Effect of different zinc solubilizing microbial cultures and zinc levels on soil chemical properties and quality of spinach

YA Waghmare, AL Dhamak and Syed Ismail

DOI: https://doi.org/10.22271/chemi.2020.v8.i1ao.8673

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 quality parameter and chemical properties of soil. Experiment consists of sixteen treatments 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. The quality attributes improved significantly with inoculation of Pseudomonas striata + 30 kg ZnSO₄ ha⁻¹. The pH, EC, organic carbon and calcium carbonate after harvest of spinach indicates non – significant interactive results.

Keywords: Spinach, zinc levels, zinc solubilizers

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 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) [13]. 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) [1]. However, only some bacterial species of the genera Bacillus, Acinetobacter, Gluconacetobacter, and Pseudomonas have been reported (Simine, et al. 1998) [9]. 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 oxido-reductive 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, Thiothecillus 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 (ZnCO₃) and zinc sulphide (ZnS) instead of costly zinc sulphate (Mahdi et al. 2010) [3].

**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**

**A) Quality parameters**

**Moisture content**

Data narrated in Table 1 and Fig.1 related to moisture percentage in spinach indicates significant effect of zinc solubilizers and graded level of zinc on moisture percentage in spinach. Zinc solubilizers influence the moisture percentage which range 86.76 to 91.30 percent for 15 DAS, 87.32 to 89.30 percent for 30 DAS and 87.90 to 91.36 percent for 45 DAS showing significantly higher moisture percentage in *Pseudomonas striata* treated plots follows by *Bacillus megaterium* and *Trichoderma viride*. Whereas significantly lower moisture percentage was noted in uninoculated control. Similarly, graded levels of zinc in the form of zinc sulphate also increase moisture percentage with each incremental dose up to 30 kg ZnSO₄ ha⁻¹. The moisture percentage influenced by zinc application ranged from 87.78 to 91.27 percent at 45 DAS. However, interaction effects did not reach to the level of significance in 15 DAS and 45 DAS stage.

**Table 1:** Effect of zinc solubilizing cultures and zinc levels on moisture content in spinach.

| Treatments | Moisture content (%) |
|------------|----------------------|
|            | 15 days after sowing | 30 days after sowing | 45 days after sowing |
| Zinc solubilizers (S) | | | |
| S0: Control       | 86.76               | 87.32               | 87.90               |
| S1: *Pseudomonas striata* | 91.30               | 89.30               | 91.36               |
| S2: *Trichoderma viride* | 88.75               | 88.19               | 89.77               |
| S3: *Bacillus megaterium* | 90.27               | 88.25               | 90.20               |
| S. Em.±          | 0.37                | 0.36                | 0.34                |
| C.D. at 5%       | 1.09                | 1.04                | 0.98                |
| Levels of ZnSO₄ (Zn) | | | |
| Zn0: ZnSO₄ 0 kg ha⁻¹ | 87.19               | 85.88               | 87.78               |
| Zn1: ZnSO₄ 10 kg ha⁻¹ | 89.38               | 87.72               | 89.51               |
| Zn2: ZnSO₄ 20 kg ha⁻¹ | 89.88               | 89.42               | 90.67               |
| Zn3: ZnSO₄ 30 kg ha⁻¹ | 90.71               | 90.03               | 91.27               |
| S. Em.±          | 0.37                | 0.36                | 0.34                |
| C.D. at 5%       | 1.09                | 1.04                | 0.98                |
| Interaction (S×Zn) | | | |
| S. Em.±          | 0.75                | 0.72                | 0.68                |
| C.D. at 5%       | NS                  | 2.08                | NS                  |

**Table 1a:** Interaction effect of zinc solubilizers and level of zinc on moisture content in spinach.

| Treatments | Zn0: ZnSO₄ 0 kg ha⁻¹ | Zn1: ZnSO₄ 10 kg ha⁻¹ | Zn2: ZnSO₄ 20 kg ha⁻¹ | Zn3: ZnSO₄ 30 kg ha⁻¹ | Mean |
|-----------|----------------------|-----------------------|-----------------------|-----------------------|------|
|           | 30 days after sowing |                       |                       |                       |      |
| S0: Control | 83.41               | 86.25                 | 89.46                 | 90.16                 | 87.31|
| S1: *Pseudomonas striata* | 86.81               | 87.88                 | 90.98                 | 91.53                 | 89.30|
| S2: *Trichoderma viride* | 85.71               | 88.62                 | 89.92                 | 89.49                 | 88.18|
| S3: *Bacillus megaterium* | 87.59               | 88.14                 | 88.32                 | 88.96                 | 88.25|
| Mean      | 85.87               | 87.72                 | 89.42                 | 90.03                 |      |
| Interaction | S                   | Zn                    | S×Zn                  |                       |      |
| S. Em.±   | 0.36                | 0.36                  | 0.72                  |                       |      |
| C.D. at 5% | 1.04                | 1.04                  | 2.08                  |                       |      |
Interaction effect of zinc solubilizing cultures and zinc levels on moisture percentage of spinach in Table 1a. Synergistic effect of both factor was recorded on each other showing significantly only in 30 DAS highest moisture percentage in *Pseudomonas striata* X ZnSO$_4$ 30 kg ha$^{-1}$ which is 91.53 percent.

Ascorbic acid content
Results given in Table 2 and Fig.2 related to ascorbic acid content of plant in spinach indicate significant effect of zinc solubilizers and graded levels of zinc. The zinc solubilizers influenced the ascorbic acid content which ranges from 11.77 to 13.46 mg 100 gm$^{-1}$ for 15 DAS, 20.89 to 24.93 mg 100 gm$^{-1}$ for 30 DAS and 29.11 to 34.53 mg 100 gm$^{-1}$ for 45 DAS showing significantly higher ascorbic acid content of plant in *Pseudomonas striata* treated plots followed by *Bacillus megaterium* and *Trichoderma viride*. Whereas, significantly lower ascorbic acid content of plant was noted in uninoculated control. Similarly graded levels of zinc in the form of zinc sulphate also increased ascorbic acid content of plant with each incremental dose up to 30 kg ZnSO$_4$ ha$^{-1}$. The ascorbic acid content influenced by Zn application ranged from 11.61 to 13.56 mg 100 gm$^{-1}$ for 15 DAS, 21.02 to 25.22 mg 100 gm$^{-1}$ for 30 DAS and 27.22 to 37.25 mg 100 gm$^{-1}$ for 45 DAS. However, interaction effects did not reach to the level of significance in 15 DAS.

![Graph](image.png)

**Fig 1:** Interaction effect of zinc solubilizers and levels of zinc on moisture content in spinach.

| Treatments | Ascorbic acid content (mg 100 gm$^{-1}$) |
|------------|-----------------------------------------|
|            | 15 days after sowing | 30 days after sowing | 45 days after sowing |
| **Zinc solubilizers (S)** | | | |
| S0: Control | 11.77 | 20.89 | 29.11 |
| S1: *Pseudomonas striata* | 13.46 | 24.93 | 34.53 |
| S2: *Trichoderma viride* | 12.37 | 22.95 | 31.89 |
| S3: *Bacillus megaterium* | 12.51 | 23.63 | 32.55 |
| S.Em.± | 0.21 | 0.29 | 0.49 |
| C.D. at 5% | 0.61 | 0.83 | 1.43 |
| **Levels of ZnSO$_4$ (Zn)** | | | |
| Zn0: ZnSO$_4$ 0 kg ha$^{-1}$ | 11.61 | 21.02 | 27.22 |
| Zn1: ZnSO$_4$ 10 kg ha$^{-1}$ | 12.26 | 22.50 | 30.37 |
| Zn2: ZnSO$_4$ 20 kg ha$^{-1}$ | 12.68 | 23.66 | 33.24 |
| Zn3: ZnSO$_4$ 30 kg ha$^{-1}$ | 13.56 | 25.22 | 37.25 |
| S.Em.± | 0.21 | 0.29 | 0.49 |
| C.D. at 5% | 0.61 | 0.83 | 1.43 |
| **Interaction (SxZn)** | | | |
| S.Em.± | 0.42 | 0.58 | 0.99 |
| C.D. at 5% | NS | 1.67 | 2.87 |
Table 2a: Interaction effect of zinc solubilizers and levels of zinc on ascorbic acid content in spinach.

| Treatments   | Zn0: ZnSO₄ 0 kg ha⁻¹ | Zn1: ZnSO₄ 10 kg ha⁻¹ | Zn2: ZnSO₄ 20 kg ha⁻¹ | Zn3: ZnSO₄ 30 kg ha⁻¹ | Mean |
|--------------|-----------------------|-----------------------|-----------------------|-----------------------|------|
| 30 days after sowing |                       |                       |                       |                       |      |
| S0: Control  | 19.72                 | 20.72                 | 21.39                 | 21.71                 | 20.88|
| S1: *Pseudomonas striata* | 21.61                 | 23.74                 | 26.63                 | 27.76                 | 24.93|
| S2: *Trichoderma viride* | 21.13                 | 22.43                 | 22.71                 | 25.53                 | 22.94|
| S3: *Bacillus megaterium* | 21.63                 | 23.10                 | 23.90                 | 25.89                 | 23.62|
| Mean         | 21.02                 | 22.49                 | 23.65                 | 25.22                 |      |
| Interaction  | S                     | Zn                   | SXZn                  |                       |      |
| SEm⁺         | 0.29                  | 0.29                  | 0.58                  |                       |      |
| CD at 5%     | 0.83                  | 0.83                  | 1.67                  |                       |      |
| 45 days after sowing |                       |                       |                       |                       |      |
| S0: Control  | 22.34                 | 28.76                 | 31.20                 | 34.13                 | 29.10|
| S1: *Pseudomonas striata* | 29.27                 | 31.68                 | 35.18                 | 42.00                 | 34.53|
| S2: *Trichoderma viride* | 27.98                 | 29.59                 | 34.02                 | 35.97                 | 31.89|
| S3: *Bacillus megaterium* | 29.28                 | 31.44                 | 32.57                 | 36.91                 | 32.55|
| Mean         | 27.21                 | 30.36                 | 33.24                 | 37.25                 |      |
| Interaction  | S                     | Zn                   | SXZn                  |                       |      |
| SEm⁺         | 0.49                  | 0.49                  | 0.99                  |                       |      |
| CD at 5%     | 1.43                  | 1.43                  | 2.87                  |                       |      |

Interaction effect of zinc solubilizing cultures and zinc levels on ascorbic acid content of spinach is given in Table 2a. Significantly highest ascorbic acid content of plant was found in *Pseudomonas striata* X ZnSO₄ 30 kg ha⁻¹ (37.76 mg 100 gm⁻¹ – 30 DAS and 42.00 mg 100 gm⁻¹ – 45 DAS). However the lower values of ascorbic acid content of plant was recorded in control plots.

Experimental result are in agreement with the reports of Vidyashree *et al.* (2018) [11] who also noted that highest content of ascorbic acid was obtained with the inoculation of *Bacillus aryabhattai* treated plots which was on par with the treatment inoculated with *Bacillus sp* (PAN-TM1). This might be due to better growth and yield attribute by this bacterial isolate.

Chlorophyll content

Results given in Table 3 related to chlorophyll content in spinach indicates significant effect of zinc solubilizers and graded level of zinc on chlorophyll content in spinach. Zinc solubilizers influence the chlorophyll content as for 15 DAS which ranged in between 2.73 to 3.48 mg gm⁻¹ for 30 DAS which ranged in between 5.96 to 6.59 mg gm⁻¹ and for 45 DAS which ranged in between 6.27 to 7.44 mg gm⁻¹ showing significantly higher chlorophyll content in *Pseudomonas striata* treated plots followed by *Bacillus megaterium* and *Trichoderma viride*. Whereas, significantly lower chlorophyll content per plot noted in uninoculated control. Similarly graded levels of zinc in the form of zinc sulphate also increase chlorophyll content with each incremental dose up to 30 kg ZnSO₄ ha⁻¹.

Interaction effect of zinc solubilizing cultures and zinc levels on chlorophyll content in spinach in Table 3a and Fig.3. Significantly synergistic effect of each factor was recorded to each other. Showing highest chlorophyll content in *Pseudomonas striata* X ZnSO₄ 30 kg ha⁻¹ (15 DAS – 4.34 mg gm⁻¹, 30 DAS – 7.16 mg gm⁻¹, 45 DAS – 8.53 mg gm⁻¹) and it was found at par with *Bacillus megaterium* X ZnSO₄ 30 kg ha⁻¹. However the lower values of chlorophyll content was recorded in without microbial culture and zinc application.
Table 3: Effect of zinc solubilizing cultures and zinc levels on chlorophyll content in spinach.

| Treatments | Chlorophyll content (mg gm\(^{-1}\)) | 15 days after sowing | 30 days after sowing | 45 days after sowing |
|------------|--------------------------------------|----------------------|----------------------|----------------------|
| Zinc solubilizers (S) | | | | |
| S0: Control | | 2.73 | 5.96 | 6.27 |
| S1: Pseudomonas striata | | 3.48 | 6.59 | 7.44 |
| S2: Trichoderma viride | | 3.36 | 6.22 | 7.10 |
| S3: Bacillus megaterium | | 3.39 | 6.26 | 7.13 |
| S.Em± | | 0.05 | 0.03 | 0.08 |
| C.D. at 5% | | 0.14 | 0.10 | 0.24 |
| Levels of ZnSO\(_4\) (Zn) | | | | |
| Zn0: ZnSO\(_4\) 0 kg ha\(^{-1}\) | | 2.75 | 5.81 | 5.98 |
| Zn1: ZnSO\(_4\) 10 kg ha\(^{-1}\) | | 3.14 | 6.11 | 6.84 |
| Zn2: ZnSO\(_4\) 20 kg ha\(^{-1}\) | | 3.36 | 6.32 | 7.39 |
| Zn3: ZnSO\(_4\) 30 kg ha\(^{-1}\) | | 3.70 | 6.79 | 7.74 |
| S.Em± | | 0.05 | 0.03 | 0.08 |
| C.D. at 5% | | 0.14 | 0.10 | 0.24 |
| Interaction (SxZn) | | | | |
| S.Em± | | 0.10 | 0.07 | 0.16 |
| C.D. at 5% | | 0.29 | 0.21 | 0.48 |

Progressive increase in photosynthetic pigments by bioinoculants could be a result of enhanced gas exchange capacity due to decreased stomatal resistances and increased transpiration fluxes. Larger leaf area also increases the number of stomata per leaf and hence better photosynthetic rate. PGPR treatment increases chlorophyll content in plants and thereby enhances the rate of photosynthesis leading to improved overall plant health. Amino acids such as methionine, Histidine and organic acids are known to have a positive effect on zinc absorption and have been used for zinc supplement (Vaid \textit{et al.} 2014) [10].

Table 3a: Interaction effect of zinc solubilizers and levels of zinc on chlorophyll content 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 |
|------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|------|
| S0: Control | 2.52 | 2.75 | 2.79 | 2.87 | 2.73 |
| S1: Pseudomonas striata | 2.88 | 3.12 | 3.58 | 4.34 | 3.47 |
| S2: Trichoderma viride | 2.90 | 3.40 | 3.50 | 3.64 | 3.36 |
| S3: Bacillus megaterium | 2.71 | 3.29 | 3.58 | 3.97 | 3.38 |
| Mean | 2.75 | 3.14 | 3.36 | 3.70 | |
| Interaction | S | Zn | SXZn |
| S.Em+ | 0.05 | 0.05 | 0.10 |
| C.D at 5% | 0.14 | 0.14 | 0.29 |

30 days after sowing

| S0: Control | 5.33 | 5.99 | 6.08 | 6.45 | 5.96 |
| S1: Pseudomonas striata | 6.20 | 6.33 | 6.69 | 7.16 | 6.57 |
| S2: Trichoderma viride | 5.84 | 5.98 | 6.31 | 6.77 | 6.22 |
| S3: Bacillus megaterium | 5.89 | 6.15 | 6.22 | 6.79 | 6.26 |
| Mean | 5.81 | 6.11 | 6.32 | 6.79 | |
| Interaction | S | Zn | SXZn |
| S.Em+ | 0.03 | 0.03 | 0.07 |
| C.D at 5% | 0.10 | 0.10 | 0.21 |

45 days after sowing

| S0: Control | 4.88 | 6.20 | 6.75 | 7.27 | 6.27 |
| S1: Pseudomonas striata | 6.34 | 7.04 | 7.85 | 8.53 | 7.43 |
| S2: Trichoderma viride | 6.25 | 6.97 | 7.52 | 7.65 | 7.09 |
| S3: Bacillus megaterium | 6.44 | 7.13 | 7.44 | 7.52 | 7.13 |
| Mean | 5.97 | 6.83 | 7.38 | 7.74 | |
B) Chemical properties of soil

Soil pH

pH is an important intrinsic property of soil which usually does not change easily particularly in Vertisols due to more buffering capacity. The results pertaining to the effect of different zinc solubilizing microbial isolates on periodical soil pH in soil are narrated in Table 4 related to soil pH in soil indicates non-significant effect of zinc solubilizers and graded zinc levels. Zinc solubilizers decrease the soil pH. The uninoculated control pH is 8.12 and after addition of bioinoculants slightly decrease in soil pH Pseudomonas striata 7.99, Bacillus megaterium 7.94 and Trichoderma viride 8.01. Addition of graded levels of zinc in the form of zinc sulphate also increase the soil pH with each incremental dose up to 30 kg ZnSO$_4$ ha$^{-1}$. The soil pH as influenced by zinc application ranged from 7.88 to 8.15. The non-significant interactive effect of zinc solubilizers and levels of zinc in present investigation may be due to one season results i.e. treatment generally not affecting the physico-chemical properties in a single season particularly in Vertisol. Similar decline in the pH of soil with inoculation of solubilizing microorganisms was reported by Sable et al. (2016) [8]. The slight decrease in soil pH with bioinoculants treatment may be ascribed by the secretion of organic acids by different bioinoculants.

Electrical conductivity

The results pertaining to the effect of different zinc solubilizing microbial isolates on electrical conductivity (EC) in soil are narrated in Table 4 related to electrical conductivity (EC) in soil indicates non-significant effect of zinc solubilizers and graded zinc levels. The uninoculated control EC was 0.20 dSm$^{-1}$ and after addition of bioinoculants Pseudomonas striata slightly increase in soil EC 0.22 dSm$^{-1}$ and for Bacillus megaterium is lowest 0.17 dSm$^{-1}$. However soil EC for Bioinoculant Trichoderma viride is 0.19 dSm$^{-1}$ and for Bacillus megaterium is lowest 0.17 dSm$^{-1}$. But addition of graded levels of zinc in the form of zinc sulphate increase the soil EC with each incremental dose up to 30 kg ZnSO$_4$ ha$^{-1}$. The soil EC as influenced by zinc application ranged from 0.18 dSm$^{-1}$ to 0.21 dSm$^{-1}$.

Table 4: Effect of zinc Solubilizing cultures and zinc levels on chemical properties of soil after harvest of spinach.

| Treatments                  | pH (1:2.5) | EC (dSm$^{-1}$) | Calcium carbonate (gkg$^{-1}$) | Organic carbon (gkg$^{-1}$) |
|-----------------------------|------------|----------------|--------------------------------|----------------------------|
| Zinc solubilizers (S)       |            |                |                                |                            |
| S0: Control                 | 8.12       | 0.20           | 21.25                          | 4.75                       |
| S1: Pseudomonas striata    | 7.99       | 0.22           | 29.50                          | 5.43                       |
| S2: Trichoderma viride      | 8.01       | 0.19           | 23.25                          | 4.98                       |
| S3: Bacillus megaterium    | 7.94       | 0.17           | 28.75                          | 5.35                       |
| S.Em.±                      | 0.03       | 0.00           | 0.97                           | 0.08                       |
| C.D. at 5%                  | 0.08       | 0.01           | 2.06                           | 0.25                       |
| Levels of ZnSO$_4$ (Zn)    |            |                |                                |                            |
| Zn0: ZnSO$_4$ 0 kg ha$^{-1}$ | 7.88       | 0.18           | 21.08                          | 4.67                       |
| Zn1: ZnSO$_4$ 10 kg ha$^{-1}$ | 7.96       | 0.19           | 25.42                          | 4.94                       |
| Zn2: ZnSO$_4$ 20 kg ha$^{-1}$ | 8.08       | 0.20           | 27.83                          | 5.25                       |
| Zn3: ZnSO$_4$ 30 kg ha$^{-1}$ | 8.15       | 0.21           | 28.42                          | 5.65                       |
| S.Em.±                      | 0.03       | 0.00           | 0.97                           | 0.08                       |
| C.D. at 5%                  | 0.08       | 0.01           | 2.80                           | 0.25                       |
| Interaction (SxZn)          |            |                |                                |                            |
| S.Em.±                      | 0.06       | 0.01           | 1.94                           | 0.17                       |
| C.D. at 5%                  | NS         | NS             | NS                             | NS                         |
Organic carbon content
A perusal of data in Table 4 shows that the organic carbon content in soil clearly indicated that there was buildup of organic carbon over initial values of spinach crop due to the inoculation of zinc solubilizing microbial strains. The results are presented in Table 4. Zinc solubilizers influenced the soil organic carbon which ranged from 4.75 to 5.43 g kg⁻¹ showing significantly higher soil organic carbon in *Pseudomonas striata* (5.43g kg⁻¹) treated plots follows by *Bacillus megaterium* (5.35 g kg⁻¹) and *Trichoderma viride* (4.98 g kg⁻¹). Whereas, significantly lower soil organic carbon per plot were noted in uninoculated control. Similarly, graded levels of zinc in the form of zinc sulphate also increase the soil organic carbon with each incremental dose up to 30 kg ZnSO₄ ha⁻¹. The soil organic carbon as influenced by zinc application ranged from 4.67 to 5.65 g kg⁻¹. Interaction effect of zinc solubilizers and zinc levels are statistically non-significant Pawar and Ismail (2016) [7] reported increased soil organic carbon due to application of recommended dose of fertilizer along with bioinoculant than the recommended dose of fertilizer. Similar findings were also reported by Jeyabal and Kurpuswamy (2003) [4], Wu et al. (2005) [12] and Sable et al. (2016) [8].

Calcium carbonate content
Table 4 related to soil calcium carbonate in spinach indicates significant effect of zinc solubilizers and non-significant effect of graded levels of zinc. The slightly increased calcium carbonate thereafter at harvest due to various zinc solubilizing microbial treatments over control. Zinc solubilizers influenced the calcium carbonate which ranged from 21.25 to 29.50 g kg⁻¹ showing significantly higher calcium carbonate in *Pseudomonas striata* (29.50 g kg⁻¹) treated plots followed by *Bacillus megaterium* (28.75 g kg⁻¹) and *Trichoderma viride* (23.25 g kg⁻¹). Whereas, significantly lower soil calcium carbonate per plot were noted in uninoculated control. Similarly, graded levels of zinc in the form of zinc sulphate also increase the calcium carbonate with each incremental dose up to 30 kg ZnSO₄ ha⁻¹. The soil organic carbon as influenced by Zn application ranged from 21.08 to 28.42 g kg⁻¹. Interaction effect of zinc solubilizers and zinc levels are statistically non-significant. Sable et al. (2016) [8] reported that slight reduction in soil calcium carbonate was observed to initial values but treatment differences were non-significant.

Conclusion
The quality parameters like moisture percentage, ascorbic acid content and chlorophyll content improved significantly due to inoculation of bioinoculants. Maximum values of this quality parameter were noted with inoculation of *Pseudomonas striata* + 30 kg ZnSO₄ ha⁻¹ over rest of the treatments. The interactive effect of various zinc solubilizing inoculants and graded levels of zinc in the form of zinc sulphate on soil pH, electrical conductivity, organic carbon and calcium carbonate content was statistically non – significant.

References
1. Gadd GM. Geomycology: Biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol. Res. 2007; 111: 3-49.
2. Goteti PK, Amalraj ELD, Desai S, Shaik MHA. Prospective zinc solubilising bacteria for enhanced nutrient uptake and growth promotion in maize (*Zea mays* L.). Int. J Microbiol. 2013, 1-7.
3. Hafeez B, Khanif YM, Saleem M. Role of Zinc in Plant Nutrition- A Review. American Journal of Experimental Agriculture. 2013; 3(2):374-391.
4. Jeyabal A, Kuppuswamy G. Recycling of organic wastes for the production of vermicomposts and its response in rice-legume cropping system and soil fertility. Eur. J Agron. 2003; 15:153.
5. Mahdi SS, Dar SA, Ahmad S, Hassan GI. Zinc availability - A major issue in agriculture. Res. J Agri. Sci. 2010; 3:78-79.
6. Panse UG, Sukhatme PV. Statistical Methods for Agricultural Workers. I.C.A.R. Pub., New Delhi, 1985.
7. Pawar Anuradha, Ismail Syed. Evaluation of Zinc Solubilization Capacity of Different Microbial Strains and their Effect on Bt Cotton. Int. J of Agri Sci. 2016; 8(24):1474-1477.
8. Sable P, Ismail Syed, Pawar A. Effect of different microbial inoculants on yield, microbial population and chemical properties in soil of groundnut grown on Vertisol. International Journal of Microbiology Research. 2016; 9(1): 831-833.
9. Simine CD, Sayed JA, Gadd GM. Solubilization of zinc phosphate by a strain Pseudomonas fluorescens isolated from a forest soil. Biol Fertil Soils. 1998; 28:87-94.
10. Vaid SK, Kumar B, Sharma A, Shukla AK, Srivastava PC. Effect of zinc solubilizing bacteria on growth promotion and zinc nutrition of rice. Journal of Soil Science and Plant Nutrition. 2014; 14(4):889-910.
11. Vidyashree DN, Muthuraj R, Panneerselvam P. Evaluation of Zinc Solubilizing Bacterial (ZSB) Strains on Growth, Yield and Quality of Tomato (*Lycopersicon esculentum*). Int. J Curr. Microbiol. App. Sci. 2018; 7(4):1493-1502.
12. Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth. Geoderma. 2005; 125(1/2):155-166.