrease soil pH, lead to increase solubility of the insoluble compound and availability of micronutrients like zinc.

Table 2: Effect of zinc solubilizing cultures and zinc levels on DTPA micronutrient in soil after harvest of spinach

Treatments	DTPA Zn (mg kg ⁻¹)	DTPA Fe (mg kg ⁻¹)	DTPA Cu (mg kg ⁻¹)	DTPA Mn (mg kg ⁻¹)
Zinc solubilizers (S)				
S0: Control	0.38	2.28	0.88	7.31
S1: <i>Pseudomonas striata</i>	0.64	3.05	0.96	7.74
S2: <i>Trichoderma viride</i>	0.51	2.53	0.92	7.58
S3: <i>Bacillus megaterium</i>	0.55	2.68	0.94	7.66
S.Em.±	0.01	0.03	0.007	0.06
C.D. at 5 %	0.03	0.10	0.02	0.19
Levels of ZnSO₄ (Zn)				
Zn0: ZnSO ₄ 0 kg ha ⁻¹	0.38	2.43	0.85	7.14
Zn1: ZnSO ₄ 10 kg ha ⁻¹	0.44	2.51	0.92	7.51
Zn2: ZnSO ₄ 20 kg ha ⁻¹	0.57	2.67	0.94	7.73
Zn3: ZnSO ₄ 30 kg ha ⁻¹	0.68	2.94	0.98	7.92
S.Em.±	0.01	0.03	0.007	0.06
C.D. at 5 %	0.03	0.10	0.02	0.19
Interaction (SxZn)				
S.Em.±	0.02	0.07	0.01	0.13
C.D. at 5 %	0.07	0.21	0.03	0.39

Further, Kumar *et al.* (2004) [12] also reported that the marked effect of FYM + ZSB on turmeric rhizome yield might have attributed in the direct role played by zinc solubilizer on enhancing the availability of Zn. Similarly, Imran *et al.*

(2014) [10] also observed that sustainable increase in soil bioavailable content of zinc has been reported in fungal inoculated soils as compared to uninoculated soils.

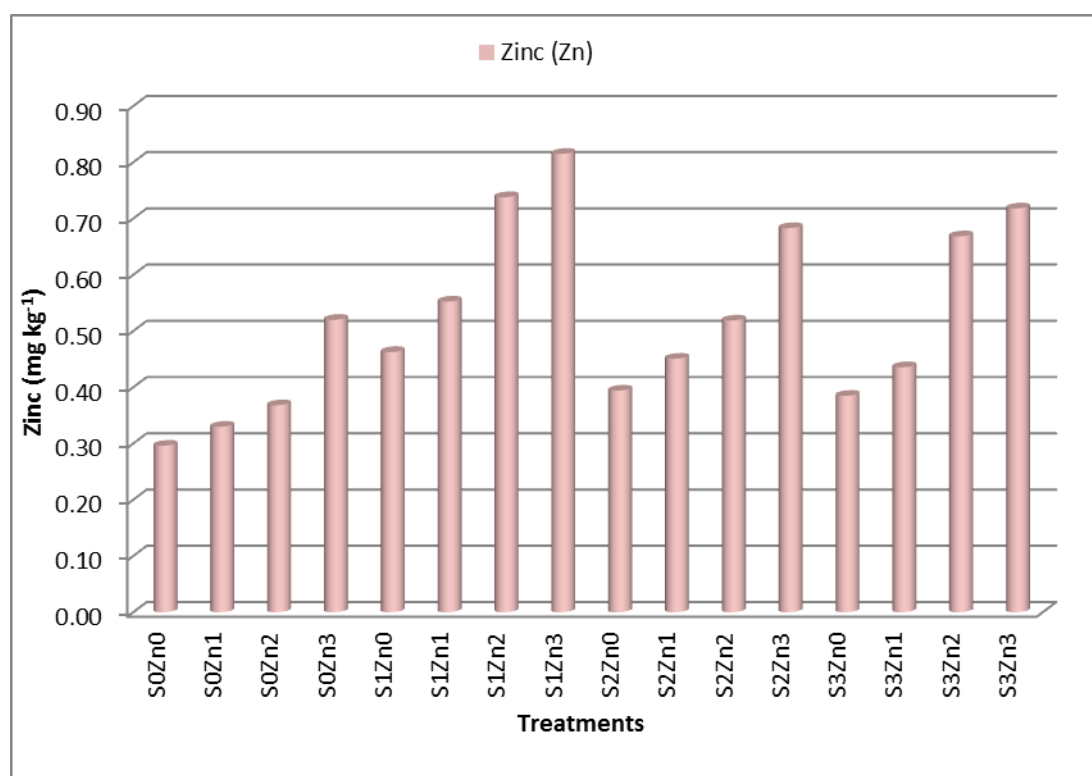


Fig. 1: Interaction effect of zinc solubilizers and levels of zinc on zinc availability in soil.

DTPA extractable iron

Results narrated in Table 2 related to DTPA iron in spinach indicate significant effect of zinc solubilizers and graded level of zinc. The zinc solubilizing cultures influenced the DTPA iron which ranged from 2.28 to 3.05 mg kg⁻¹ showing significantly higher DTPA iron in treatment *Pseudomonas striata* treated plots followed by *Bacillus megaterium* and *Trichoderma viride*. Whereas, significantly lower DTPA iron per plot were noted in uninoculated control. Similarly, graded levels of zinc in the form of zinc sulphate also increased the DTPA iron with each incremental dose up to 30 kg ZnSO₄ha⁻¹. The DTPA iron as influenced by zinc application ranged between 2.43 to 2.94 mg kg⁻¹.

Interaction effect of zinc solubilizers and zinc levels in Table 2a. Showing significantly the highest DTPA iron was noticed under in *Pseudomonas striata* X ZnSO₄ 30 kg ha⁻¹ (3.69 mg kg⁻¹) followed *Bacillus megaterium* X ZnSO₄ 30 kg ha⁻¹ (2.89 mg kg⁻¹) it was found at par with *Trichoderma viride* X ZnSO₄ 30 kg ha⁻¹. However the lower values of DTPA iron (2.14 mg kg⁻¹) was recorded in without zinc application.

Microorganisms are able to mobilize metals by formation of organic or inorganic acids (citric acid, sulphuric acid) by oxidation and reduction processes and by the excretion of complexation agents. Contents of organic acids released in the rhizosphere could be responsible for increased Fe solubilization. As earlier reported, the release of organic acid that both sequester cation and acidity the microenvironment

near roots is thought to be a major mechanism of solubilization of P as well as Fe in soils. The similar results were quoted by Birgita *et al.* (2000) [4], Kumar *et al.* (2004) [12] and Adriana *et al.* (2010) [1].

DTPA extractable copper

A perusal of data given in Table 2 revealed that DTPA extractable copper content in soil was influenced significantly with different zinc solubilizing microbial strains. Zinc solubilizers influenced the DTPA copper which ranged from 0.88 to 0.96 mg kg⁻¹ have showing significantly higher DTPA copper in treatment *Pseudomonas striata* treated plots follows by *Bacillus megaterium* and *Trichoderma viride*. Whereas, significantly lower DTPA copper per plot were noted in uninoculated control. Similarly, applied zinc levels in the form of zinc sulphate also increase the DTPA copper with each incremental dose up to 30 kg ZnSO₄ ha⁻¹. The DTPA copper as influenced by Zn application ranged from 0.85 to 0.98 mg kg⁻¹.

Interaction effect of zinc solubilizers and zinc levels on DTPA Cu in Table 2a. Showing significantly the highest DTPA copper was noticed under in *Pseudomonas striata* X ZnSO₄ 30 kg ha⁻¹ (1.03 mg kg⁻¹) it was found at par with both *Trichoderma viride* and *Bacillus megaterium* X ZnSO₄ 30 kg ha⁻¹. However the lower values of DTPA copper (0.77 mg kg⁻¹) was recorded in without microbial culture and zinc application.

Table 2a: Interaction effect of zinc solubilizers and levels of zinc on DTPA micronutrient in soil after harvest of spinach

Treatments	Zn0:ZnSO ₄ 0 kg ha ⁻¹	Zn1:ZnSO ₄ 10 kg ha ⁻¹	Zn2:ZnSO ₄ 20 kg ha ⁻¹	Zn3:ZnSO ₄ 30 kg ha ⁻¹	Mean
DTPA Zn (mg kg⁻¹)					
S0: Control	0.296	0.330	0.368	0.520	0.378
S1: <i>Pseudomonas striata</i>	0.463	0.552	0.738	0.815	0.642
S2: <i>Trichoderma viride</i>	0.394	0.451	0.519	0.683	0.512
S3: <i>Bacillus megaterium</i>	0.385	0.436	0.668	0.718	0.552
Mean	0.384	0.442	0.573	0.684	
Interaction	S	Zn	SXZn		
SEm+	0.013	0.013	0.026		
CD at 5%	0.038	0.038	0.076		
DTPA Fe (mg kg⁻¹)					
S0: Control	2.14	2.17	2.27	2.55	2.281
S1: <i>Pseudomonas striata</i>	2.57	2.76	3.18	3.69	3.050
S2: <i>Trichoderma viride</i>	2.47	2.49	2.54	2.62	2.531
S3: <i>Bacillus megaterium</i>	2.54	2.60	2.70	2.89	2.683
Mean	2.430	2.505	2.673	2.937	
Interaction	S	Zn	SXZn		
SEm+	0.037	0.037	0.074		
CD at 5%	0.106	0.106	0.213		
DTPA Cu (mg kg⁻¹)					
S0: Control	0.77	0.88	0.92	0.95	0.878
S1: <i>Pseudomonas striata</i>	0.89	0.93	0.97	1.03	0.956
S2: <i>Trichoderma viride</i>	0.86	0.92	0.93	0.97	0.918
S3: <i>Bacillus megaterium</i>	0.88	0.93	0.95	0.98	0.937
Mean	0.850	0.915	0.944	0.979	
Interaction	S	Zn	SXZn		
SEm+	0.007	0.007	0.014		
CD at 5%	0.02	0.02	0.039		
DTPA Mn(mg kg⁻¹)					
S0: Control	6.44	7.22	7.66	7.93	7.314
S1: <i>Pseudomonas striata</i>	7.52	7.66	7.75	8.04	7.743
S2: <i>Trichoderma viride</i>	7.41	7.56	7.64	7.73	7.583
S3: <i>Bacillus megaterium</i>	7.19	7.58	7.86	7.99	7.655
Mean	7.140	7.506	7.727	7.923	
Interaction	S	Zn	SXZn		
SEm+	0.068	0.068	0.135		
CD at 5%	0.196	0.196	0.391		

These results are in line of findings of Jayant Raman (2012) [11] who found the highest availability of Cu in soil with the inoculation of treatment combination of *Pseudomonas striata* + *Trichoderma viride* and *Azotobacter chroococcum*. The release of organic acid that both sequester cation and acidity the microenvironment near roots is thought to be a major mechanism of solubilization of Cu in soils as reported by Altomare *et al.* (1999) [3].

DTPA extractable manganese

Inoculation of zinc solubilizing microbial inoculants enhanced the DTPA manganese content in soil as compared to initial values. Zinc mobilizing cultures influenced the DTPA manganese which ranged between 7.31 to 7.74 mg kg⁻¹ showing significantly higher DTPA manganese in *Pseudomonas striata* treated plots follows by *Bacillus megaterium* and *Trichoderma viride*. Whereas, significantly lower DTPA manganese per plot were noted in control plots. Similarly, application of zinc levels in the form of zinc sulphate also increased the DTPA manganese with each incremental dose up to 30 kg ZnSO₄ ha⁻¹. The DTPA manganese as influenced by Zn application ranged from 7.14 to 7.92 mg kg⁻¹.

Interaction effect of zinc solubilizers and zinc levels on DTPA manganese in Table 2a. Increase the DTPA manganese significantly synergistic effect of each factor was recorded on each other showing significantly the highest DTPA manganese was noticed under in *Pseudomonas striata* X

ZnSO₄ 30 kg ha⁻¹ (8.04 mg kg⁻¹) it was found at par with *Bacillus megaterium* X ZnSO₄ 30 kg ha⁻¹. However, the lower values of DTPA manganese (6.44 mg kg⁻¹) was recorded in without microbial culture and zinc application.

The DTPA Mn increased might be due to transformation of oxidized form of Mn to reduced form. Similar results are also registered by Altomare *et al.* (1999) [3]. Kumar *et al.* (2004) [12] reported that the availability of Mn in the soil, brought on the positive effect of Zn and Fe added alone or along with FYM and FYM + ZSB.

Conclusion

Significantly maximum nutrient availability in soil after harvest of spinach crop was also registered with zinc solubilizers. Significantly highest values of available N, P₂O₅, K₂O and S were recorded maximum in treatment receiving the inoculation of *Pseudomonas striata* + 30 kg ZnSO₄ ha⁻¹ over rest of the treatments. The DTPA micronutrients (Zn, Fe, Mn and Cu) in soil after harvest of spinach were also found significantly influenced by bioinoculants. DTPA micronutrients (Zn, Fe, Mn and Cu) were note maximum in treatment receiving the inoculation of *Pseudomonas striata* + 30 kg ZnSO₄ ha⁻¹.

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