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EFFECT OF INTEGRATED USE OF CHEMICAL, ORGANIC AND BIOFERTILIZER ON GROWTH OF SPINACH (SPINACIA OLERACEA L.)

IRUM NAZ
PhD, Department of Botany, Hazara, University, Mansehra, Pakistan. Email: enn.k2015@gmail.com

ALIA GUL
Lecturer, Department of Botany, Hazara, University, Mansehra, Pakistan. Corresponding author Email: aliagulbotanist@gmail.com.

NOSHEEN SHAFQATH
Assistant Professor, Department of Agriculture, Hazara, University, Mansehra, Pakistan. Email: noshinshafqat@gmail.com

MUHAMMAD ISLAM
Assistant Professor, Department of Biotechnology and Genetic Engineering, Hazara, University, Mansehra, Pakistan. ORCID: 0000-0003-2514-1585 .Email: mislamhu@hu.edu.pk

YASMEEN SHAKIR
Department of Biochemistry, Hazara, University, Mansehra Pakistan. Email: yasmeenshakir@hotmail.com, y.shakir@bit.edu.cn

Dr. FAZAL HADI
Lecturer, Department of Botany, University of Peshawar, Pakistan. Email: hadibotany@uop.edu.pk

TARIQ AZIZ
MPhil Scholar, Department of Botany, Hazara, University, Mansehra, Pakistan.
Email: tariqbot2018@gmail.com, tariqazizafdmdk@gmail.com

ABDUL BASIT
BS student Department of Botany, University of Peshawar, Pakistan. Email: abdulbasitraza440@gmail.com

HINA GUL
MPhil Scholar, Department of Botany, Hazara, University, Mansehra, Pakistan.

WAQAR ALI
PhD scholar, Department of Pharmacy, University of Peshawar, Pakistan. Email: aliwaqar241@gmail.com

Abstract

The present research work was done at NARC Islamabad, to search the potential of combine organic application, bio fertilizers & chemicals for improving micronutrient concentration in spinach. Different treatments were designed to compare with NP fertilizer, bio fertilizers and Organic fertilizer to observe micronutrient concentration in shoot and root at various stages of development & growth. Highest concentration of iron shoot was noted with isolates Azorhizobium, Rhizobium and PSM-Q. At different stages of growth & development. Maximum content of root Iron was noted with Azorhizobium and Rhizobium. Among the isolates, Azorhizobium, Rhizobium and PSM-QA performed relatively better in enhancing zinc content in spinach shoot at all the three stages of growth. Highest root zinc concentration was recorded with inoculum Azorhizobium and PSM-QA. The results suggested that bacterial inoculation significantly increased the micronutrient content of spinach as compared with the application of chemical

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fertilizers, organic fertilizer and the control. We communicate sound proofs to recommend bacterial inoculation as a suitable and most reliable substitute of chemical fertilizers that promote/enhance the growth of spinach along with improving the rhizosphere for field crop production. As a result, it may be argued that microbial inoculation enhanced efficient nutrient uptake, resulting in higher-quality plants.

Index terms: Integrated; Organic; Chemical; Bio fertilizers; Azorhizobium; Rhizobium; Spinach; Growth.

1. INTRODUCTION

Modern agricultural practices have emphasized the widespread use of fertilizer and this approach has certainly increased grain yields in many countries in the last three decades. But use of chemical fertilizers for a long term can led to decline fertility of soil and yield of crops in a cropping system. There is the evidence that both ground and surface water concentration were increased by different nutrients of plants due to over fertilization, therefore different health hazards were produce. Depending on their activity of mobilising different nutrients, biofertilizers offer a less expensive, low-capital, and environmentally friendly way to increase farm productivity. Integrated Fertilizer Management (INM) holds a lot of potential for meeting intensive agriculture's increased nutrient demands while also sustaining higher crop output and improving the quality of the resource base. Thus the combined use of fertilizer (bio & organic) can improved chemical fertilizers, which increase and maintain the fertility of soil and also improved production of crop. It makes the high productivity more sustainable mono cropping cereals [1]. Organic fertilizers have the ability to increase the fertility of soil due to their holding capacity and local availability [2]. Organic materials require to increase the fertility and quantity of soil under low input agriculture system [3]. Various types of microorganisms which convert unavailable elements to available by the biological processes is known as bio fertilizers [4]. They boost crop productivity by creating a key components of the nutrients supply system. Different types of bio fertilizers were developed i.e. potassium solubilizer, phosphate solubilizer, nitrogen fixer and arbuscular mycorrhizal [5]. Chelation, exchange reaction and acidification by phosphate solubilizing bacteria can convert insoluble phosphate to soluble phosphate [6].

Micronutrients play an important role in vegetables production. Iron play important role in the formation of chlorophyll and process of photosynthesis [7]. Iron also required for photosynthesis, respiration and various cellular processes like synthesis of DNA, nitrogen fixation and production of hormone. Usually iron is abundant elements of nature but unavailable, because it insoluble in the complexes of ferric hydroxide [8]. Shortage of iron in plant can produce sickness plant called limited induced chlorosis. Shortage of iron also disturb deficiency of manganese. Usually deficiency of iron in soil is rare, but not available for absorption due to the pH of soil between 5-6.5. Mostly deficiency of iron can produce when the pH of soil is above 6.5 (too alkaline). Over fertilization of soil can led iron deficiency [9]. Among micronutrients, zinc effect the auxin synthesis by disturbing tryptophane, but on other hand zinc also improve the height of plant [10]. Concentration of zinc were noted various in different plant material, like 169mg/kg were documented in

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wheat, 24 & 63 mg/kg were reported in dry plants material [11]. Death of child (small than five years) was occur due to the deficiency of zinc [12].

2. MATERIALS AND METHODS

The present research work was done in the Soil Biology & Biochemistry Group (SBB), Land Resources Research Institute (LRRI), and National Agricultural Research Centre (NARC) Islamabad. Spinach is noted as one of the important leafy vegetables, which produce worldwide and consumed through the year [13]. Spinach contain vitamins, iron and other health improving product that improve immune system and another biological functions [14]. During the research work sown the spinach on 15/11/2011 having average temperature was 1ºC-36ºC, with 430 mm total rainfall, 80% was relative humidity, 10-13 hours' average day length were noted. Limited period of the selected plant growth in the region is April and November. The research work was done in randomized complete block design (RCBD) having three replications.

Treatments were as follow:

T1= Uninoculated control

T2= Azorhizobium

T3= Tax-psm1

T4= Rhizobium

T5= PSM-QA

T6= SP.OL

T7= ½ NP@23:10Kg/ha

T8= NP @46:20 Kg/ha

T9= Humic Acid

T10= Indole Acetic Acid

2.1 Inocula preparation and Inoculation method

Five different PGPR bacteria were isolated and cultured in the media, these isolated bacteria are as follow Rhizobium, Azorhizobium, P SM-QA, Tax PSM1 and SP.OL. Incubated culture about 2-3 days, until population of cells were reached $10x10^{5-9}$ ml in every inoculum, maintained the above using spectronic having 550nm optical density. Then shifted the above culture into a bio fertilizers carrier (organic matter rich soil) at NARC. Coated the Spinach seeds with slurry (mixing the packet of bio fertilizers with ten percent sugar solution). PGPR and carrier Combination was made as desired in treatments. @6Kg h^{-1} was humic acid treatment, while the treatment of IAA was made by soaking the seeds in 0.01% for two hours.

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Applied the above both fertilizers during sowing time. Applied single super phosphate (SSP) and urea as a source of P and N respectively. Collected the sample of above and below ground parts of plants for zinc and iron analysis at 70,110 and 130 days after emergence (DAE). Taken the sample of seed (0.25g) and other ground parts of plant, then added to mixed acid of 10 ml (nitric & perchloric acid 1:2). Then give 100-330 ^{0}C temperature till colorless of sample solution (2-3ml). Removed the flask and again gave cooling. Transferred the digest into volumetric flask of 50ml, and made a volume of 50ml with distilled water. Then determined both micronutrients in simple extract in Atomic Absorption Spectrophotometer.

2.2 Statistical analysis

The obtained data of the selected work was analysed using STATISTIX, arranged as a randomized complete block (RCBD) and means were compared by LSD test at 5% ($p < 0.05$) level of significance.

3. Results

In this study micronutrient concentration was significantly affected by bacterial inoculation treatments over chemical fertilizers and organic fertilizers.

3.1 Different treatment effect on the concentration of iron at spinach shoot

Diazotrophic bacteria namely Azorhizobium Rhizobium, and phosphate solubilizing bacteria PSM-QA, Tax-psm1, and SP-OL along with chemical fertilizers NP, ½ NP, and organic fertilizers Humic Acid & Indole Acetic Acid were analyzed from the spinach plants at active growth stage viz. 70 days after emergence for iron content, is shown in **(Fig: 01)**, there are various statistically values for different treatment (p 0.00). Maximum shoot Iron concentrations were recorded with Rhizobium (495.73mg kg^{-1}) which was followed by PSM-QA (481.87 mg kg^{-1}) with a significant difference. Azorhizobium (460.67mg kg^{-1}) occupied the third position. Generally, significant differences were observed among the treatments Tax-psm1 (446.47mg kg^{-1}), ½ NP (409.53 mg kg^{-1}), Humic Acid (373.07mg kg^{-1}), Indole Acetic Acid (339.53mg kg^{-1}) except SP-OL (389.47mg kg^{-1}) and NP (401.93mg kg^{-1}) which had non-significant differences. The lowest content was observed with un inoculated control (344.47mg kg^{-1}). Iron concentration by spinach at 110 DAE is shown in **(Fig: 02).**

There are various statistically values for different treatment (p 0.00). The highest values of iron content were recorded in Azorhizobium (469mg kg^{-1}), Rhizobium (454.47mg kg^{-1}), PSM-QA (467.93mg kg^{-1}), and Tax-psm1 (451.4mg kg^{-1}) which had non-significant differences. In general, there were no significant differences across the treatment NP (361mg kg^{-1}), ½ NP (370.8mg kg^{-1}), Humic Acid (367.33mg kg^{-1}), Indole Acetic Acid (358.67mg kg^{-1}) and un inoculated control (364.13mg kg^{-1}). The highest values of iron content were recorded in Azorhizobium (378.07mg kg^{-1}) **(Fig: 03)** at 130 DAE. Rhizobium (306.33mg kg^{-1}) & PSM-QA (307.73mg kg^{-1}) ranked second with non-significant differences. Tax-psm1 (295.8mg kg^{-1}) and SP-OL (285.93mg kg^{-1}) occupied the third

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position. In general, there were no significant differences across the treatment NP (282.53 mg kg^{-1}), ½ NP (274.6mg kg^{-1}), Humic Acid (274.6mg kg^{-1}), Indole Acetic Acid (276.07mg kg^{-1}). The lowest content was observed with un inoculated control (222.27mg kg^{-1}).

Figure 01: Iron content (mg kg^{-1}) of Spinach shoot as Affected by different inoculants at 70 DAE. Bars Sharing the same letter (s) are statistically non-Significant (≤ 0.05)

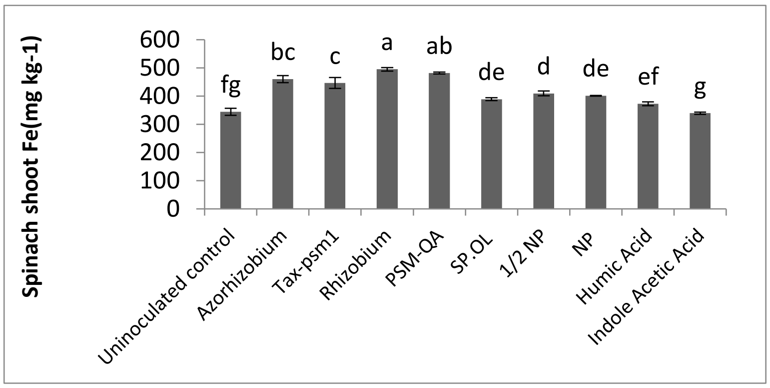


Figure 02: Iron content (mg kg^{-1}) of Spinach shoot as Affected by different inoculants at 110 DAE. Bars sharing the same letter (s) are statistically non-significant (≤0.05)

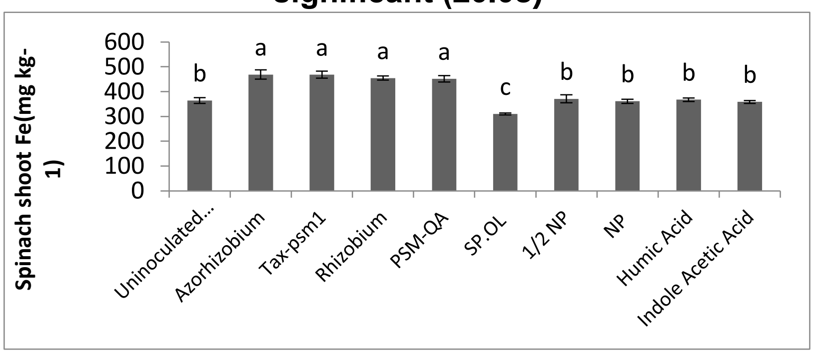
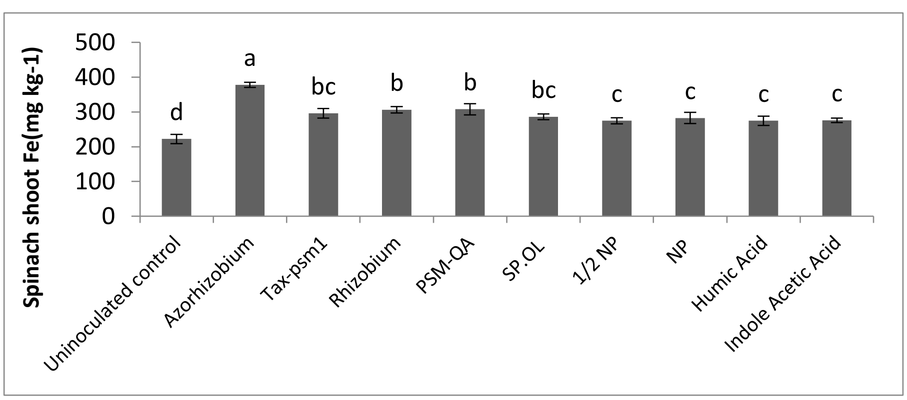


Figure 03: Iron content (mg kg^{-1}) of Spinach shoot as affected by different inoculants at 130 DAE. Bars the same letter (s) Are statistically non-significant (≤ 0.05). Significant (≤ 0.05)



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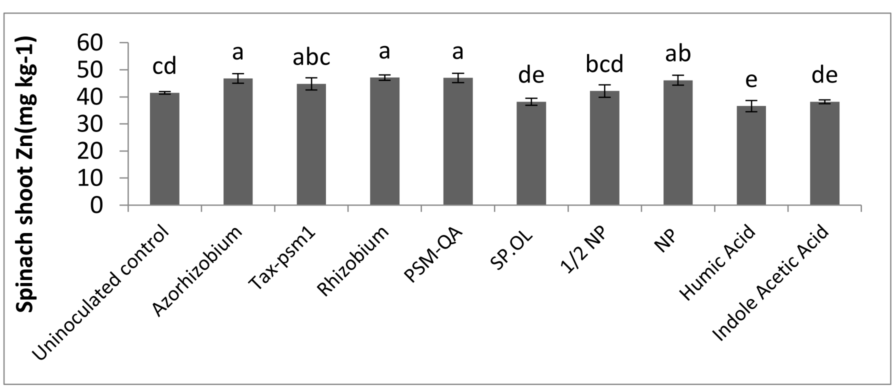
3.2 Different treatments effect on concentration of Zinc at spinach shoot

The zinc analysis for spinach on the performance of biofertilizers in comparison with chemical fertilizers and organic crop was performed as in Figure 4. there are various statistically values for different treatment (p 0.00). The maximum values of zinc content at 70 DAE was observed in Azorhizobium (46.8 mg kg^{-1}), Rhizobium (47.13mg kg^{-1}) & PSM-Q (47mg kg^{-1}) with non-significant differences **(Fig: 04).** Full dose of chemical fertilizers ranked second (46.13mg kg [1]). Generally, significant differences were observed among the treatments Tax-psm1 (44.8mg kg^{-1}), SP-OL (38.2mg kg^{-1}), ½ NP (42.13mg kg^{-1}), Humic Acid (36.6mg kg^{-1}), Indole Acetic Acid (38.2mg kg^{-1}) and un inoculated control (41.46mg kg^{-1}).

Zinc concentration by spinach at 110 DAE is shown in (**Fig: 05)**, there are various statistically values for different treatment (p 0.00). The maximum values of zinc content were noted with Azorhizobium (47.6mg kg^{-1}), Tax-psm1 (46.86mg kg^{-1}), Rhizobium (47.46mg kg^{-1}), & PSM-QA (48.46mg kg^{-1}) with non-significant differences. SP-OL (37.13mg kg^{-1}), NP (32.66mg kg^{-1}), ½ NP (37.86mg kg^{-1}), Humic Acid (33.66mg kg^{-1}), Indole Acetic Acid (35.93mg kg^{-1}) and un inoculated control (37.53mg kg^{-1}) had significant differences in zinc concentration at this stage.

The highest values of zinc content were recorded in PSM-QA (37.2mg kg^{-1}) whereas Azorhizobium (35.66mg kg^{-1}) ranked second **(Fig: 06),** at 130 DAE with significant difference. There are various statistically values for different treatment (p 0.00). Rhizobium (34mg kg [1]) ranked third. In general, there were no significant differences across the treatment Tax-psm1 (32.86mg kg^{-1}), SP-OL (29.73mg kg^{-1}), NP (30.66mg kg^{-1}), ½ NP (31.93mg kg^{-1}), Humic Acid (27.26mg kg^{-1}), Indole Acetic Acid (30.2mg kg^{-1}) and un inoculated control (29.06mg kg^{-1}).

Figure 4: Zinc content (mg kg^{-1}) of Spinach shoot as affected by different inoculations at 70 DAE. Bars sharing the same Letter (s) are statistically non-significant ((≤ 0.05))



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E-Publication: Online Open Access
Vol: 65 Issue 09 | 2022
DOI 10.17605/OSF.IO/F5ZU4

Figure 05: Zinc content (mg kg^{-1}) of Spinach shoot as Affected by different inoculants at 110 DAE. Bars sharing the same letter (s) are statistically significant (≤ 0.05)

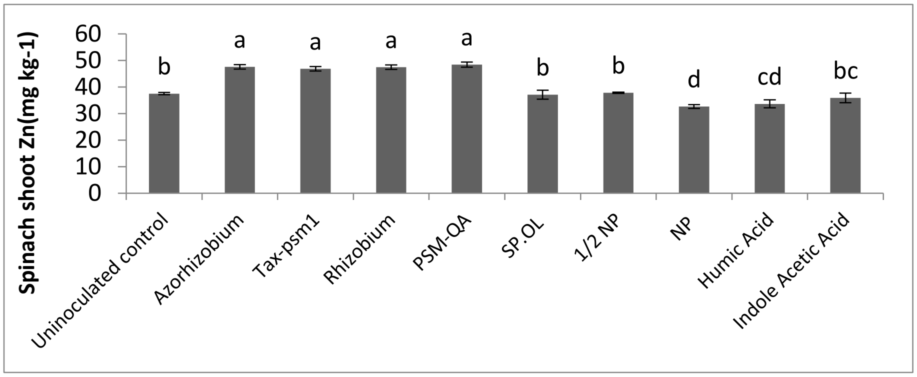
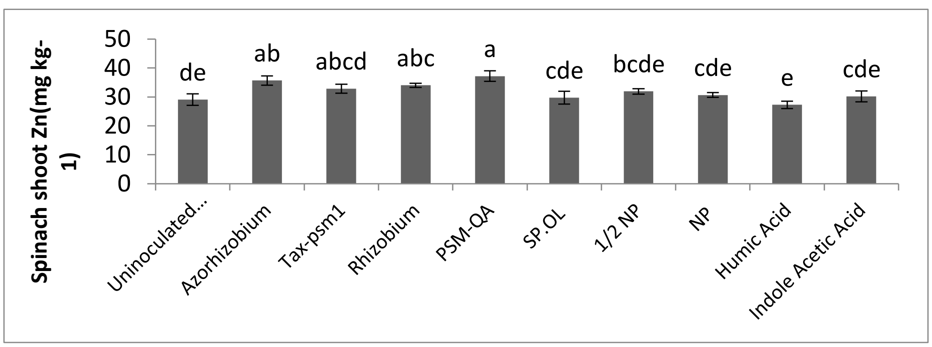


Figure 06: Iron content (mg kg^{-1}) of Spinach shoot as Affected by different inoculants at 130 DAE. Bars sharing the same letter(s) are statistically non-significant (≤ 0.05)



3.3 Different treatments effect on Iron concentration in spinach root

Fig: 07 showed that all treatments differ significantly with respect to Iron in spinach root at 70 DAE there are various statistically values for different treatment at (p 0.00). When compared to the Uninoculated control, all inoculation treatments considerably enhanced the iron content of the roots. However, the maximum iron concentration was obtained under Rhizobium (358.27mg kg^{-1}), followed by Azorhizobium (320mg kg^{-1}), with significant difference. Treatments PSM-QA (312.8mg kg^{-1}), NP (318.87mg kg^{-1}), & ½ NP (317.8mg kg^{-1}) ranked next with non-significant differences. Tax-psm1 (309.93mg kg^{-1}), SP-OL (287.47mg kg^{-1}), Humic Acid (281.6mg kg^{-1}), Indole Acetic Acid (277.27mg kg^{-1}) and Uninoculated control (222.8mg kg^{-1}) had significant differences in iron concentration at this stage.

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Vol: 65 Issue 09 | 2022
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Iron concentration by spinach at 110 DAE is show in **(Fig: 8),** there are various statistically values for different treatment (p 0.00). The maximum iron concentration was obtained by Azorhizobium (357.8mg kg^{-1}), whereas Rhizobium (326.93mg kg^{-1}), Tax-psm1 (320.13mg kg^{-1}), & PSM-QA (319.93mg kg^{-1}), ranked second with non-significants difference. Treatments SP-OL (218.2mg kg^{-1}), NP (266.13mg kg^{-1}), & ½ NP (242.6mg kg^{-1}), Humic Acid (268.07mg kg^{-1}), Indole Acetic Acid (242.4mg kg^{-1}) and un inoculated control (225.07mg kg^{-1}) had significant differences in iron concentration.

Iron concentration by spinach at 130 DAE is show in **(Fig: 9)**, there are various statistically values for different treatment (p 0.00). Azorhizobium (387.07mg kg^{-1}) had highest iron content whereas Rhizobium (306.33mg kg^{-1}) & PSM-QA (307.73mg kg^{-1}) ranked second with non-significant difference. Tax-psm1 (295.8mg kg^{-1}) & SP-OL (285.93mg kg^{-1}) ranked third with non-significant difference. NP (282.53mg kg^{-1}), ½ NP (274.6mg kg^{-1}), Humic Acid (274.6mg kg^{-1}) & Indole Acetic Acid (274.07mg kg^{-1}) ranked next with non-significant difference. Un inoculated control (222.27mg kg^{-1}) had lowest value for iron concentration at this stage.

Figure 07: Iron content (mg kg^{-1}) of Spinach root as Affected by different inoculants at 70 DAE. Bars Sharing the same letter (s) are statistically non-Significant (≤ 0.05)

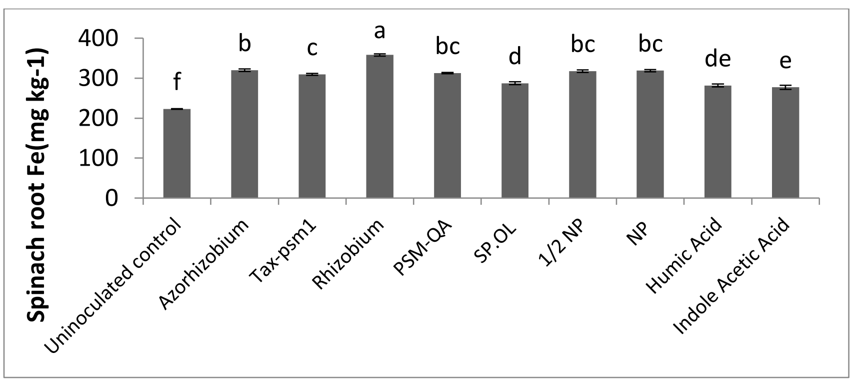
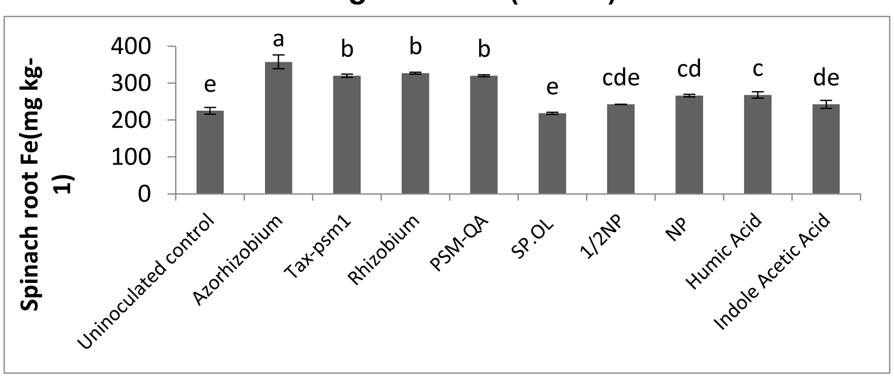


Figure 08: Iron content (mg kg^{-1}) of Spinach root as affected by different inoculants at 110 DAE. Bars sharing the same letter (s) are statistically non-significant (≤ 0.05)



Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
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Vol: 65 Issue 09 | 2022
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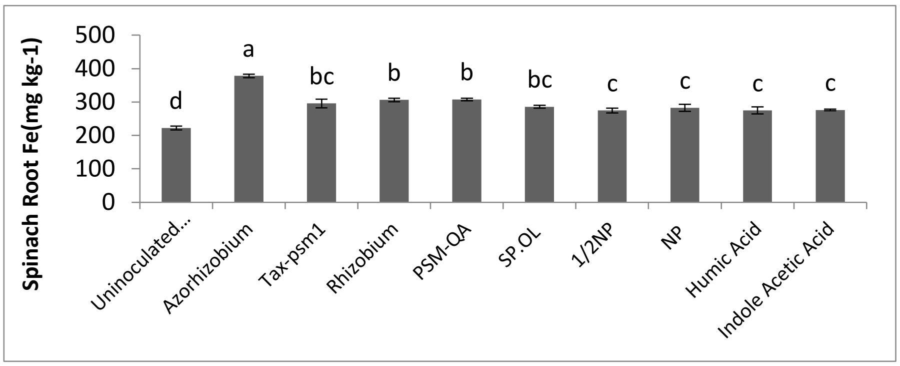
Figure 09: Iron content (mg kg^{-1}) of Spinach root as affected by Different inoculants at 130 DAE. Bars sharing the same letter (s) are statistically non-significant (≤ 0.05)



3.4 Different treatments effect on Zinc concentration in spinach root

The zinc analysis for spinach root was performed as in **(Fig: 10).** There are various statistically values for different treatment (p 0.00). The maximum values of zinc content at 70 DAE was observed in Azorhizobium (36.2mg kg^{-1}), whereas Rhizobium (33.6mg kg^{-1}) & Tax-psm1 (33.73mg kg^{-1}) ranked second with non-significant difference. Chemical fertilizers NP (32.6mg kg^{-1}), & ½ NP (32.8mg kg^{-1}) ranked next with non-significant difference. In general, there were no significant differences across the therapies PSM-QA (31.26mg kg^{-1}), SP-OL (25.06mg kg^{-1}), Humic Acid (27.46mg kg^{-1}), Indole Acetic Acid (27.26mg kg^{-1}) & UN inoculated control (28.73mg kg^{-1}) at this stage of growth.

Zinc concentration by spinach root at 110 DAE is show in (**Fig: 11),** there are various statistically values for different treatment (p 0.00). Highest concentration of zinc was noted with PSM-QA (36.73mg kg^{-1}), whereas Azorhizobium (34.6mg kg^{-1}) & ½ NP (34.8mg kg^{-1}) ranked second with non-significant difference. Rhizobium (32.86mg kg^{-1}), PSM-QA (36.73mg kg^{-1}) & Indole Acetic Acid (32.06mg kg^{-1}) ranked third with non-significant difference. Full dose of chemical fertilizers (30.46mg kg^{-1}) and Humic Acid (31.2mg kg^{-1}) ranked next with non-significant difference. In general, there were no significant differences among the treatments SP-OL (24.13mg kg^{-1}) and un inoculated control (28.8mg kg^{-1}).

The highest values of zinc content were recorded in PSM-QA (37.2mg kg^{-1}), whereas Azorhizobium (35.66mg kg^{-1}) ranked second and Rhizobium (34 mg kg^{-1}) ranked third **(Fig: 12),** at 130 DAE with significant differences. there are various statistically values for different treatments (p 0.00). In general, there were no significant differences among the treatments Tax-psm1 (32.86mg kg^{-1}), SP-OL (29.73mg kg^{-1}), NP (30.66mg kg^{-1}), & ½ NP (31.93mg kg^{-1}), Humic Acid (27.26mg kg^{-1}), Indole Acetic Acid (30.2mg kg^{-1}), & Uninoculated control (29.06mg kg^{-1}), at this stage of growth.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
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Vol: 65 Issue 09 | 2022
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Figure 10: Zinc content (mg kg^{-1}) of Spinach root as affected by different inoculants at 70 DAE. Bars sharing the same Letter (s) are statistically non-significant (≤ 0.05)

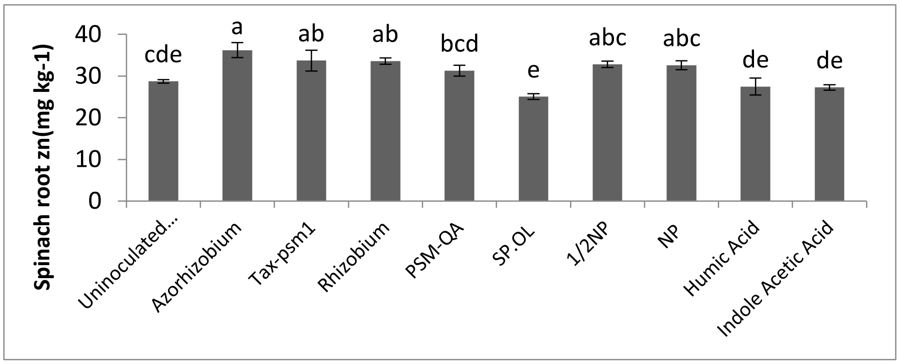


Figure 11: Zinc content (mg kg^{-1}) of Spinach root as Affected by different inoculants at 110 DAE. Bars sharing the same letter (s) are statistically non-significant (≤ 0.05)

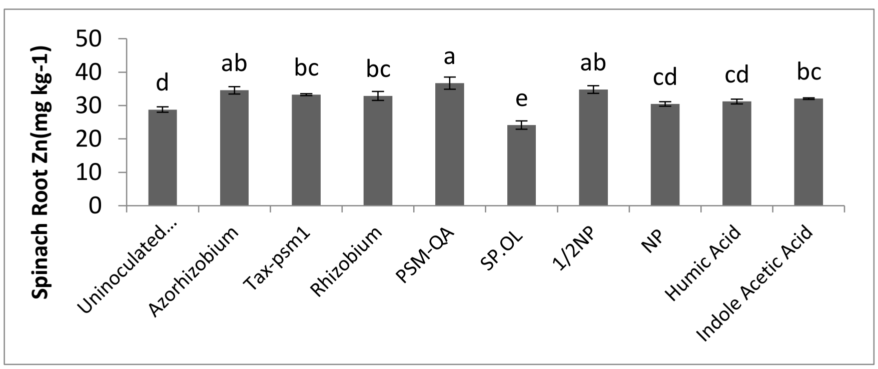
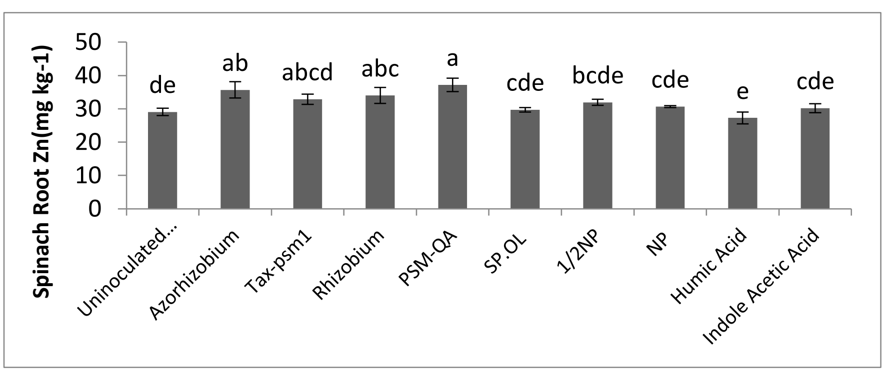


Figure 12: Zinc content (mg kg^{-1}) of Spinach root as affected by different inoculants at 130 DAE. Bars sharing the same letter (s) are statistically non-significant (≤ 0.05)



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4. DISCUSSION

The soil bacteria not only use in biogeochemical cycle but also play important role in production of plants. These bacteria also improve growth of plant due to their colonization in the rhizosphere [15]. The proper applications of rhizobia along with PGPR/PGRs is effective and also an approach of environmental friendly, which usually improved the yield of crops under suitable condition [16]. Inoculations of bacteria were also improved the total uptake of zinc per pot (52.5%), concentration of methionine in grain (38.8%) [17]. Usually rhizobacteria promote plant growth by various mechanism (direct or indirect), like fixation of nitrogen (N_2), solubilisation of phosphate and production of phytochrome [18]. As we know that bio fertilizers are eco-friendly with nature, but on other hand we can get higher productivity and yields [19]. Our present research work is similar to [20], they also documented the growth and survival of rhizobial bacteria in the soil. Therefore, root system was enlarged due to fabrication of hormones by improving uptake of nutrients. According to [21], rate of root elongation, various minerals like K, P, N and absorption of other micronutrients were improved due to inoculation of microbial. Application of A. chroococcum and PSM were increased the uptake of few trace elements like Zn and Fe, and also the production of plant growth promoting elements [22]. Our present work is also similar to those of [23], they documented that culture of Rhizobium were increased the uptake of Zn, Mn and N in urd bean. Isolation of PGPR were also positive effect on the uptake of plant micronutrient such as Fe, Zn, Mn and Cu on the shoot and root growth of Triticum “[24, 25]”. Reduction of Fe^{3+}--Fe^{2+} due to Rhizospheric bacteria. Uptake of Iron in Oryza sativa were increased by 15-64% due to rhizobial inoculation [26]. Rhizospheric bacteria were also effect the availability of iron, manganese and sulphur due to redox reaction [27]. According to [28], PGPR improved the uptake of Zn, Mn and Fe in Healianthus species.

5. CONCLUSION AND RECOMMENDATIONS

Comparison of NP fertilizer, bio fertilizers and Organic fertilizer for micronutrient concentration in different stages of growth and development in spinach resulted highest shoot Iron concentration with isolates Rhizobium, Azorhizobium and PSM-QA. While maximum root Iron content was recorded with Rhizobium and Azorhizobium. Among the isolates, Azorhizobium, Rhizobium and PSM-QA performed relatively better in enhancing zinc content in spinach shoot at all the three stages of growth. Highest root zinc concentration was recorded with inoculum Azorhizobium and PSM-QA. It was concluded that bacterial inoculation significantly increased the micronutrient content of spinach as compared with the application of chemical fertilizers, organic fertilizer and the control and recommend bacterial inoculation as a suitable and most reliable substitute of chemical fertilizers that promote/enhance the growth of spinach along with improving the rhizosphere for field crop production. Therefore, it can be concluded that microbial inoculation facilitated efficient nutrient’s uptake which ultimately produce plants of superior quality.

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