International Journal of Plant & Soil Science

Volume 35, Issue 17, Page 238-253, 2023; Article no.IJPSS.101137
ISSN: 2320-7035

Microbial Biofortification of Zn by Plant Growth Promoting Microorganisms in Wheat (*Triticum aestivum*)

R. R. Bagmare [a*] and Syed Ismail [a]

[a] *Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Agricultural University, Parbhani 431 402, Maharashtra, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i173204

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/101137

Original Research Article

Received: 17/04/2023
Accepted: 19/06/2023
Published: 06/07/2023

ABSTRACT

A lab experiment was conducted during 2021 and field experiment was conducted during the *Rabi* seasons of 2021 and 2022 to study the biofortification of Zn by plant growth-promoting microorganisms in wheat (*Triticum aestivum*). The results emerged from the lab experiment indicated that *Pseudomonas striata, Bacillus megaterium* and *Trichoderma viride* showed significantly highest colony diameter, clearing zone, halozone diameter, solubilization index and solubilization efficiency. Biofortification of zinc *i.e.* highest increase zinc content in grain and in straw over control and only RDF was reported in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (19.52 and 15.76 percent respectively)

Keywords: Biofortification; solubilization; microorganisms.

**Corresponding author: E-mail: rakesh.bagmare@yahoo.com;*

1. INTRODUCTION

Modern agriculture must continue feeding the expanding world population. To support the ever-growing populations, strategies have been employed to maximize biomass production. One of the famous examples is the “green revolution” which has significantly boosted crop yields to combat hunger. Besides the yield in biomass, the nutritional values of crops are another important consideration for providing proper nutrition. Apart from caloric intake and macronutrients such as N, P, K and micronutrients Fe, Zn humans also depend on food crops for obtaining certain micronutrients. Malnutrition due to insufficient dietary intake of micronutrients such as minerals and vitamins is regarded as “hidden hunger”. The rhizosphere is an important interface between plant roots and the soil, contributing to sustainable agriculture when the interaction between plants and beneficial bacteria is considered. About 35 years ago, Kloepper first described the role of plant growth-promoting rhizobacteria (PGPR) in plant growth and defense [1]. PGPR plays a major role in the direct or indirect promotion of plant growth when associated with plant roots. Biofertilization and phytostimulation are the direct plant growth promoter mechanisms, that simultaneously minimize the use of chemical fertilizers and promote plant growth, and bacteria with both biocontrol and phytostimulation properties to enhance nutrient supply and disease control in plants. The current scenario exemplifies work in the area of plant-microbe interactions that has focused on the biofortification of staple crops using these PGPR. The WHO has acknowledged micronutrients that are essential for the proper functioning of the human body, *i.e*. selenium (Se), iron (Fe) and zinc (Zn), and making for a significant portion of the current research on PGPR-mediated biofortification [2].

Wheat is an important source of carbohydrates. Globally, it is the leading source of vegetable protein in human food when eaten as whole grain, wheat is a source of multiple nutrients and dietary fiber [3]. In 100 grams, wheat provides 327 kilocalories of food energy and is a rich source of multiple essential nutrients, such as protein, dietary fiber, zinc, iron, manganese, phosphorus and niacin. Several B vitamins and other dietary minerals are in significant content. Wheat is 13% water, 71% carbohydrates and 1.5% fat. Its 13% protein content is mostly gluten. Wheat proteins have a low quality for human nutrition, according to the new protein quality method (DIAAS) promoted by the Food and Agriculture Organization [4]. Though they contain adequate amounts of the other essential amino acids, at least for adults, wheat proteins are deficient in the essential amino acid, lysine [5].

Zinc (Zn) is the only metal resident in all enzyme classes and characteristically the most abundant transition metal in living organisms after Fe. It plays a critical role in human health and strengthening of the immune system. Being involved in protein synthesis, metabolic homeostasis and modulation of gene expression it plays a critical role in male fertility with Zn deficiency resulting in inhibition of spermatogenesis and abnormal sperm production [6].

Biofortification is the process of adding essential micronutrients and other health-promoting compounds to crops or foods to improve their nutritional value. This is imperative as the diets of over two-thirds of the world’s population lack one or more essential mineral elements and the three staple crops, rice, maize, and wheat, which provide nearly half of the calories consumed by humans, are deficient in micronutrients. Plant growth-promoting rhizobacteria (PGPR) represent a wide variety of microorganisms, growing in association with plants. They lead to stimulation of growth, due to the increased solubilization, mobility, uptake, and enrichment of nutrients in the plant. Their significance in improving the nutrient use efficiency of applied fertilizers and improving nutrient uptake in problematic soils or denuded lands is well established.

Large quantities of fertilizers are commonly used to obtain high yields in many crops. But these chemical fertilizers compromise human and animal health and pollute the environment. Microbes represent a promising input, which possesses an array of mechanisms to sequester macro- and micronutrients from soil or water, which can be also made available to plants. Therefore, the application of microbial isolates needs to be advocated as a possible strategy not only to improve yields on infertile or nutrient-poor soils but also to increase nutrient concentrations and values in edible parts of the crop. Based of this, the present study was carried out to provide a biological platform by investigating the involvement of plant growth-promoting rhizobacteria in biofortification of wheat. This was done by studying zinc solubilization in *in vitro* conditions and analyzed for plant growth

promotion by monitoring growth and zinc content in wheat.

2. MATERIALS AND METHODS

2.1 Laboratory Experiment

Eight microbial isolates (*Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus thuringiensis, Pseudomonas striata, Trichoderma viride, Azotobacter chroococcum* and *Azospirillum lipoferum*) were selected based on the zinc solubilizing zone formation were procured from Department of Plant Pathology and All India Network Project on Soil Biodiversity-Biofertilizers Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The zinc solubilization potential was evaluated in both plate and broth media assays.

To determine the zinc solubilization capacity of the microbial strains, they were subjected to a PKV medium supplemented with D-glucose and different insoluble zinc compounds by following the protocol of Bapiri et al., [7]. The microbial isolates were inoculated into modified PKV medium (ingredients g L^{-1}) glucose-10.0 g; Ammonium Sulphate-1.0 g; Potassium Chloride-0.2 g; Dipotassium Hydrogen Phosphate-0.2 g; Magnesium Sulphate-0.1g; Yeast-0.2 g; distilled water -1000 ml, pH 7.0) containing 0.1% insoluble zinc compounds. In plate assay, the PKV medium was separately supplemented with insoluble zinc compounds at a concentration of 0.1 %, as zinc oxide [1.244 g/L], zinc carbonate [1.913 g/L] and zinc phosphate [5.904 g/L]. After sterilization and plating, fresh cultures of microbial species were inoculated on the media using sterile toothpicks in three replications. The inoculated plates were incubated at 28°C for 3 days in the dark for observing halo zone formation around colonies. Further, for broth assay, take 100ml of liquid PKV medium in 250 ml Erlenmeyer flasks, which was separately supplemented with three insoluble zinc compounds at 0.1% zinc in the form of zinc oxide, zinc carbonate and zinc phosphate. Then, the media was steam sterilized for 30 minutes in an autoclave. Thereafter it was inoculated with 0.1 ml aliquot of each microbial culture. Three flasks were maintained with an uninoculated control for each treatment. Experiments were done in three replications. The inoculated media was kept for 5^{th} and 7^{th} days. After 5^{th} and 7^{th}days the samples were withdrawn, centrifuged to remove the debris and cells. One ml of this solution was directly fed to Atomic Absorption Spectrophotometer (AAS) to determine the soluble Zn content. The pH of the isolates and the uninoculated samples were determined after 5^{th} and 7^{th}days of inoculation. The culture was filtered using Whatman No. 42 filter paper. The pH was measured using pH meter.

2.2 Field Experiment

The experiments on wheat were conducted to study the “Microbial biofortification of zinc by plant growth promoting microorganisms in wheat”. Field experiments on wheat were conducted at the same site in two successive years during *Rabi* 2020-21 and *Rabi* 2021-22 at Research Field, Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The experiment was laid out in randomized block design with three replications and ten treatments. Based on the laboratory experiment *i.e.* determination of zinc solubilization, these microbial isolates were exposed to field conditions as a seed treatment. Biofortification of Zn, enhancement of growth, nutrient content and nutrient uptake in wheat was determined by field experiment.

2.3 Statistical Analysis

The data obtained from the field experiment was done by completely randomized design as per the methods described in “Statistical Methods for Agricultural Workers” by Panse and Sukhatme (1985). The appropriate standard error (S.E.) and critical differences (C.D.) at 5% level were worked out as and when necessary and used for data interpretation.

3. RESULTS AND DISCUSSION

3.1 Experiment 1 (Laboratory Experiment)

3.1.1 Zinc solubilization activity of various microorganisms under different insoluble zinc sources in solid media

Evaluation of zinc solubilization activity of eight plant growth-promoting microorganisms from different insoluble zinc sources in solid media was undertaken and the results obtained are presented in Tables 1, 2 and 3. The inoculation of growth-promoting microorganisms in modified PKV medium (Pikovskaya, 1948) using zinc oxide, zinc carbonate and zinc phosphate as insoluble zinc sources showed significant variation in zinc solubilization in laboratory conditions.

3.1.2 Colony diameter

Inoculation of *Pseudomonas striata* in modified PKV medium shows significantly higher colony diameter in ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ amended media, (1.26, 1.28 and 1.62 cm respectively) which was at par with *Bacillus megaterium* and *Trichoderma viride*. However significantly lowest colony diameter was found in medium inoculated with *Azospirillum lipoferum*.

3.1.3 Clearing zone

Modified PKV medium with inoculation of *Pseudomonas striata* and insoluble zinc sources like ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ shows significantly higher size of clearing zone (2.37, 2.49 and 2.15 respectively) which was at par with *Bacillus megaterium* and *Trichoderma viride*. However, significantly lowest clearing zone was found in a medium inoculated with *Bacillus thuringiensis*.

3.1.4 Halozone diameter

Modified PKV medium supplemented with ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ and inoculated with *Pseudomonas striata* shows significantly higher halozone diameter (3.43, 3.65 and 3.70 respectively) which was at par with *Bacillus mgaterium* and *Trichoderma viride.* However significantly lowest halozone diameter was found in a medium inoculated with *Bacillus thuringiensis* when the medium was amended with ZnO and $ZnCO_3$ and when $Zn_3(PO_4)_2$ was used zinc source the lowest halozone diameter was found in strain *Azospirillum lipoferum.*

3.1.5 Solubilization index and solubilization efficiency

The solubilization index and solubilization efficiency of various plant growth-promoting microorganisms in plate assay were significantly influenced and presented in Tables 1, 2 and 3. Significantly highest solubilization index and solubilization efficiency in modified PKV medium supplemented with ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ as an insoluble zinc source was inoculated with *Pseudomonas striata* (3.72 ZNI; 271.58%, 3.86 ZNI; 285.91% and 3.29 ZNI; 228.72% by using ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ respectively) which was at par with inoculation of strain *Bacillus megaterium* and inoculation of strain *Trichoderma viride.*

In plate assay, all the microbial isolates produced a clear solubilization halo on PKV medium supplemented separately with three insoluble zinc compounds [ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$] at 0.1% zinc concentration. The mechanism of acquisition of zinc by these strains to form insoluble zinc compounds might be a consequence of H^+ extrusion and production of organic acids of microbial origin possibly in a nonspecific way leading to solubilization of zinc and thereby enhancing the bioavailability of zinc. Such solubilization of zinc compounds mediated through the production of organic acids and subsequent release of zinc in the external environment and bioaccumulation of zinc inside cells of bacterial species has also been confirmed [8]. In fungal system, it has been reported that ZnO has a good buffering capacity neutralizing protons close to the plasma membrane of H^+-AT Pase where they are generated. Production of H^+ and organic acids appear to be the most significant mechanisms for heterotrophic metal solubilization. Our results revealed that the efficiency to solubilize insoluble Zn compounds varied between the strains in *in vitro* conditions. *Pseudomonas aeruginosa* showed the best solubilization and solubilized both zinc oxide and zinc phosphate in their lab experiment by Fasim *et al.* [9]. Saravanan *et al.* [10] also found the greater zinc solubilizing activity of *Bacillus sp*. and *Pseudomonas sp*. using zinc oxide, zinc sulphide (Sphalerite) and zinc carbonate in plate and broth tests. Moreover, Sharma *et al.* [11] showed that *Pseudomonas spp*. were discovered to be promising bacterial isolates since they solubilized both inorganic zinc sources given independently in Tris minimum medium. Ramesh *et al.* [12] also reported that *Bacillus aryabhattai* exhibited a higher distinct halo zone with a solubilization diameter of >10 mm, halo diameter and had the best solubilization efficiency and inoculation of *Bacillus* strains resulting in significantly increased soluble zinc concentration in liquid broth than the un-inoculated control. Hussain *et al*. [13] reported *Bacillus sp*. maximal zinc solubilization and possessed a variety of growth-promoting characteristics as well as the ability to generate organic acids. Our results are also found in collaborate with Pawar *et al.* [14] showed that zinc oxide produced the highest clear halo zone and halo zone diameter with the highest zinc solubilization efficiency and solubilization index. Though colony diameter was found highest in $ZnCO_3$ amended media. However, in the case of microbial inoculants, *Pseudomonas striata* and *Trichoderma viride* produced the highest clear halozone with a halozone diameter. *Pseudomonas striata* and *Trichoderma viride*

also showed the highest solubilization efficiency. Similarly, Ingole [15] also reported that *Pseudomonas striata*, *Bacillus megaterium and Trichoderma viride reported* significantly highest colony diameter, clearing zone, halozone diameter, solubilization efficiency and solubilization index.

3.1.6 Zinc solubilization activity of various microorganisms under different insoluble zinc sources in liquid media

Screened by plate assay, plant growth promoting microorganisms supplemented with Zn compound at 0.1% Zn concentration were selected for both broth assay to measure the soluble Zn concentration at different growth periods, given in Table 4. The amount of total soluble Zn present in the culture supernatant as measured by AAS at 5^{th} and 7^{th} day of growth and reduction in broth pH was presented in Table 4. Maximum reduction of supernatant pH and higher solubilization of Zn with insoluble zinc sources was observed on 7^{th} day as compared to 5^{th} day. Among the Zn sources on 5^{th} and 7^{th} day $ZnCO_3$ show the highest solubilization followed by ZnO and $Zn_3(PO_4)_2$ showed the lowest.

Table 1. Zinc solubilization activity of various plant growth-promoting microorganisms under different insoluble zinc sources in solid media (ZnO)

Sr no.	Microbial Inoculants	ZnO				
		Clearing zone (cm)	Colony Diameter (cm)	Halozone Diameter (cm)	Solubilization index (ZNI)	Solubilization efficiency (%)
1	*Uninoculated Control*	Nd	Nd	Nd	Nd	Nd
2	*Bacillus subtilis*	0.65	1.13	1.72	2.52	152.49
3	*Bacillus licheniformis*	0.87	1.18	2.09	2.78	178.22
4	*Bacillus megaterium*	1.88	1.24	3.35	3.71	270.68
5	*Bacillus thuringiensis*	0.59	1.15	1.71	2.50	150.08
6	*Pseudomonas striata*	2.37	1.26	3.43	3.72	271.58
7	*Trichoderma viride*	1.64	1.22	3.20	3.63	263.22
8	*Azotobacter chroococcum*	0.78	1.19	2.00	2.68	168.15
9	*Azospirillum lipoferum*	0.69	1.10	1.73	2.58	158.44
	SEm ±	0.05	0.03	0.05	0.07	7.00
	CD at 5%	0.16	0.09	0.16	0.21	21.24
	CV	7.87	4.46	3.86	4.02	6.02

Table 2. Zinc solubilization activity of various plant growth promoting microorganisms under different insoluble zinc sources in solid media ($ZnCO_3$)

Sr no.	Microbial Inoculants	$ZnCO_3$				
		Clearing zone (cm)	Colony Diameter (cm)	Halozone Diameter (cm)	Solubilization index (ZNI)	Solubilization efficiency (%)
1	*Uninoculated Control*	Nd	Nd	Nd	Nd	Nd
2	*Bacillus subtilis*	0.88	0.95	1.72	2.82	181.66
3	*Bacillus licheniformis*	1.17	1.12	2.18	2.96	196.22
4	*Bacillus megaterium*	2.23	1.25	3.52	3.83	283.24
5	*Bacillus thuringiensis*	0.75	0.75	1.42	2.90	190.41
6	*Pseudomonas striata*	2.49	1.28	3.65	3.86	285.91
7	*Trichoderma viride*	2.15	1.21	3.31	3.74	274.35
8	*Azotobacter chroococcum*	0.81	0.89	1.70	2.92	191.79
9	*Azospirillum lipoferum*	0.76	0.80	1.52	2.93	193.07
	SEm±	0.06	0.05	0.06	0.11	10.87
	CD at 5%	0.18	0.16	0.18	0.33	32.96
	CV	7.46	8.98	4.35	5.80	8.38

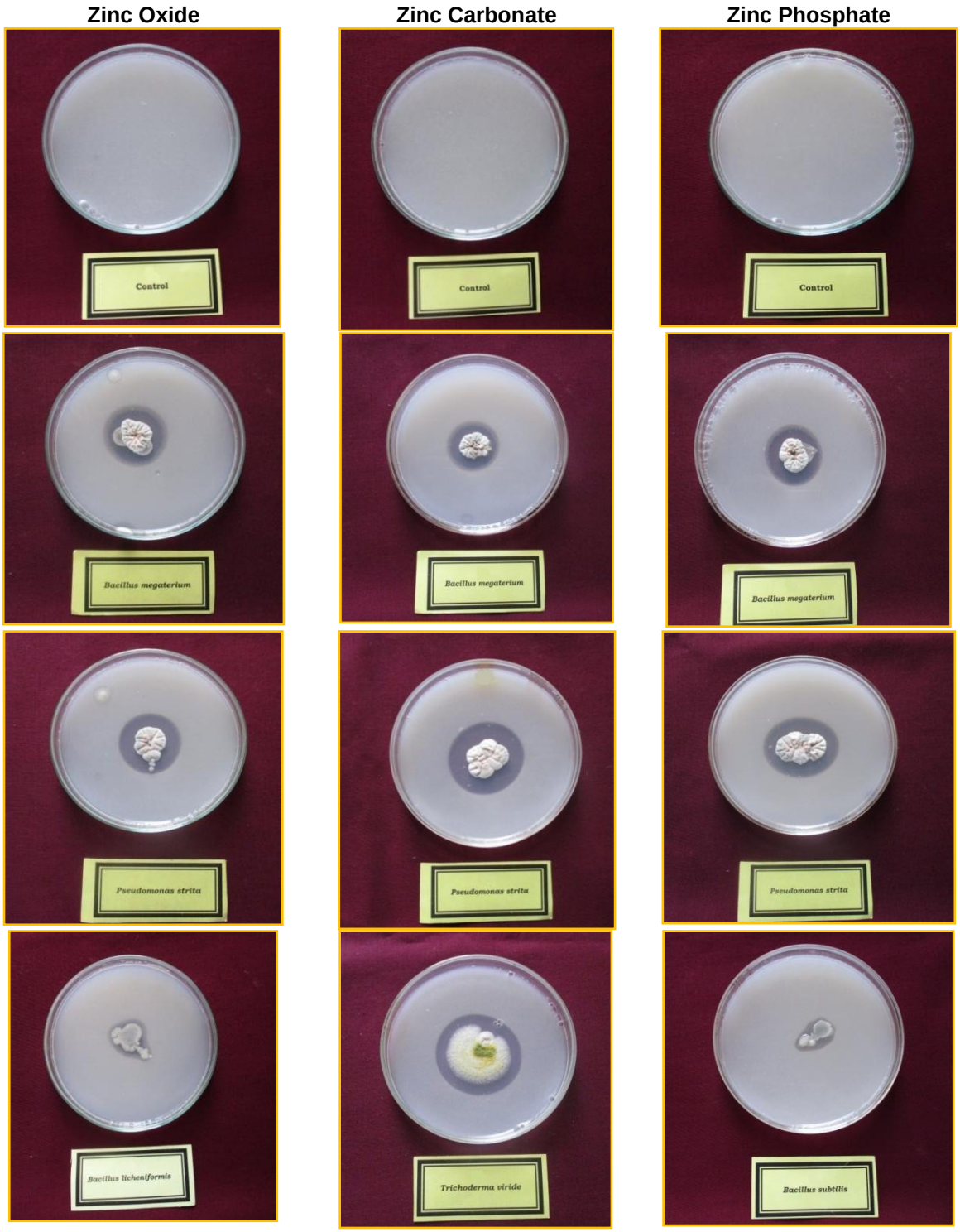

Plate 1. Zinc solubilization potential of different plant growth promoting microorganisms using Pikovskayas agar medium with ZnO, $ZnCO_3$ and $Zn(PO_4)_2$ insoluble zinc source

Modified PKV medium-liquid assay with ZnO and inoculated with plant growth promoting microorganism *Pseudomonas striata* shows more reduction in pH at 5th and 7th day (5.52 and 4.51) and higher zinc solubilization (188.00 to 227.89 mg lit^{-1}) at 5th and 7th day respectively, which was followed by inoculation of plant growth promoting microorganism *Bacillus megaterium* and *Trichoderma viride*. However significantly higher pH and lowest zinc solubilization were

found in the uninoculated control treatment. Similarly modified PKV medium-liquid assay using $ZnCO_3$ as an insoluble zinc source inoculated with plant growth promoting microorganism *Pseudomonas striata* shows the highest reduction in pH at 5th and 7th day (5.12 and 4.27) and higher zinc solubilization (215.61 to 239.80 mg lit^{-1}) at 5th and 7th day respectively, which was followed by inoculation of plant growth promoting microorganism *Bacillus megaterium* and *Trichoderma viride.* However, significantly higher pH and lowest zinc solubilization were found in the uninoculated control treatment. Also $Zn_3(PO_4)_2$ as a insoluble Zn sources in modified PKV medium inoculated with plant growth promoting strain *Pseudomonas striata* show significant lower pH and higher zinc solubilization (pH 5.45 and 4.59; zinc solubilization 177.37 to 201.54 mg lit^{-1} at 5th and 7th day respectively) which was followed by inoculation of plant growth promoting microorganism *Bacillus megaterium* and *Trichoderma viride*. However significantly higher pH and lowest zinc solubilization was found in uninoculated control treatment. The results showed a variation in solubilization potential that were found among the different microorganisms in zinc oxide, zinc carbonate and zinc phosphate-containing media. This might be related to differences in genomics and plasmid properties of different microorganisms that are affected by the location from which they were isolated. All microbial isolates showed higher solubilizing ability in zinc carbonate-containing medium which may be attributed to the fact that these strains were isolated from calcareous soils, presently a higher potential than other zinc-containing chemical substrates making these adherences with the carbonate particles capable of solubilizing zinc-carbonate and also it depends on the chemical properties of zinc carbonate that easier than others affected by acidic exudates of bacteria as earlier reported by Bapiri *et al.* [7]. From the results, after 5 days after inoculation, the data show that the amount of zinc that was available increased as the pH decreased. The synthesis of organic acids like gluconic acid may be the cause of the dissolution of the metals $ZnCO_3$, ZnO and $Zn_3(PO_4)_2$. Our data are compatible with several researchers [16-18] who reported that zinc can be dissolved by various methods, including the excretion of metabolites such as organic acids, proton extrusion, or the formation of chelating agents. Furthermore, the formation of inorganic acids such as sulphuric acid, nitric acid, and carbonic acid might aid in the solubilization process also plant growth promoting compounds such as biofilm and chitinase enzymes are produced by *Bacillus megaterium var. Phosphaticum* (strong), gibberellic acids and siderophore (moderate) and indole acetic acid (weak) that can enhance the solubilization process in soil. Anuradha and Ismail [19] who reported that *Pseudomonas striata, Bacillus megaterium*, and *Trichoderma viride* formed the highest zinc solubilization potential in *in vitro* conditions. Moreover, Martino *et al.* [20] reported the *ericoid* fungal strain with the greatest capacity for zinc solubilization from polluted soils also demonstrated a metal solubilization mechanism, as evidenced by the formation of insoluble Zn crystals and a drop in pH beneath the fungal strains.

Table 3. Zinc solubilization activity of various plant growth promoting microorganisms under different insoluble zinc sources in solid media $Zn_3(PO_4)_2$

Sr no.	Microbial Inoculants	$Zn_3(PO_4)_2$				
		Clearing zone (cm)	Colony Diameter (cm)	Halozone Diameter (cm)	Solubilization index (ZNI)	Solubilization efficiency (%)
1	*Uninoculated Control*	Nd	Nd	Nd	Nd	Nd
2	*Bacillus subtilis*	0.88	1.05	1.85	2.78	177.88
3	*Bacillus licheniformis*	1.22	1.20	2.32	2.95	194.70
4	*Bacillus megaterium*	1.96	1.58	3.58	3.27	226.92
5	*Bacillus thuringiensis*	0.78	1.00	1.71	2.73	172.89
6	*Pseudomonas striata*	2.15	1.62	3.70	3.29	228.72
7	*Trichoderma viride*	1.67	1.40	3.09	3.22	221.59
8	*Azotobacter chroococcum*	1.02	1.03	2.03	2.99	199.04
9	*Azospirillum lipoferum*	0.79	0.93	1.62	2.75	174.77
	SEm±	0.05	0.06	0.06	0.12	12.28
	CD at 5%	0.16	0.18	0.19	0.37	37.25
	CV	7.07	8.23	4.35	7.10	10.66

Table 4. Zinc solubilization activity of various plant growth promoting microorganisms under different insoluble zinc sources in liquid broth media

Sr no	Microbial Inoculants	Broth Assay											
		ZnO				$ZnCO_3$				$Zn_3(PO_4)_2$			
		pH		Zinc solubilization (mg lit^{-1})		pH		Zinc solubilization (mg lit^{-1})		pH		Zinc solubilization (mg lit^{-1})	
		5Day	7Day	5Day	7Day	5Day	7Day	5Day	7Day	5Day	7Day	5Day	7Day
1	*Uninoculated Control*	6.75	6.40	34.42	63.03	6.03	5.78	57.57	75.32	6.12	5.99	37.33	45.42
2	*Bacillus subtilis*	6.45	6.11	136.72	160.51	5.68	5.08	150.29	182.55	5.90	5.38	120.27	152.42
3	*Bacillus licheniformis*	6.38	6.05	152.66	173.55	5.62	5.01	172.35	200.37	5.76	5.29	138.61	160.60
4	*Bacillus megaterium*	5.54	4.80	175.59	212.70	5.15	4.62	210.25	230.42	5.61	4.82	170.39	188.54
5	*Bacillus thuringiensis*	6.69	6.35	135.81	148.41	5.79	5.26	152.28	182.56	5.95	5.58	117.25	140.40
6	*Pseudomonas striata*	5.52	4.51	188.00	227.89	5.12	4.27	215.61	239.80	5.45	4.59	177.37	201.54
7	*Trichoderma viride*	5.79	4.96	174.52	203.39	5.28	4.74	205.50	223.63	5.68	5.06	169.07	175.55
8	*Azotobacter chroococcum*	6.35	6.01	144.71	165.76	5.53	5.02	170.41	194.47	5.80	5.33	138.88	160.44
9	*Azospirillum lipoferum*	6.60	6.08	130.68	140.51	5.58	5.07	160.40	193.26	5.90	5.38	124.38	144.55
	SEm±	0.06	0.05	4.45	3.91	0.05	0.06	5.85	5.99	0.04	0.05	5.94	5.85
	CD at 5%	0.17	0.15	13.33	11.72	0.15	0.18	17.55	17.95	0.12	0.16	17.80	17.54
	CV	1.60	1.53	5.44	4.07	1.56	2.14	6.10	5.42	1.15	1.72	7.76	6.66

3.2 Experiment 2 (Field Experiment)

3.2.1 Effect of plant growth promoting microorganisms on yield attributes of wheat

The magnitude pooled mean data of grain and straw yield over control and RDF influenced due to inoculation of growth promoting microorganisms in wheat crop and narrated in Table 5. The highest increase in grain yield and straw yield over only RDF was reported in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* and it was 25.34 and 17.88 percent respectively which was followed by treatment T_7 receiving RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and it was 23.26 and 16.84 percent respectively and T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride* and it was 22.50 and 15.34 per cent respectively. The lowest grain and straw yield was observed in treatment T_1 absolute control.

The rhizosphere the soil environment where the plant root is available and is a zone of maximum microbial activity resulting in a confined nutrient pool in which essential macro and micronutrients are extracted. Plant growth regulators, also termed plant exogenous hormones, are synthetic substances that are similar to natural plant hormones. One of the terms for the prominent modes of action for growth promotion by PGPR is Phytostimulation or plant growth regulator. They are used to regulate the growth of plants and are important measures for boosting agricultural production. Similar findings have been reported by Mohmoud and Abd-alla [21] who stated that coinoculation of siderophore producing bacterial isolates significantly and positively enhanced the growth habitat, nodulation, and dry matter build up of mung bean crop. Inoculation of treatment *Bradyrhizobium+ P. Chrysogenium* increased the fresh weight, shoot dry matter yield and roots dry matter yield and increased mung bean production significantly. Similarly, Singh *et al.*, [22] showed that inoculation of *Rhizobium* on *Kharif* cowpea along with 30 kg N and 60kg P_2O_5ha^{-1}owing to improved growth and yield attributing characteristics, increased grain and straw yields significantly as compared to control. Shanmugaiah *et al.* [23], Omidvari *et al*. [24], Amalraj*et al.* [16] and Ahmad *et al.* [25] reported that coinoculation of *Rhizobium* and *Pseudomonas strains* boost the mung bean growth and production (*Vigna radiate L*) in comparison to uninoculated control. Co-inoculation enhanced shoot fresh weight (145%), root fresh weight (173%), number of pods plant (150%), pod fresh weight (182%), total dry matter (269%), relative water content (19%), water use efficiency. Siderophore producing plant growth promoting rhizobacteria (*Pseudomonas fluorescens*) play an important role in plant nutrition and as a result, in plant growth promotion, resulting in healthy plants that use microbial siderophore for iron nutrition and which is beneficial to plants by Parmar and Chakraborty [26]. Bhosle [27] and Jasrotia et al. [28] also reported that when PGPR strains were inoculated with 50 percent of the recommended NPK fertilizer dosage (RDF) showed that *Pseudomonas otitidis sp.* and *Enterobacter sp*. showed the greatest increase in root length and substantial increases the yield of paddy as compared to uninoculated control.

Table 5. Effect of plant growth promoting microorganisms on percent increase seed and straw yield over control and only RDF of wheat (two years pooled)

Sr No	Treatment	Yield (kg ha^{-1})		Percent increase over RDF	
		Grain	Straw	Grain	Straw
T_1	Absolute Control	1694.42	2718.21	-51.69	-51.40
T_2	Only RDF	3507.07	5592.63	-	-
T_3	RDF + *Azotobacter chroococcum*	4091.98	5877.16	16.68	5.09
T_4	T_3+ *Bacillus subtilis*	4263.58	5998.15	21.57	7.25
T_5	T_3+ *Bacillus licheniformis*	4271.60	6343.21	21.80	13.42
T_6	T_3+ *Bacillus thuringiensis*	4232.10	6289.51	20.67	12.46
T_7	T_3+ *Bacillus megaterium*	4322.84	6534.15	23.26	16.84
T_8	T_3+ *Pseudomonas striata*	4395.68	6592.59	25.34	17.88
T_9	T_3+*Azospirillum lipoferum*	4203.70	6195.06	19.86	10.77
T_{10}	T_3+*Trichoderma viride*	4296.30	6450.62	22.50	15.34
	SEm±	47.95	72.50	-	-
	CD at 5%	136.69	206.68	-	-
	CV	2.99	3.03	-	-

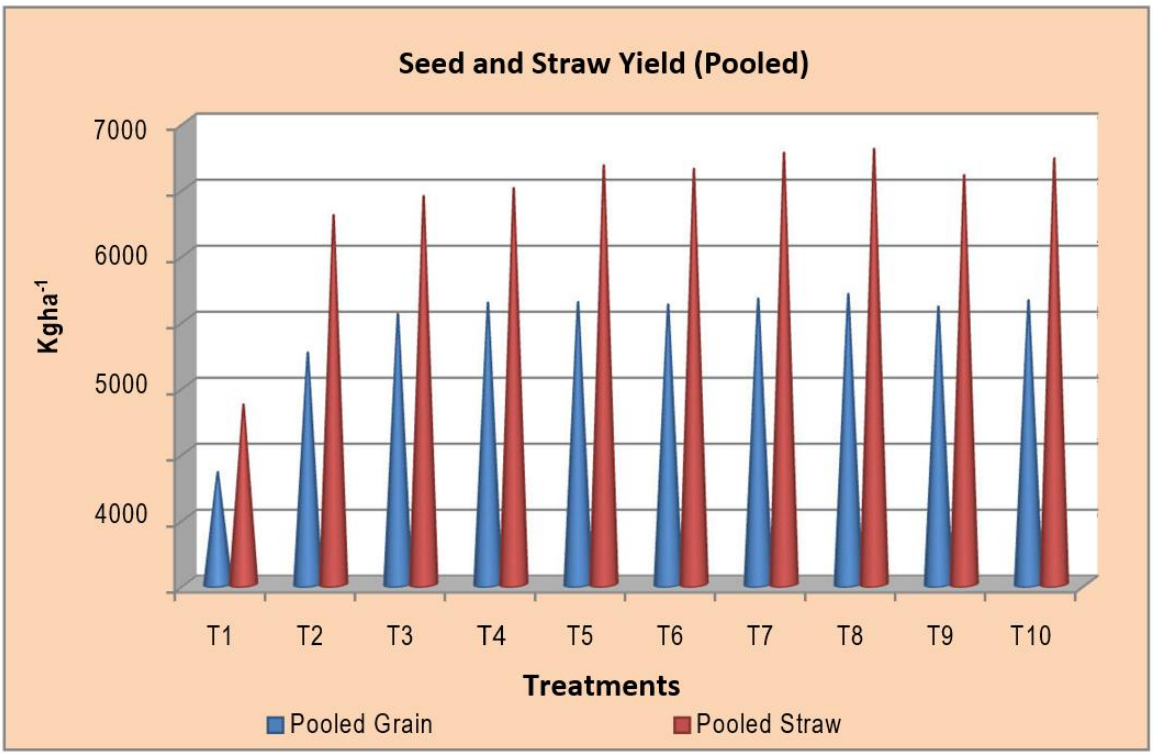

Fig. 1. Effect of plant growth promoting microorganisms on yield attributes of wheat

3.2.2 Zinc concentration of wheat straw and grain

A perusal of data presented in Table 6 shows that biofortification of Zn by plant growth promoting microorganisms in wheat straw and grains respectively. The zinc concentration in leaves and straw was enhanced due to the coinoculation of different plant growth promoting microorganisms in wheat. The Zinc concentration of leaves was estimated periodically at tillering, flowering and at harvest of crop in both seasons *Rabi* 2020-21, 2021-22 and pooled mean was calculated.

The highest Zn concentration at tillering stage was noticed in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (91.53 mg kg^{-1}) which was followed by treatment T_4 receiving RDF + *Azotobacter chroococcum* + *Bacillus subtilis* and treatment T_6 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus thuringiensis*. In was calculated the highest Zn concentration was noticed in treatment T_7 RDF + *Azotobacter chroococcum* + *Bacillus megaterium* (74.13 mg kg^{-1}) which was at par with treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* and treatment T_4 receiving RDF + *Azotobacter chroococcum* + *Bacillus subtilis*. While at harvest stage the highest Zn concentration was noticed in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (33.41 mg kg^{-1}) which was at par with treatment T_7 receiving RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride*. The Zn concentration in grain was positively and significantly influenced by different plant growth promoting microorganisms in wheat. The highest Zn concentration in grain was noticed in treatment T_7 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* (73.77 mg kg^{-1}) which was at par with treatment T_8 receiving RDF + *Azotobacter chroococcum* + *Pseudomonas striata* and treatment T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride*. While Zn concentration of wheat leaves at tillering stage in season *Rabi* 2021-22 was significantly influenced by inoculation of growth promoting microorganisms. The highest Zn concentration at tillering stage was noticed in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (87.79 mg kg^{-1}) which was followed by treatment T_4 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus subtilis* and treatment T_6 receiving RDF + *Azotobacter chroococcum* + *Bacillus thuringiensis*. In flowering stage the highest Zn concentration was noticed in treatment T_8 *i.e.* RDF + *Azotobacter*

chroococcum + *Pseudomonas striata* (69.97 mg kg^{-1}) which was at par with treatment T_7*i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_4 receiving RDF + *Azotobacter chroococcum* + *Bacillus subtilis*. While at harvest stage the highest Zn concentration was noticed in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (30.71 mg kg^{-1}) which was at par with treatment T_7 *i.e.* RDF + *Azotobacter chroococcum*+ *Bacillus megaterium* and treatment T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride*. The Zn concentration in grain significantly influenced by different plant growth promoting microorganisms in wheat. The highest Zn concentration in grain was noticed in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (70.24 mg kg^{-1}) which was followed by treatment T_7 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride*. The lowest Zn concentration in straw and grain was noticed in treatment T_1 Absolute Control. While pooled data of Zn concentration of wheat leaves significantly influenced by coinoculation of growth promoting microorganisms. At tillering stage ranges from 73.00 to 89.66 mg kg^{-1}. The highest Zn concentration at tillering stage was noticed in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (89.66 mg kg^{-1}) which was followed by treatment T_4*i.e.* RDF + *Azotobacter chroococcum*+ *Bacillus subtilis* and treatment T_6 receiving RDF + *Azotobacter chroococcum* + *Bacillus thuringiensis*. In the flowering stage, the highest Zn concentration was noticed in treatment T_7 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* which was at par with treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* and treatment T_4 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus subtilis*. While at harvest stage the highest Zn concentration was noticed in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (32.06 mg kg^{-1}) which was at par with treatment T_7 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride*. The biofortification of Zn concentration in grain was significantly influenced by different plant growth promoting microorganisms in wheat. The highest Zn concentration in grain was noticed in treatment with treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (71.34 mg kg^{-1}) which was followed by treatment T_7 receiving RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride*.

The increase in zinc content ofleaves and grain might be due to increased zinc availability in soil due to the solubilisation effect of *Pseudomonas striata, Bacillus megaterium, Trichoderma viride* and other plant growth-promoting microorganisms *in vitro* conditions. Our laboratory experimental results show that higher zinc solubilization by *Pseudomonas striata, Bacillus megaterium and Trichoderma viride* results increased available Zn in soil. Also enhanced the concentration of Zn in leaves and grain. The increased nutrient concentration might be due to greater availability of nutrient through inorganic, organic and biological sources by enhancing the activity of root hair, root proliferation and cell development in the root surface area. The increased content was also due to added supply of nutrients and well-developed root system under balanced nutrient application resulting in better absorption of nutrients. Our results have been confirmed by Gamit and Tank [29] showed that inoculation of *Pseudomonas pseudoalcaligenes*, which produces siderophores, has additional benefits beyond iron nutrition. Inoculation of *Pseudomonas pseudoalcaligenes* along with RDF enhanced the uptake of Fe, Cu, Mn, Zn, Co, Ni and Al in the plant's pot-grown shoot and root parts in *Cajanas cajan.* Similarly, Parewa *et al.*, [30] reported that rhizosphere soil consists of both harmful and beneficial microorganisms. The use of beneficial microbes has increased significantly. Zn solubilizing bacteria and P-solubilizing microorganisms successfully reduce the demand for fertilizer requirements. PGPRs are rhizobacteria that promote plant development and enhanced the nutrient concentration and uptake by crop plants. Further, Kumar *et al.* [31] stated that Zn solubilizing microbial isolates enhanced yield, nutrients content, nutrient uptake and quality of soybean and reported that nutrient concentration and uptake of soybean increased with the application of microbial culture *i.e.* RDF + *Rhizobium* + *Trichoderma viride*. Haroon *et al.* [32] showed that Zn biofortification of crops using these heterotrophic organisms such as strains of *Gluconacetobacter sp., Acinetobacter sp., Burkholderia sp., Klebsiella sp., Ralstonia sp., Bacillus sp., Pseudomonas sp., Serratia sp.* and *Ericoid mycorrhizal* boost the uptake of Zn by plants.

Table 6. Biofortification of zinc by plant growth promoting microorganisms in wheat straw and grain

Sr No	Treatment	Zinc content (mg kg^{-1})											
		2020-21				2021-22				Pooled			
		Till	Flow	AH	Grain	Till	Flow	AH	Grain	Till	Flow	AH	Grain
T_1	Absolute Control	75.30	60.00	17.73	61.30	70.70	56.44	13.31	58.08	73.00	58.22	15.52	59.69
T_2	Only RDF	78.90	63.87	20.92	63.70	75.13	59.95	18.89	59.55	77.01	61.91	19.91	61.63
T_3	RDF +*Azotobacter chroococcum*	84.10	65.77	24.80	65.80	79.96	61.24	22.49	61.12	82.03	63.50	23.65	63.46
T_4	T_3+ *Bacillus subtilis*	88.23	71.82	30.42	66.53	83.43	65.93	28.75	62.14	85.83	68.87	29.58	64.34
T_5	T_3+ *Bacillus licheniformis*	82.03	66.53	25.93	68.46	78.19	61.58	24.15	63.43	80.11	64.06	25.04	65.95
T_6	T_3+ *Bacillus thuringiensis*	87.37	67.27	26.17	64.50	83.28	62.15	24.54	60.87	85.33	64.71	25.36	62.68
T_7	T_3+ *Bacillus megaterium*	81.03	74.13	32.47	73.77	76.56	69.45	29.82	68.49	78.80	71.79	31.14	71.13
T_8	T_3+ *Pseudomonas striata*	91.53	73.53	33.41	72.43	87.79	69.97	30.71	70.24	89.66	71.75	32.06	71.34
T_9	T_3+*Azospirillum lipoferum*	86.63	63.70	27.20	67.93	82.54	58.94	25.66	63.78	84.59	61.32	26.43	65.86
T_{10}	T_3+*Trichoderma viride*	82.90	67.37	31.08	70.27	79.43	61.71	29.49	65.40	81.16	64.54	30.28	67.84
	SEm±	0.57	1.18	0.83	0.58	0.67	1.16	0.94	0.68	0.40	0.76	0.59	0.45
	CD at 5%	1.70	3.50	2.47	1.73	1.98	3.46	2.79	2.03	1.15	2.17	1.69	1.29
	CV	1.18	3.02	5.34	1.49	1.45	3.21	6.55	1.87	1.21	2.87	5.62	1.69

Till- Tillering, Flow- Flowering, AH- At harvest

Table 7. Effect of plant growth promoting microorganisms on percent increase in zinc in wheat grain and straw over control and only RDF of wheat

Sr No	Treatment	Zinc content (mg kg^{-1})			Zinc content (mg kg^{-1})		
		Grain (Pooled)	% increased over control	% increased over RDF	Straw (Pooled)	% increased over control	% increased over RDF
T_1	Absolute Control	59.69	-	-3.15	15.52	-	-22.05
T_2	Only RDF	61.63	3.25	-	19.91	28.29	-
T_3	RDF +*Azotobacter chroococcum*	63.46	6.32	2.97	23.65	52.38	18.78
T_4	T_3+ *Bacillus subtilis*	64.34	7.79	4.40	29.58	90.59	48.57
T_5	T_3+ *Bacillus licheniformis*	65.95	10.49	7.01	25.04	61.34	25.77
T_6	T_3+ *Bacillus thuringiensis*	62.68	5.01	1.70	25.36	63.40	27.37
T_7	T_3+ *Bacillus megaterium*	71.13	19.17	15.41	31.14	100.64	56.40
T_8	T_3+ *Pseudomonas striata*	71.34	19.52	15.76	32.06	106.57	61.02
T_9	T_3+*Azospirillum lipoferum*	65.86	10.34	6.86	26.43	70.30	32.75
T_{10}	T_3+*Trichoderma viride*	67.84	13.65	10.08	30.28	95.10	52.08
	SEm±	0.45	-	-	0.45	-	-
	CD at 5%	1.29	-	-	1.29	-	-
	CV	1.69	-	-	1.69	-	-

The magnitude pooled mean data of zinc in straw and grain over control and RDF influence due to coinoculation of growth promoting microorganism in wheat. Table 7 above shows the effect of plant growth promoting microorganisms on the percent increase in zinc content in grain over control and RDF of wheat. Highest increase zinc content in grain over control and only RDF was reported in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (19.52 and 15.76 per cent respectively) which was followed by treatment T_7 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* (19.17 and 15.41 percent respectively) and treatment T_{10} receiving RDF + *Azotobacter chroococcum* + *Trichoderma viride* (13.65 and 10.08 per cent respectively). Table 7 above shows that the highest increase zinc content in straw over control and only RDF was reported in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (106.57 and 61.02 percent respectively) which was followed by treatment T_7 receiving RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride*. Moreover, the highest increase in iron content in straw over control and only RDF was reported in treatment T_8 receiving RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (105.62 and 69.93 percent respectively) which was followed by treatment T_7 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_9 *i.e.* RDF + *Azotobacter chroococcum* + *Azospirillum lipoferum*.

The enhancement of macro and micro-nutrient content and uptake by plants by inoculation with PGPR may be due to their effect on initiation and development of lateral roots increased root weight and nutrient uptake also increased in iron content of leaves and grain might be due to increased iron availability in soil due to siderophore producing ability of *Pseudomonas striata, Bacillus megaterium, Bacillus subtilis, Trichoderma viride* and other plant growth promoting microorganisms *in vitro* condition. Our laboratory experimental results show that higher siderophore production by *Pseudomonas striata, Bacillus megaterium and Bacillus subtilis* results in increased available Fe in soil and make available to wheat. Our findings have been similar to Sharma *et al*. [33] they reported that PGPRs include *Pseudomonas putida*, *Pseudomonas fluorescens* and *Azospirillum lipoferum*, which were isolated from rhizospheric soils and applied on rice plants as a seed treatment. They found that iron content in plants and grain virtually increased after inoculation. In addition, after treatment, the iron's ability to move from roots to shoots to grains was improved. Similarly, Gamit and Tank [29] showed that siderophore producing *Pseudomonas pseudoalcaligenes* promote the solubility of nutrients such as Fe and increase the uptake of Fe in *Cajanas cajan*. Kumar *et al.* [31] showed that coinoculation of *Rhizobium* and *Trichodermaviride* along with RDF enhanced the yield, nutrient content, nutrient uptake and quality of soybean crop. Bagmare *et al.* [34] reported that siderophore production of different plant growth promoting rhizobacteria and its impact on nutrient concentration and uptake by plant. They stated that maximum production of siderophore was found in *Pseudomonas fluorescens*, *Azospirillum lipoferum* and *Pseudomonas striata* which increased the Fe availability in soil and boosted the Fe content and uptake by green gram [35,36].

4. CONCLUSION

4.1 Laboratory Experiment

A laboratory experiment was carried out to find out the zinc solubilization of different plant growth promoting microorganisms under *invitro* conditions. Zinc solubilization potential of different plant growth promoting microorganisms under different zinc sources in solid and liquid media. In plate assay, among the different insoluble zinc compounds, zinc carbonate supplemented medium found greater colony diameter, clearing zone, halozone diameter, solubilization index and solubilization efficiency as compared to zinc phosphate and zinc oxide amended media. As such *Pseudomonas striata, Bacillus megaterium* and *Trichoderma viride* reported significantly highest colony diameter, clearing zone, halozone diameter, solubilization index and solubilization efficiency as compared to other growth promoting microorganisms in all insoluble zinc sources. Similarly, in broth assay, among the insoluble zinc compounds, zinc carbonate supplemented shows the highest zinc solubilization as campared to zinc phosphate and zinc oxide. As such among the different microorganisms *Pseudomonas striata, Bacillus megaterium* and *Trichoderma viride* significantly highest zinc solubilization and maximum reduction in supernatant pH as compared to other growth promoting microorganisms in all insoluble zinc compound sources.

4.2 Field Experiment

Grain and straw yield was significantly influenced by inoculation of plant growth promoting microorganisms in wheat. The greater grain yield was noted in treatmentT_8 receiving RDF + *Azotobacter chroococcum* + *Pseudomonas striata* which was at par with treatment T_7 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_{10} *i.e.*RDF + *Azotobacter chroococcum* + *Trichoderma viride* and higher straw yield was noted in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* which was atpar with T_7 receiving RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride*. The magnitude pooled mean data of grain and straw yield over control and RDF affected due coinoculation of growth promoting microorganism in wheat crop. The highest increase in grain yield and straw yield over only RDF was reported in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* and it was 25.34 and 17.88 per cent respectively which was followed by treatment T_7 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and it was 23.26 and 16.84 percent respectively and T_{10} RDF + *Azotobacter chroococcum* + *Trichoderma viride* and it was 22.50 and 15.34 percent respectively. The lowest grain and straw yield was observed in treatment T_1 absolute control.

Highest Zn concentration of wheat straw was noticed at harvest stage *i.e.* biofortification of Zn in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* which was at par with treatment T_7 receiving RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride* while highest Zn concentration in grain *i.e.* biofortification of Zn in grain was noticed in treatment T_8 receiving RDF + *Azotobacter chroococcum* + *Pseudomonas striata* which was at par with treatment T_7*i.e.*RDF + *Azotobacter chroococcum* + *Bacillus megaterium* and treatment T_{10}*i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride*.

The magnitude pooled mean data of zinc concentration in straw and grain over control and RDF influenced due to inoculation of growth promoting microorganisms in wheat. Biofortification of zinc *i.e.* highest increase zinc content in grain over control and only RDF was reported intreatment T_8 *i.e.*RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (19.52 and 15.76 percent respectively) which was followed by treatment T_7 *i.e.* RDF + *Azotobacter chroococcum* + *Bacillus megaterium* (19.17 and 15.41 per cent respectively) and treatment T_{10} receiving RDF + *Azotobacter chroococcum* + *Trichoderma viride* (13.65 and 10.08 per cent respectively). The highest increase zinc content in straw over control and only RDF was reported in treatment T_8 *i.e.* RDF + *Azotobacter chroococcum* + *Pseudomonas striata* (106.57 and 61.02 per cent respectively) which was followed by treatment T_7 receiving RDF + *Azotobacter chroococcum* + *Bacillus megaterium* (100.64 and 56.40 per cent respectively) and treatment T_{10} *i.e.* RDF + *Azotobacter chroococcum* + *Trichoderma viride* (95.10 and 52.08 percent respectively).

ACKNOWLEDGEMENT

We feel grateful to Department of Plant Pathology and ICAR-All India Network Project on Soil Biodiversity-Biofertilizers, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. Also grateful to National Collection of Industrial Microorganisms **(**NCIM) Pune for providing the microbial strains for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kloepper JW, Schroth MN, Miller TD. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology. 1980;70(11):1078-1082.
2. Kumar A, Maurya BR, Raghuwanshi R, Meena VS, Tofazzal Islam M. Co-inoculation with *Enterobacter* and Rhizobacteria on yield and nutrient uptake by wheat (*Triticumaestivum L.*) in the alluvial soil under indo-gangetic plain of India. Journal of plant growth Regulation. 2017;36(3):608-617.
3. Consultation FE. Dietary protein quality evaluation in human nutrition. FAO food and. Nutrition. Paper. 2011;92:1-66.
4. Wolfe RR. Update on protein intake: importance of milk proteins for health status of the elderly. Nutrition Reviews. 2015;73(1):41-47.

5. Shewry PR, Hey SJ. The contribution of wheat to human diet and health. Food and Energy Security. 2015;4(3):178-202.
6. Tariq M, Hameed S, Malik KA, Hafeez FY. Plant root associated bacteria for zinc mobilization in rice. Pakistan Journal of Botany. 2007;39(1):245.
7. Bapiri A, Asgharzadeh A, Mujallali H, Khavazi K, Pazira E. Evaluation of Zinc solubilization potential by different strains of *Fluorescent pseudomonads*. Journal of Applied Sciences and Environmental Management. 2012;16(3):295 – 298.
8. Agusto de Costa AC, Duta FP. Bioaccumulation of copper, zinc, cadmium and lead by *Bacillus sp*., *Bacillus cereus*, *Bacillus sphaericus* and *Bacillus subtilis*. Brazil Journal of Microbiology. 2011;32: 1-5.
9. Fasim F, Ahmed N, Parsons R, Gadd GM. Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. FEMS Microbiology Letters. 2002;213(1):1-6.
10. Saravanan VS, Subramoniam SR, Raj SA. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. Brazilian Journal of Microbiology. 2004;35:121-125.
11. Sharma P, Kunawat KC, Kaur S, Kaur N. Assessment of zinc solubilization by endophytic bacteria in legume rhizosphere. Indian Journal of Applied Research. 2014;4(6):439-441.
12. Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP. Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. Applied Soil Ecology. 2014;73:87–96.
13. Hussain A, Arshad M, Zahir ZA, Asghar M. Prospects of zinc solubilizing bacteria for enhancing growth of maize. Pakistan Journal of Agricultural Sciences. 2015;52(4):87-95.
14. Pawar A, Syed Ismail, Sable P. Impact of zinc mobilizing microorganisms on growth enhancement in Btcotton grown on Vertisol. International Journal of Microbiology Research. 2017;9(1):823-825.
15. Ingole AJ. Effect of promising microbial growth promoting agents on guava (*Psidium guajava*) grown on Vertisol. Ph.D. Thesis, Vasantrao Naik Marathwada Agricultural University, Parbhani; 2021.
16. Amalraj DL, Maiyappan S, John Peter A. In vivo and *in vitro* studies of *Bacillus megaterium var. phosphaticum* on nutrient mobilization, antagonism and plant growth promoting traits. Journal of Ecology and Biotechnology. 2012;4(1):35-42.
17. Goteti PK, Amalraj, ELD, Desai S, Shaik MHA. Prospective zinc solubilizing bacteria for enhanced nutrient uptake and growth promotion in maize (*Zea mays L.).* International Journal of Microbiology. 2013;7(1):1-7.
18. Patel T, Desai PB. Study on rhizosphericmicroflora of wild and Transgenic varieties of *Gossypium* species in monsoon. Research Journal of Recent Sciences. 2014;3:42-51.
19. Anuradha P, Syed Ismail. Evaluation of Zinc Solubilization Capacity of Different Microbial Strains and their Effect on *Bt* Cotton. International Journal of Agriculture Sciences. 2016;8(24):1474-1477.
20. Martino E, Perotto S, Richard P, Gadd GM. Solubilization of insolubleinorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. Soil Biology and Biochemistry. 2003;35:133–141.
21. Mahmoud A, Abd-Alla M. Siderophore production by some microorganisms and their effect on *Bradyrhizobium*-mung bean symbiosis. International Journal of Agriculture and Biology. 2001;3(2):157-162.
22. Singh AK, Tripathi PN, Singh R. Effect of *Rhizobium* inoculation, nitrogen and phosphorus levels on growth, yield and quality of *kharif* cowpea [*Vigna unguiculata* (L.)]. Crop Research, Hisar. 2007;33(13):71.
23. Shanmugaiah V, Balasubramanian N, Gomathinayagam S, Manoharan PT, Rajendran A. Effect of single application of *Trichoderma viride* and *Pseudomonas fluorescens* on growth promotion in cotton plants. African Journal of Agriculture Residence. 2009;4(11):1220-1225.
24. Omidvari M, Sharifi RA, Ahmadzadeh M, Dahaji PA. Role of *fluorescent pseudomonads* siderophore to increase bean growth factors. Journal of Agricultural Science. 2010;2(3):242.
25. Ahmad M, Zahir ZA, Asghar HN, Arshad M. The combined application of rhizobial strains and plant growth promoting

rhizobacteria improves growth and productivity of mung bean (*Vigna radiata L.*) under salt-stressed conditions. Annals of Microbiology. 2012;62(3):1321-1330.
26. Parmar HY, Chakraborty H. Effect of siderophore on plant growth promotion. International Journal of Applied and Pure Science and Agriculture. 2016;2(3):60-68.
27. Bhosle A. Enhancement of drought tolerance in wheat (*Triticumaestivum*) and soil health using promising microbial cultures in Vertisol. Ph.D. Thesis, submitted to Vasantrao Naik Marathwada Agricultural University, Parbhani; 2021.
28. Jasrotia S, Salgotra RK, Samnotra RK. Identification of basmati rice (*OryzasativaL.*) rhizobacteria and their effect on plant growth traits for sustainable development in agriculture. Proceedings of the Indian National Science Academy. 2021;87(3):469-486.
29. Gamit DA, Tank SK. Effect of siderophore producing microorganism on plant growth of *Cajanus cajan* (Pigeon pea). International Journal of Research in Pure and Applied Microbiology. 2014;4(1):20-27.
30. Parewa HP, Yadav J, Rakshit A, Meena VS, Karthikeyan N. Plant growth promoting rhizobacteria enhance growth and nutrient uptake of crops. Agronomy for Sustainable Development. 2014;2(2):101-116.
31. Kumar B, Kranthi, Ismail Syed, Pawar A, Manasa K. Effect of zn solubilizing microbial cultures on yield, nutrient uptake and quality of soybean. Ecology Environment and Conservation. 2016;22: 339–346.
32. Haroon M, Khan ST, Malik A. Zinc-solubilizing bacteria: an option to increase zinc uptake by plants. In Microbial Biofertilizers and Micronutrient Availability. Springer. 2022:207-238
33. Sharma A, Shankhdhar D, Shankhdhar SC. Enhancing grain iron content of rice by the application of plant growth promoting rhizobacteria. Plant, Soil and Environment. 2013;59(2):89-94.
34. Bagmare RR, Ismail S, Swami YP, Ghode MK. Effect of siderophore producing microorganisms on plant growth promotion in green gram. International Journal of Chemical Studies. 2019;7(4): 620-624.
35. Panse UG, Sukhatme PV. Statistical Methods for Agricultural Workers. I.C.A.R. Pub., New Delhi; 1985.
36. Pikovskaya RI. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya. 1948;17:362-370.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/101137