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Effect of levels of zinc and various zinc solubilizing microorganisms on growth and yield of 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 solubilizes and graded levels of zinc on growth and yield. Experiment consists of sixteen treatment in which four zinc solubilizes (*Pseudomonas striata*, *Bacillus megaterium*, *Trichoderma viride* and Control) and four graded levels of $ZnSO_4$ (0 kg $ZnSO_4$ ha^{-1},10 kg $ZnSO_4$ ha^{-1}, 20 kg $ZnSO_4$ ha^{-1} and 30 kg $ZnSO_4$ 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. Significant effect of zinc solubilizing culture particularly *Psuedomonas striata* and levels of zinc up to 30 kg $ZnSO_4$ ha^{-1} was noted on plant height, number of leaves per plant, dry matter yield per plant and leaf area index over other treatments. Also higher dry weight and fresh weight yield of spinach was noted with *Psuedomonas striata* + 30 kg $ZnSO_4$ ha^{-1}.

Keywords: spinach, plant height, number of leaves, leaf area index, yield

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, playhouse and hygroscopic system as well. It can be grown for daily use in pots, containers, backyards also in field. Spinach belongs to family of '*Chenopodiaceous*' 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 increases. 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) [4]. 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) [2]. However, only some bacterial species of the genera *Bacillus*, *Acinetobacter*, *Gluconacetobacter*, and *Pseudomonas* have been reported (Simine, *et al.* 1998) [12]. 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, $ZnCO_3$ 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) [3]. Microorganisms are capable for zinc solubilization *viz*., *B. subtilis*, *Thiobacillus thioxidans* 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 ($ZnCO_3$) and zinc sulphide (ZnS) instead of costly zinc sulphate (Mahdi *et al*. 2010) [6].

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 solubilizes (*Pseudomonas striata*, *Bacillus megaterium*, *Trichoderma viride* and Control) and four graded levels of $ZnSO_4$ (0 kg $ZnSO_4$ ha^{-1}, 10 kg $ZnSO_4$ ha^{-1}, 20 kg $ZnSO_4$ ha^{-1} and 30 kg $ZnSO_4$ 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-

1. Plant height

Plant height of spinach taken during 15, 30 and 45 (at harvest) days after sowing was significantly influenced by various zinc solubilizing microbial strains and graded levels of $ZnSO_4$. The data presented in Table 1 and depicted in Fig.1 shows that plant height increased significantly due to zinc solubilizing microbial cultures over control but both the strains *Pseudomonas striata* (S1) and *Bacillus megaterium* (S2) were found statistically at par with each other in increasing plant height. Levels of $ZnSO_4$ also increased the plant height in spinach up to 30 kg $ZnSO_4$ ha^{-1} significantly.

Table 1: Effect of zinc solubilizing cultures and zinc levels on plant height in spinach.

Treatments	Plant height (cm)		
	15 days after sowing	30 days after sowing	45 days after sowing
Zinc solubilizes (S)			
S0: Control	5.87	22.60	30.92
S1: *Pseudomonas striata*	7.27	24.71	35.74
S2: *richoderma viride*	6.66	24.07	31.26
S3: *Bacillus megaterium*	7.24	24.15	33.60
S.Em. ±	0.07	0.16	0.12
C.D. at 5 %	0.21	0.47	0.37
Levels of $ZnSO_4$ (Zn)			
Zn0: $ZnSO_4$ 0 kg ha^{-1}	6.27	21.54	30.41
Zn1: $ZnSO_4$ 10 kg ha^{-1}	6.42	22.92	32.66
Zn2: $ZnSO_4$ 20 kg ha^{-1}	7.02	24.89	33.63
Zn3: $ZnSO_4$ 30 kg ha^{-1}	7.32	26.18	34.82
S.Em.±	0.07	0.16	0.12
C.D. at 5 %	0.21	0.47	0.37
Interaction (Sx Zn)			
S. Em.±	0.14	0.33	0.25
C.D. at 5 %	0.45	0.95	0.74

The interaction between zinc solubilizers and zinc levels was found significant for plant height (Table 1a). Significantly highest plant height was recorded in *Pseudomonas striata* X $ZnSO_4$ 30 kg ha^{-1} treatment for 15 DAS was 8.17 cm, for 30 DAS was 26.79 cm and for 45 DAS was 38.39 cm. However the lowest plant height was recorded in without microbial culture and zinc application.

Table 1a: Interaction effect of zinc solubilizers and levels of zinc on plant height in spinach.

Treatments	Zn0:$ZnSO_4$0 kg ha^{-1}	Zn1:$ZnSO_4$0 kg ha^{-1}	Zn2:$ZnSO_4$ 20 kg ha^{-1}	Zn3:$ZnSO_4$ 30 kg ha^{-1}	Mean
15 days after sowing					
S0: Control	5.29	5.33	6.25	6.60	5.86
S1:*Pseudomonas striata*	6.62	6.80	7.48	8.17	7.26
S2:*Trichoderma viride*	6.43	6.44	6.85	6.92	6.65
S3: *Bacillus megaterium*	6.76	7.09	7.49	7.61	7.23
Mean	6.27	6.41	7.02	7.32	
Interaction	S	Zn	SXZn		
S.Em+	0.07	0.07	0.14		
CD at 5%	0.21	0.21	0.42		

30 days after sowing					
S0: Control	8.92	21.56	24.57	25.33	22.59
S1:*Pseudomonas striata*	22.79	23.68	25.59	26.79	24.71
S2:*Trichoderma viride*	21.84	23.10	24.95	26.39	24.06
S3: *Bacillus megaterium*	22.61	23.33	24.44	26.20	24.14
Mean	21.54	22.91	24.88	26.17	
Interaction	S	Zn	SX Zn		
S.Em +	0.16	0.16	0.33		
CD at 5%	0.47	0.47	0.95		
45 days after sowing					
S0: Control	28.89	31.01	31.79	31.8	30.91
S1:*Pseudomonas striata*	33.66	34.69	36.21	38.9	35.73
S2:*Trichoderma viride*	28.53	31.37	31.75	33.38	31.25
S3: *Bacillus megaterium*	30.54	33.56	34.77	35.51	33.59
Mean	30.40	32.65	33.62	34.8	
Interaction	S	Zn	SX Zn		
S.Em+	0.12	0.12	0.25		
CD at 5%	0.37	0.37	0.74		

It was noticed that application of various zinc solubilizes and graded levels of zinc up to 30 kg ha^{-1} recorded significantly higher plant height at various growth stages. These shows that application of recommended dose of fertilizers and solubilization zinc through zinc solubilizers resulted in increase in plant height. These results are incompliance with the findings of Subramanian and Vijay Kumar *et al.* (2001), Sengupta *et al.* (2002) [11], Sajan *et al.* (2002) [10].

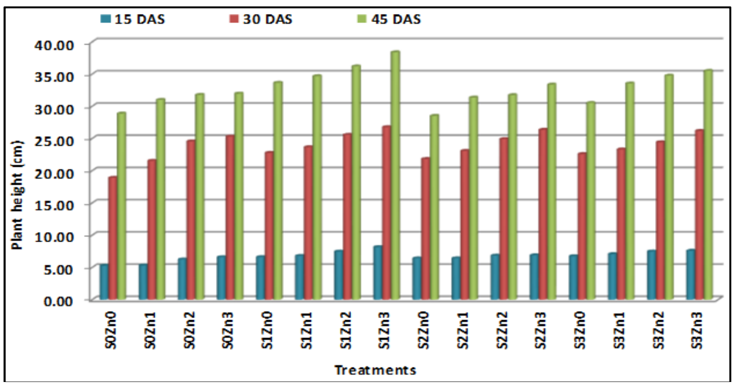

Fig 1: Interaction effect of zinc solubilizers and levels of zinc on plant height in spinach.

2. Number of leaves

Results given in Table 2 and Fig.2 related to number of leaves in spinach indicates significant effect of zinc solubilizers and graded level of zinc on number of leaves in spinach. Zinc solubilizers influence the number of leaves as for 15 DAS which ranged in between 4.68 to 5.57 for 30 DAS which ranged in between 7.05 to 7.54 and for 45 DAS which ranged in between 10.27 to 12.01 showing significantly higher number of leaves in *Pseudomonas striata* treated plots follows by *Bacillus megaterium* and *Trichoderma viride*. Whereas significantly lower number of leaves per plot noted in uninoculated control. Similarly graded levels of zinc in the form of zinc sulphate also increase number of leaves with each incremental dose up to 30 kg $ZnSO_4$ ha^{-1}.

Table 2: Effect of zinc solubilizing cultures and zinc levels on number of leaves in spinach.

Treatments	Number of leaves/plant		
	15 days after sowing	**30 days after sowing**	**45 days after sowing**
Zinc solubilizers(S)			
S0: Control	4.68	7.05	10.27
S1: *Pseudomonas striata*	5.57	7.54	12.01
S2: *Trichoderma viride*	4.76	7.28	10.46
S3: *Bacillus megaterium*	5.29	7.43	11.01
S.Em.±	0.02	0.07	0.07
C.D. at 5%	0.07	0.20	0.20
Levels of $ZnSO_4$ (Zn)			
Zn0: $ZnSO_4$ 0 kg ha^{-1}	4.81	6.70	10.09
Zn1: $ZnSO_4$ 10 kg ha^{-1}	5.03	7.12	10.63
Zn2: $ZnSO_4$ 20 kg ha^{-1}	5.14	7.50	11.27
Zn3: $ZnSO_4$ 30 kg ha^{-1}	5.33	7.98	11.76
S.Em.±	0.02	0.07	0.07
C.D. at 5%	0.07	0.20	0.20
Interaction (Sx Zn)			
S.Em.±	0.05	0.14	0.14
C.D. at 5 %	0.15	0.41	0.41

Interaction effect of zinc solubilizing cultures and zinc levels on number of leaves in spinach in Table 2a. Significantly synergistic effect of each factor was recorded to each other. Showing highest number of leaves in *Pseudomonas striata* X $ZnSO_4$ 30 kg ha^{-1} (15 DAS – 5.85, 30 DAS – 8.33, 45 DAS – 12.77) and it was found at par with *Bacillus megaterium* X $ZnSO_4$ 30 kg ha^{-1}. However the lower values of number of leaves was recorded in control.

Table 2a: Interaction effect of zinc solubilizers and levels of zinc on number of leaves in spinach.

Treatments	Zn0:$ZnSO_4$0 kg ha^{-1}	Zn1:$ZnSO_4$10 kg ha^{-1}	Zn2:$ZnSO_4$20kg ha^{-1}	Zn3:$ZnSO_4$ 30 kg ha^{-1}	Mean
15 days after sowing					
S0: Control	4.38	4.65	4.74	4.97	4.68
S1:*Pseudomonas striata*	5.38	5.51	5.55	5.85	5.57
S2: *Trichoderma viride*	4.61	4.66	4.82	4.96	4.76
S3: *Bacillus megaterium*	4.86	5.31	5.45	5.55	5.29
Mean	4.80	5.03	5.14	5.33	
Interaction	S	Zn	SX Zn		
SEm+	0.02	0.02	0.05		
CD at 5%	0.07	0.07	0.15		
30 days after sowing					
S0: Control	6.10	7.07	7.43	7.60	7.05
S1:*Pseudomonas striata*	7.07	7.23	7.53	8.33	7.54
S2: *Trichoderma viride*	6.70	6.83	7.63	7.97	7.28
S3: *Bacillus megaterium*	6.93	7.33	7.40	8.03	7.42
Mean	6.70	7.11	7.50	7.98	
Interaction	S	Zn	SX Zn		
SEm+	0.07	0.07	0.14		
CD at 5%	0.20	0.20	0.41		
45 days after sowing					
S0: Control	9.37	10.10	10.77	10.83	10.26
S1:*Pseudomonas striata*	11.17	11.73	12.37	12.77	12.00
S2: *Trichoderma viride*	9.70	9.80	10.80	11.54	10.46
S3: *Bacillus megaterium*	10.13	10.90	11.13	11.88	11.01
Mean	10.09	10.63	11.26	11.75	
Interaction	S	Zn	SX Zn		
SEm+	0.07	0.07	0.14		
CD at 5%	0.20	0.20	0.41		

The number of leaves per plant is one of the growth parameter related to physiological development of the crop. The inoculation of zinc solubilizers and graded levels of zinc proved significantly to increase number of leaves per plant over uninoculated control. Ramandeep *et al.* (2018) reported that maximum number of leaves per plant in potato with inoculation of biofertilizer, Azotobactor and phosphate solubilizing bacteria along with recommended dose of fertilizers. These results are in line with the findings of Bairva *et al.* (2012) [1] and Singh *et al.* (2014) [13].

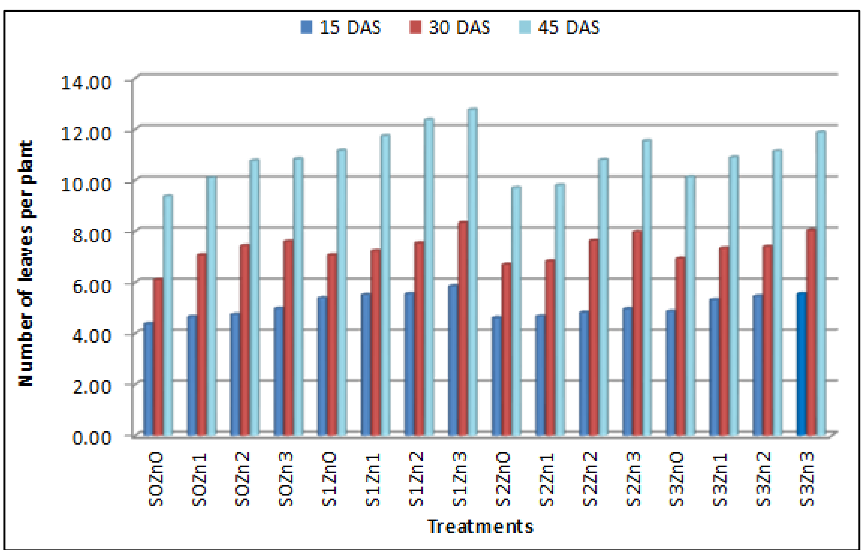

Fig 2: Interaction effect of zinc solubilizers and levels of zinc on number of leaves in spinach.

3. Dry matter weight

Results given in Table 3 and depicted in Fig.3 related to dry matter weight of plant in spinach indicates significant effect of zinc solubilizers and graded levels of zinc. The zinc solubilizers influenced the dry matter weight which ranges from 0.17 to 0.22 gm/plant for 15 DAS, 1.39 to 2.03 gm/plant for 30 DAS and 1.88 to 2.21 gm/plant for 45 DAS showing significantly higher dry matter weight of plant in

Pseudomonas striata treated plots followed by *Bacillus megaterium* and *Trichoderma viride*. Whereas, significantly lower dry matter weight of plant was noted in uninoculated control. Similarly graded levels of zinc in the form of zinc sulphate also increased dry matter weight of plant with each incremental dose up to 30 kg $ZnSO_4$ ha^{-1}. The dry matter weight influenced by Zn application ranged from 0.18 to 0.22 gm/plant for 15 DAS, 1.50 to 2.00 gm/plant for 30 DAS and 1.77 to 2.45 gm/plant for 45 DAS.

Table 3: Effect of zinc solubilizing cultures and zinc levels on dry matter weight in spinach.

Treatments	Dry matter weight (gm/plant)		
	15 days after sowing	**30 days after sowing**	**45 days after sowing**
Zinc solubilizers (S)			
S0: Control	0.17	1.39	1.88
S1: *Pseudomonas striata*	0.22	2.03	2.21
S2: *Trichoderma viride*	0.19	1.73	2.05
S3: *Bacillus megaterium*	0.21	1.89	2.11
S. Em.±	0.002	0.03	0.04
C.D. at 5 %	0.006	0.11	0.11
Levels of $ZnSO_4$ (Zn)			
Zn0: $ZnSO_4$ 0 kg ha^{-1}	0.18	1.50	1.77
Zn1: $ZnSO_4$ 10 kg ha^{-1}	0.19	1.71	1.90
Zn2: $ZnSO_4$ 20 kg ha^{-1}	0.20	1.82	2.14
Zn3: $ZnSO_4$ 30 kg ha^{-1}	0.22	2.00	2.45
S. Em.±	0.002	0.03	0.04
C.D. at 5 %	0.006	0.11	0.11
Interaction (SX Zn)			
S. Em.±	0.004	0.07	0.08
C.D. at 5 %	0.01	0.22	0.23

Interaction effect of zinc solubilizing cultures and zinc levels on dry matter weight of spinach is given in Table 3a. Significantly highest dry matter weight of plant was found in *Pseudomonas striata* X $ZnSO_4$ 30 kg ha^{-1} (0.24 gm/plant – 15 DAS, 2.49 gm/plant – 30 DAS and 2.71 gm/plant – 45 DAS) and it was found at par with *Bacillus megaterium* X $ZnSO_4$ 30 kg ha^{-1}. However the lower values of dry matter weight of plant was recorded in control plots.

Table 3a: Interaction effect of zinc solubilizers and levels of zinc on dry matter weight in 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
15 days after sowing					
S0: Control	0.14	0.16	0.16	0.20	0.16
S1:*pseudomonas striata*	0.21	0.21	0.22	0.24	0.21
S2:*Trichoderma viride*	0.18	0.19	0.20	0.20	0.19
S3: *Bacillus megaterium*	0.20	0.20	0.21	0.22	0.20
Mean	0.18	0.18	0.19	0.21	
Interaction	S	Zn	SX Zn		
SEm+	0.002	0.002	0.004		
CD at 5%	0.006	0.006	0.013		
30 days after sowing					
S0: Control	1.23	1.36	1.46	1.51	1.39
S1:*Pseudomonas striata*	1.58	1.91	2.13	2.49	2.02
S2: *Trichoderma viride*	1.62	1.70	1.73	1.86	1.72
S3: *Bacillus megaterium*	1.58	1.88	1.94	2.15	1.88
Mean	1.50	1.71	1.81	2.00	
Interaction	S	Zn	SX Zn		
SEm+	0.03	0.03	0.07		
CD at 5%	0.11	0.11	0.22		
45 days after sowing					
S0: Control	1.59	1.81	1.84	2.29	1.88
S1:*Pseudomonas striata*	1.82	1.88	2.35	2.71	2.21
S2: *Trichoderma viride*	1.86	1.89	1.99	2.44	2.04
S3: *Bacillus megaterium*	1.79	2.03	2.26	2.37	2.11
Mean	1.76	1.90	2.15	2.45	
Interaction	S	Zn	SXZn		
SEm+	0.04	0.04	0.08		
CD at 5%	0.11	0.11	0.23		

The increase in dry matter weight per plant with increasing age of the plant with application of various zinc solubilizers and graded levels of zinc in the present study was in accordance with the findings reported by Melek Ekinci*et al.*

(2014) [16] who observed that application of *Bacilllus megaterium* recorded significantly higher dry matter yield in cauliflower as compared to the uninoculated control. The results are in agreement with Pandey *et al.* (2009) [8], Bairva *et al.* (2012) [1] and Tanwar *et al.* (2013) [15].

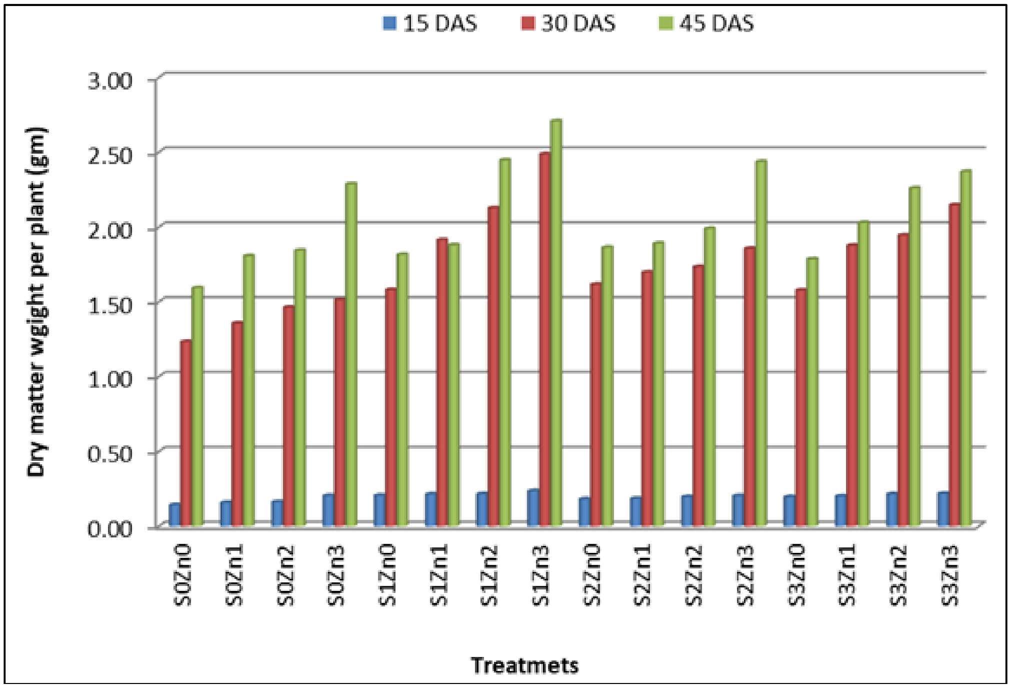

Fig 3: Interaction effect of zinc solubilizers and levels of zinc on dry matter weight in spinach.

4. Leaf area index

Data narrated in Table 4 and depicted in Fig.4 related to leaf area index of spinach indicates significant effect of zinc solubilizers and graded level of zinc on leaf area index in spinach. Zinc solubilizers influence the leaf area index which ranges 17.82 to 23.08 cm^2 for 15 DAS, 83.39 to 93.28 cm^2 for 30 DAS and 103.54 to 114.30 cm^2 for 45 DAS showing significantly higher leaf area index in *Pseudomonas striata* treated plots follows by *Bacillus megaterium* and *Trichoderma viride*. Whereas significantly lower leaf area index per plot was noted in uninoculated control. Similarly graded levels of zinc in the form of zinc sulphate also increase leaf area index with each incremental dose up to 30 kg $ZnSO_4$ ha^{-1}. The leaf area index influenced by zinc application ranged from 99.99 to 119.28 cm^2 at 45 DAS.

Table 4: Effect of zinc solubilizing cultures and zinc levels on leaf area index in spinach.

Treatments	Leaf area index (cm^2)		
	15 days after sowing	30 days after sowing	45 days after sowing
Zinc solubilizers (S)			
S0: Control	17.82	83.39	103.54
S1: *Pseudomonas striata*	23.08	93.28	114.30
S2: *Trichoderma viride*	21.62	89.25	110.69
S3: *Bacillus megaterium*	22.95	91.38	111.02
S. Em.±	0.20	0.58	0.70
C.D. at 5 %	0.57	1.68	2.02
Levels of $ZnSO_4$ (Zn)			
Zn0: $ZnSO_4$ 0 kg ha^{-1}	19.16	85.72	99.99
Zn1: $ZnSO_4$ 10 kg ha^{-1}	20.50	88.53	106.65
Zn2: $ZnSO_4$ 20 kg ha^{-1}	22.00	89.97	113.63
Zn3: $ZnSO_4$ 30 kg ha^{-1}	23.82	93.48	119.28
S. Em.±	0.20	0.58	0.70
C.D. at 5 %	0.57	1.68	2.02
Interaction (Sx Zn)			
S. Em.±	0.40	1.16	1.40
C.D. at 5 %	1.15	3.36	4.05

Interaction effect of zinc solubilizing cultures and zinc levels on leaf area index of spinach in Table 4a. Synergistic effect of both factor was recorded on each other showing significantly highest leaf area index in *Pseudomonas striata* X $ZnSO_4$ 30 kg ha^{-1} (15 DAS – 27.12 cm^2, 30 DAS – 98.98 cm^2 and 45 DAS -125.04 cm^2) and it was found at par with *Bacillus megaterium* X $ZnSO_4$ 30 kg ha^{-1} but for 45 DAS values are at par with *Trichoderma viride* X $ZnSO_4$ 30 kg ha^{-1}. However the lower values of leaf area index was recorded in without microbial culture and zinc application.

Table 4a: Interaction effect of zinc solubilizers and levels of zinc on leaf area index in spinach.

Treatments	Zn0:ZnSO₄0 kg ha-1	Zn1:ZnSO₄10 kg ha-1	Zn2:ZnSO₄ 20 kg ha-1	Zn3:ZnSO₄ 30 kg ha-1	Mean
	15 days after sowing				
S0: Control	16.74	17.51	18.08	18.96	17.82
S1: *eudemons striata*	20.76	21.21	23.25	27.12	23.08
S2: *richoderma viride*	18.82	21.43	22.02	24.21	21.62
S3: *Bacillus megaterium*	20.31	21.85	24.66	24.96	22.94
Mean	19.15	20.49	22.00	23.81	
Interaction	S	Zn	SX Zn		
S.Em+	0.20	0.20	0.40		
CD at 5%	0.57	0.57	1.15		
	30 days after sowing				
S0: Control	80.85	81.32	83.19	88.19	83.38
S1: *Pseudomonas striata*	85.95	93.66	94.52	98.98	93.27
S2: *richoderma viride*	87.37	88.53	89.83	92.87	89.65
S3: *Bacillus megaterium*	88.70	90.60	92.36	93.86	91.38
Mean	85.71	88.52	89.97	93.47	
Interaction	S	Zn	SX Zn		
S.Em+	0.583	0.58	1.16		
CD at 5%	1.68	1.68	3.36		
	45 days after sowing				
S0: Control	91.44	100.86	108.35	113.51	103.54
S1: *Pseudomonas striata*	104.36	109.69	118.13	125.04	114.30
S2: *richoderma viride*	98.74	109.60	114.49	119.91	110.68
S3: *Bacillus megaterium*	105.41	106.45	113.54	118.68	111.01
Mean	99.98	106.64	113.62	119.28	
Interaction	S	Zn	SX Zn		
S.Em+	0.70	0.70	1.40		
CD at 5%	2.02	2.02	4.05		

The increased leaf area index caused by inoculation of various microbial cultures depends on the ability of microorganisms to survive and develop in the rhizospheric. The possible mechanism for increased plant growth in an increase in nutrient transfer from soil to root. The resulted increase in growth parameters could be due to certain plant growth hormones and secondary metabolites produced by different zinc solubilizers which may act as auxin like compounds. Our results are in agreement with the findings of Tanwar *et al.* (2013) [15]. Growth enhancement due to inoculation was also reported by Turan *et al.* (2014) [16] and Singh *et al.* (2015) [14].

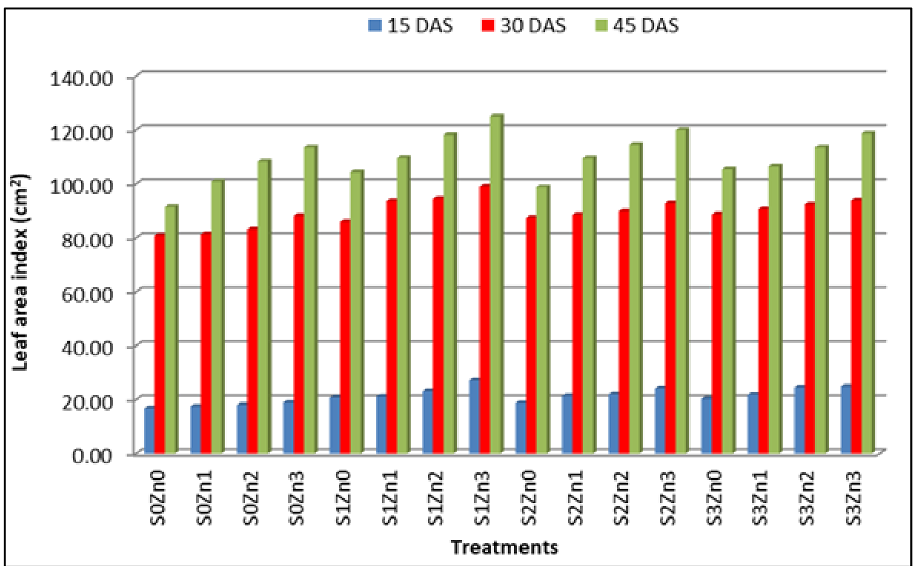

Fig 4: Interaction effect of zinc solubilizers and levels of zinc on leaf area index in spinach.

5. Yield

There was significant increase in dry and fresh weight yield of spinach due to inoculation of zinc solubilizing microbial cultures and graded levels of zinc as compared to uninoculated control (Table 5 and Fig.5). Significantly highest dry and fresh weight yield of spinach was recorded with *Pseudomonas striata* (S1) inoculation 29.25 q ha^{-1} and 188.65 q ha^{-1} dry and fresh weight yield respectively over control but *Bacillus megaterium* (S3) was found at par with S1 treatment. Further, graded levels of zinc application in the form of $ZnSO_4$ also increased the dry and fresh weight yield upto 30 kg ha^{-1}.

Table 5: Effect of zinc solubilizing cultures and zinc levels on yield of spinach.

Treatments	Dry weight yield (qha^{-1})	Fresh weight yield (qha^{-1})
Zinc solubilizers (S)		
S0: Control	24.67	160.39
S1: *Pseudomonas striata*	29.25	188.65
S2: *Trichoderma viride*	26.82	177.16
S3: *Bacillus megaterium*	27.55	180.74
S. Em.±	0.35	2.39
C.D. at 5 %	1.03	6.91
Levels of $ZnSO_4$ (Zn)		
Zn0: $ZnSO_4$ 0 kg ha^{-1}	24.40	153.57
Zn1: $ZnSO_4$ 10 kg ha^{-1}	26.26	167.91
Zn2: $ZnSO_4$ 20 kg ha^{-1}	27.60	184.42
Zn3: $ZnSO_4$ 30 kg ha^{-1}	30.03	201.04
S. Em.±	0.35	2.39
C.D. at 5 %	1.03	6.91
Interaction (Sx Zn)		
S. Em.±	0.71	4.78
C.D. at 5 %	2.06	13.82

The interaction between zinc solubilizers and levels of zinc was noted statistically significant showing highest dry (32.59 q ha^{-1}) and fresh weight (215.40 q ha^{-1}) yield of spinach in treatment having *Pseudomonas striata* treatment along with 30 kg $ZnSO_4$ ha^{-1} (Table 5a). However the lower values of dry and fresh weight yield was recorded in without microbial culture and zinc application.

Table 5a: Interaction effect of zinc solubilizers and levels of zinc on yield 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
Dry weight yield (q ha^{-1})					
S0: Control	21.37	23.15	24.58	29.56	24.66
S1: *Pseudomonas striata*	25.91	28.10	30.58	32.39	29.24
S2: *Trichoderma viride*	24.64	27.17	27.96	27.52	26.82
S3: *Bacillus megaterium*	25.66	26.63	27.27	30.66	27.55
Mean	24.39	26.26	27.59	30.66	
Interaction	S	Zn	SX Zn		
SEm+	0.35	0.35	0.71		
CD at 5%	1.03	1.03	2.06		
Fresh weight yield (q ha^{-1})					
S0: Control	138.94	150.47	159.81	192.34	160.38
S1: *Pseudomonas striata*	168.75	172.40	198.04	215.40	188.64
S2: *Trichoderma viride*	144.41	176.62	191.01	196.58	177.15
S3: *Bacillus megaterium*	162.17	172.15	188.80	199.85	180.74
Mean	153.56	167.91	184.41	201.04	
Interaction	S	Zn	SX Zn		
SEm+	2.39	2.39	4.78		
CD at 5%	6.91	6.91	13.82		

In this experiment, the use of zinc solubilizing microbial inoculants increases the dry weight and fresh weight by *Pseudomonas striata* and *Bacillus megaterium* strains. These strains enhances the growth characters and yield based on increasing capacity of roots to mobilize and take up nutrient and substances for overall reproductive plant fitness and also due to production of IAA by bacteria enhances the development of host plant root system and this helps in growth of crops. Pandey *et al.* (2009) [8] reported that inoculation of different microbial cultures significantly increased the yield of okra over control. Similarly Bairva *et al.* (2012) [1] found that yield of fenugreek was significantly influenced by the dual inoculation of seed with Rhizobium + PSB. Further, Singh (2014) [13] registered that combination of biofertilizer along with organic and inorganic sources of nutrient increased yield of coriander as compared to single application of biofertilizer. Thereafter, Kumar *et al.* (2016) [5] indicate significant impact of Zn solubilizing microorganisms on yield of chilli.

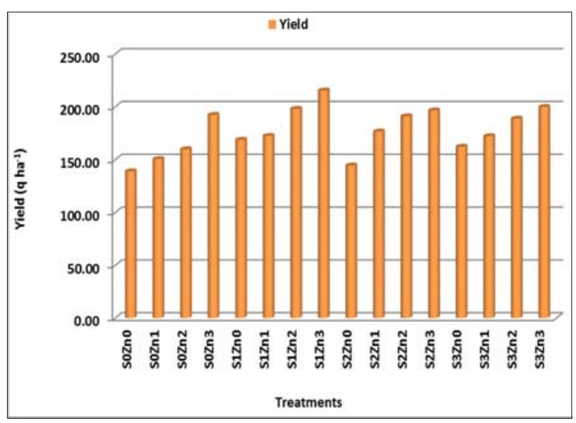

Fig 5: Interaction effect of zinc solubilizers and levels of zinc on yield of spinach.

Conclusion

Significant effect of zinc solubilizing cultures Psuedomonas striata and zinc levels 30 kg ha-1 was noted on growth parameters like Plant height, number of leaves per plant, dry matter weight per plant and leaf area index were registered in Psuedomonas striata + 30 kg ZnSO4 ha-1 over rest of the treatments. Also maximum dry weight yield and fresh weight yield of spinach was recorded in inoculated plots with Psuedomonas striata + 30 kg ZnSO4 ha-1. However treatment Bacillus megaterium was at par with superior treatment.

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