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**Priya Satwadhar**  
 Department of Soil Science and  
 Agricultural Chemistry,  
 Vasant Rao Naik Marathwada  
 Krishi Vidyapeeth, Parbhani,  
 Maharashtra, India

**Syed Ismail**  
 Department of Soil Science and  
 Agricultural Chemistry,  
 Vasant Rao Naik Marathwada  
 Krishi Vidyapeeth, Parbhani,  
 Maharashtra, India

**Dhamak AL**  
 Department of Soil Science and  
 Agricultural Chemistry,  
 Vasant Rao Naik Marathwada  
 Krishi Vidyapeeth, Parbhani,  
 Maharashtra, India

**Chavan RV**  
 Department of Agricultural  
 Economics, Vasant Rao Naik  
 Marathwada Krishi  
 Vidyapeeth, Parbhani,  
 Maharashtra, India

**Corresponding Author:**  
**Priya Satwadhar**  
 Department of Soil Science and  
 Agricultural Chemistry,  
 Vasant Rao Naik Marathwada  
 Krishi Vidyapeeth, Parbhani,  
 Maharashtra, India

## Impact of iron and zinc agronomic biofortification on yield, concentration and uptake of nutrients in wheat as affected by iron and zinc solubilizing microbial inoculants

Priya Satwadhar, Syed Ismail, Dhamak AL and Chavan RV

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### Abstract

Field experiment was conducted to improve Fe and Zn concentration and yield of wheat in response to varying Fe and Zn solubilizers and fertilizer application rates. The field experiment was carried out during *rabi* season of 2021-22 at research farm Department of Soil Science and Agricultural Chemistry, Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani. Experiment consists of thirty six treatment combinations which includes four iron and zinc solubilizing cultures (Control, *Pseudomonas striata*, *Pseudomonas fluorescens*, and *Bacillus megaterium*), three levels of iron sulphate (0, 20 and 40 kg FeSO<sub>4</sub> ha<sup>-1</sup>) and three levels of zinc sulphate (0, 20 and 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup>) in factorial randomized block design. The results emerged out found significant effect of iron and zinc solubilizing microbial inoculants particularly *Pseudomonas striata* and in levels of iron sulphate and zinc sulphate upto 40 kg ha<sup>-1</sup> on yield and concentration of iron and zinc. Significant increase in grain yield was noted with the application of *Pseudomonas striata* (2968 kg ha<sup>-1</sup>) over control. Higher grain yield found with application of 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (2975 kg ha<sup>-1</sup>) and 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (2971 kg ha<sup>-1</sup>). Similarly, maximum straw yield noted with application of *Pseudomonas striata* (5331 kg ha<sup>-1</sup>) over control. Further, in levels of iron sulphate and zinc sulphate higher straw yield found with application of 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (5612 kg ha<sup>-1</sup>) and 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (5530 kg ha<sup>-1</sup>). Higher concentration of macro nutrients and micronutrients noted with application of *Pseudomonas striata* + 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> + 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup>. Similarly, higher uptake of macro and micro nutrients found with microbial inoculant particularly *Pseudomonas striata* and with application of 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> and 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> along with recommended dose of fertilizers.

**Keywords:** Yield, uptake, concentration, wheat, zinc and iron

### Introduction

Over 2 billion people worldwide suffer from micronutrient deficiencies, primarily as a result of eating monotonous diets which is dominated in low-nutrient foods. (Bouis and Satzman 2017) [9]. Iron deficiency is the most widespread nutritional problem. It mostly impacts children's learning capacity as well as their physical and mental development. (Talpur *et al.* 2018) [30]. Deficiency of micronutrients mainly affects women and children. An estimated 45% of deaths of children under the age of 5 are linked to micronutrient malnutrition (Gernand *et al.* 2016) [13]. Iron deficiency can lead to a serious condition like anemia, which is also a prime cause of women's death during childbirth (Anuradha *et al.* 2017) [5]. The most popular diet among people living in developing nations, such as India, is vegetarianism, which mostly consists of cereals and legumes with restricted access to fruits, meat, eggs, and dairy products. etc. (Puranik *et al.* 2017) [26]. This low nutrient diet is the main cause of micronutrient deficiency. Further, zinc is essential for the proper development of the human body and its deficiency is ranked as the 5<sup>th</sup> major risk factor for impairment (Anuradha *et al.* 2017) [5]. Its deficiency is particularly prevalent in children under 5 years old as they have a comparatively huge demand for zinc to sustain development (Black *et al.* 2008) [8]. Deficiency of zinc causes physiological problems such as hindrance in brain development, improper growth and augmented vulnerability to infectious diseases like pneumonia and diarrhea and low birth outcomes in pregnant women (Hambidge and Krebs 2007) [15]. The two most vital elements in the human body are iron and zinc.

A vegetarian person depends mainly on cereal based diet which is low in of Fe and Zn. Biofortification is an economical and sustainable way to challenge the micronutrient malnutrition problem worldwide (Tripti *et al.* 2022) [32]. Wheat is one of the most important staple food crops grown in India. It is important to increase Fe and Zn concentration in wheat. Agronomic biofortification is a strategy to increase micronutrient content in the edible part of food crops through the application of mineral fertilizers (White and Broadley 2009) [34]. This approach can enrich food crops with multiple elements at a time and can reach resource poor rural populations, providing they have access to fertilizers. Food crop micronutrient concentrations can be enhanced through agronomic biofortification, with the potential to reduce micronutrient deficiencies among rural population (Teklu *et al.* 2023) [31].

## Materials and Methods

The present investigations were conducted at research farm of Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The crop was sown on fixed layout in the months of November and was harvested in month of March in during both growing seasons. The crop variety used was NIAW-1994. The recommended dose of chemical fertilizers was applied @ 100:50:50 N and P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O kg ha<sup>-1</sup> through urea, SSP and MOP. A basal dose of fertilizer was applied as per treatment at the time of sowing to wheat. Irrigation was given as per crop need. The recommended package of practices was followed. The grain and straw yields were recorded from net plot area at maturity stage of the crop. Experimental study consists of four iron and zinc solubilizing cultures (Control, *Pseudomonas striata*, *Pseudomonas fluorescens*, and *Bacillus megaterium*), three levels of iron sulphate (0, 20 and 40 kg FeSO<sub>4</sub> ha<sup>-1</sup>) and three levels of zinc sulphate (0, 20 and 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup>) replicated twice in factorial randomized block design. Seed inoculation was done with iron and zinc solubilizing microbial inoculants as seed treatment before sowing. Nutrient content in plant sample and grain were analyzed for nitrogen (Micro Kjeldhals method (AOAC, 1990), phosphorus (Vanadomolybdate phosphoric acid yellow colour method by Jackson, 1973) [18], potash estimated by Flame photometer (Jackson, 1973) [18], iron and zinc by Atomic Absorption Spectrophotometer. The results data obtained were analyzed using standard statistical procedure given by Panse and Sukhatme (1967) [23].

## Results

### Grain yield

The data in respect of grain yield of wheat as influenced by Fe and Zn solubilizing microbial inoculants and fertilizers application is presented in Table 1. Microbial inoculants positively and significantly influenced on grain yield which ranged between 2454-2869 kg ha<sup>-1</sup>. The higher grain yield was noted in plots inoculated with *Pseudomonas striata* (M<sub>1</sub>) over control i.e. 2869 kg ha<sup>-1</sup>. There was significant increase in the grain yield of wheat due to graded levels of iron and zinc sulphate as compared to control treatment. Grain yield of wheat influenced with graded levels of iron up to 40 kg ha<sup>-1</sup> ranged between 2394-2881 kg ha<sup>-1</sup>. The highest grain yield was noted in plots treated with 40 kg FeSO<sub>4</sub> kg ha<sup>-1</sup> (2881 kg ha<sup>-1</sup>). Similarly, the grain yield influenced with application of zinc sulphate which ranged between 2404-

2876 kg ha<sup>-1</sup>. Maximum yield was noted in plots treated with 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (2876 kg ha<sup>-1</sup>)

**Straw yield:** Data narrated in Table 1. related to straw yield of wheat was significantly influenced due to microbial inoculants and graded levels of iron sulphate and zinc sulphate. The significantly maximum straw yield was recorded in treatment with inoculation of *Pseudomonas striata* (M<sub>1</sub>) (5698 kg ha<sup>-1</sup>), it was statistically at par with treatment M<sub>3</sub> (*Bacillus megaterium*) (5604 kg ha<sup>-1</sup>). Further, graded levels of iron and zinc in the form of FeSO<sub>4</sub> and ZnSO<sub>4</sub> also significantly influenced on straw yield of wheat. The significantly highest straw yield was recorded in the application of 40 kg FeSO<sub>4</sub> and ZnSO<sub>4</sub> ha<sup>-1</sup>. Straw yield influenced with application of iron sulphate which ranged between 5111-5922 kg ha<sup>-1</sup>. The maximum straw yield was noted in plots treated with 40 kg FeSO<sub>4</sub> kg ha<sup>-1</sup> (5922 kg ha<sup>-1</sup>). Similarly, the straw yield influenced with application of zinc sulphate which ranged between 5245-5757 kg ha<sup>-1</sup>. Higher straw yield was noted in plots treated with 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (5757 kg ha<sup>-1</sup>).

### Macro nutrient content (NPK)

**Nitrogen content (%):** Iron and zinc solubilizing microbial inoculants and fertilizers showed significant effect on nitrogen content of straw and grain of wheat presented in Table 1. Microbial treatments influenced on nitrogen content which was ranged between 1.00-1.18 and 2.77-3.17% of straw and grain of wheat respectively. The highest nitrogen content in straw and grain noted with application of *Pseudomonas striata* i.e. 1.18% and 3.17% respectively. The levels of iron sulphate and zinc sulphate also influenced on nitrogen content. The iron sulphate up to 40 kg ha<sup>-1</sup> influenced on nitrogen content which ranged between 0.95-1.25 and 2.72-3.14% of straw and grain respectively. The higher nitrogen content was noted in 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (1.25 and 3.14% of straw and grain respectively). Similarly, levels of zinc sulphate up to 40 kg ha<sup>-1</sup> influenced on nitrogen content which was ranged between 0.99-1.18 and 2.72-3.10% of straw and grain respectively. The higher nitrogen content was noted in 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (1.18 and 3.10% of straw and grain respectively).

**Phosphorus content:** It was evidence from data presented in Table 2. That phosphorus content of wheat straw and grain was positively and significantly influenced by microbial inoculation and graded levels of iron and zinc sulphate. The microbial inoculants influence on phosphorus content and highest phosphorus content noted with application of *Pseudomonas striata* i.e. 0.39% for straw and for grain 0.84%. Scrutiny of data further revealed that, graded levels of iron and zinc in the form of FeSO<sub>4</sub> and ZnSO<sub>4</sub> also influenced the phosphorus content in straw and grain up to 40 kg ha<sup>-1</sup>. For iron sulphate up to 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> influenced on phosphorus content of straw and grain and ranged between 0.25-0.46% and 0.67-0.91% respectively. For zinc sulphate up to 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> influenced on phosphorus content and ranged between 0.29-0.40 and 0.72-0.85% for straw and grain respectively. Highest phosphorus content of straw and grain noted with 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (0.46 and 0.91% for straw and grain respectively) and 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (0.40 and 0.85% for straw and grain respectively).

**Table 1:** Effect of microbial inoculants and graded levels of iron sulphate and zinc sulphate on grain and straw yield (kg ha<sup>-1</sup>)

Treatments	Grain yield (kg ha <sup>-1</sup> )	Straw yield (kg ha <sup>-1</sup> )
<b>Microbial inoculants (M)</b>		
M0 (Control)	2454	5194
M1 ( <i>Pseudomonas striata</i> )	2869	5698
M2 ( <i>Pseudomonas fluorescens</i> )	2608	5428
M3 ( <i>Bacillus megaterium</i> )	2569	5604
SE ±	33.51	65.66
CD at 5%	68.08	133.39
<b>Levels of FeSO<sub>4</sub> (Fe)</b>		
Fe0 (FeSO <sub>4</sub> 0 kg ha <sup>-1</sup> )	2394	5111
Fe1 (FeSO <sub>4</sub> 20 kg ha <sup>-1</sup> )	2600	5411
Fe2 (FeSO <sub>4</sub> 40 kg ha <sup>-1</sup> )	2881	5922
SE±	29.03	56.86
CD at 5%	58.97	115.52
<b>Levels of ZnSO<sub>4</sub> (Zn)</b>		
Zn0 (ZnSO <sub>4</sub> 0 kg ha <sup>-1</sup> )	2404	5245
Zn1 (ZnSO <sub>4</sub> 20 kg ha <sup>-1</sup> )	2594	5441
Zn2 (ZnSO <sub>4</sub> 40 kg ha <sup>-1</sup> )	2876	5757
SE ±	29.03	56.86
CD at 5%	58.97	115.52

**Potassium content:** Microbial treatments influenced on potassium content which was ranged between 1.05-1.23% and 0.55-0.73% of straw and grain of wheat respectively (Table 2). The highest potassium content in straw and grain influenced by *Pseudomonas striata* i.e. 1.23% and 0.73% respectively. The levels of iron sulphate and zinc sulphate also influenced on potassium content. The iron sulphate up to 40 kg ha<sup>-1</sup> influenced on potassium content which ranged between 1.01-1.29 and 0.51-0.80% of straw and grain respectively. The higher potassium content was noted in 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (1.29 and 0.80% of straw and grain respectively). Similarly, levels of zinc sulphate up to 40 kg ha<sup>-1</sup> influenced on potassium content which was ranged between 1.04-1.23 and 0.53-0.75% of straw and grain respectively. The higher potassium content was noted in 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (1.23 and 0.75% of straw and grain respectively).

#### Micronutrient content (Fe and Zn)

**Iron concentration:** The microbial fortification influence on iron concentration and ranged between 201.53-204.90 and 426.03-431.90 mg kg<sup>-1</sup> for straw and grain respectively. The higher iron concentration noted with microbial inoculants particularly *Pseudomonas striata* (204.90 and 431.90 mg kg<sup>-1</sup> for straw and grain respectively). Further, graded levels of iron and zinc in the form of FeSO<sub>4</sub> and ZnSO<sub>4</sub> also influenced the iron concentration in straw and seed up to 40 kg ha<sup>-1</sup>. For iron sulphate up to 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> influenced on iron concentration of straw and grain and ranged between 200.45-206.23 mg kg<sup>-1</sup> for straw and for grain ranged between 426.41-432.33 mg kg<sup>-1</sup>. Higher iron concentration in straw and grain found in treatment receiving 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (206.23 mg kg<sup>-1</sup> and 432.33 mg kg<sup>-1</sup> for straw and grain respectively). For zinc sulphate upto 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> influenced on iron concentration and ranged between 201.02-205.45 mg kg<sup>-1</sup> for straw and for grain 426.90-431.71 mg kg<sup>-1</sup> respectively. Maximum iron concentration noted with application of 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (205.45 mg kg<sup>-1</sup> and 431.71 mg kg<sup>-1</sup> for straw and grain respectively).

**Zinc concentration:** The data pertaining to biofortification of Zn by microbial inoculation and graded levels of iron sulphate and zinc sulphate presented in Table 2. Microbial treatments influenced on zinc concentration which was ranged between 38.55-43.59 and 50.71-56.37 mg kg<sup>-1</sup> of straw and grain of wheat respectively. The highest zinc concentration in straw influenced by *Pseudomonas striata* i.e. 43.59 mg kg<sup>-1</sup> of straw and in grain (56.37 mg kg<sup>-1</sup>). The levels of iron sulphate and zinc sulphate also influenced on zinc concentration. The iron sulphate up to 40 kg ha<sup>-1</sup> influenced on zinc concentration which ranged between 39.72-44.71 and 51.74-56.57 mg kg<sup>-1</sup> of straw and grain respectively. The higher zinc concentration was noted in 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (44.71 and 56.57 mg kg<sup>-1</sup> of straw and grain respectively). Similarly, levels of zinc sulphate up to 40 kg ha<sup>-1</sup> influenced on zinc concentration which was ranged between 39.47-44.93 and 51.58-56.93 mg kg<sup>-1</sup> of straw and grain respectively. The higher zinc concentration was noted in 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (44.93 and 56.93 mg kg<sup>-1</sup> of straw and grain respectively).

**Nitrogen uptake:** Microbial treatments influenced on nitrogen uptake of straw and grain which was ranged between 52.62-68.00 and 68.24-92.67 kg ha<sup>-1</sup> respectively. For total nitrogen uptake of wheat influenced by microbial inoculation and ranged between 120.87-160.67 kg ha<sup>-1</sup>. The higher nitrogen uptake influenced by *Pseudomonas striata* i.e. 68.00, 92.67 and 160.67 kg ha<sup>-1</sup> for straw, grain and total respectively. The levels of iron sulphate and zinc sulphate also influenced on nitrogen uptake. The iron sulphate up to 40 kg ha<sup>-1</sup> influenced on nitrogen uptake of straw and grain which ranged 48.62-74.50 and 65.56-91.56 kg ha<sup>-1</sup> respectively. For total uptake of nitrogen ranged between 114.18-166.06 kg ha<sup>-1</sup>. The higher nitrogen uptake was reported in 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (74.50, 91.56 and 166.06 kg ha<sup>-1</sup> for straw, grain and total respectively). Similarly, levels of zinc sulphate upto 40 kg ha<sup>-1</sup> influenced on nitrogen uptake which was ranged between 52.20-68.62, 65.56-90.83 and 117.76-159.45 kg ha<sup>-1</sup> for straw, grain and total respectively. The higher nitrogen uptake was noted in 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (68.62, 90.83 and 159.45 kg ha<sup>-1</sup> at straw, grain and total respectively).

**Table 2:** Effect of microbial inoculants and graded levels of iron sulphate and zinc sulphate on macronutrient and micronutrient content of wheat straw and grain

Treatments	Nitrogen content (%)		Phosphorus content (%)		Potassium content (%)		Iron content (mg kg <sup>-1</sup> )		Zinc content (mg kg <sup>-1</sup> )	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
<b>Microbial inoculants (M)</b>										
M0 (Control)	1.00	2.77	0.30	0.73	1.05	0.55	201.53	426.03	38.55	50.71
M1 ( <i>Pseudomona striata</i> )	1.18	3.17	0.39	0.84	1.23	0.73	204.90	431.90	43.59	56.37
M2 ( <i>Pseudomona fluorescens</i> )	1.04	2.88	0.34	0.78	1.10	0.61	202.83	430.66	43.27	55.82
M3 ( <i>Bacillus megaterium</i> )	1.14	2.91	0.35	0.79	1.18	0.68	203.65	428.33	42.54	53.40
SE ±	0.029	0.035	0.015	0.013	0.030	0.023	0.552	0.581	0.565	0.543
CD at 5%	0.060	0.070	0.031	0.026	0.062	0.047	1.122	1.180	1.147	1.104
<b>Levels of FeSO<sub>4</sub> (Fe)</b>										
Fe0 (FeSO <sub>4</sub> 0 kg ha <sup>-1</sup> )	0.95	2.72	0.25	0.67	1.01	0.51	200.45	426.41	39.72	51.74
Fe1 (FeSO <sub>4</sub> 20 kg ha <sup>-1</sup> )	1.07	2.93	0.33	0.77	1.12	0.62	203.00	428.96	41.53	53.92
Fe2 (FeSO <sub>4</sub> 40 kg ha <sup>-1</sup> )	1.25	3.14	0.46	0.91	1.29	0.80	206.23	432.33	44.71	56.57
SE±	0.025	0.030	0.013	0.011	0.026	0.020	0.478	0.503	0.489	0.471
CD at 5%	0.052	0.061	0.027	0.023	0.053	0.041	0.972	1.022	0.994	0.956
<b>Levels of ZnSO<sub>4</sub> (Zn)</b>										
Zn0 (ZnSO <sub>4</sub> 0 kg ha <sup>-1</sup> )	0.99	2.72	0.29	0.72	1.04	0.53	201.02	426.90	39.47	51.58
Zn1 (ZnSO <sub>4</sub> 20 kg ha <sup>-1</sup> )	1.11	2.98	0.35	0.79	1.15	0.65	203.21	429.09	41.57	53.71
Zn2 (ZnSO <sub>4</sub> 40 kg ha <sup>-1</sup> )	1.18	3.10	0.40	0.85	1.23	0.75	205.45	431.71	44.93	56.93
SE ±	0.025	0.030	0.013	0.011	0.026	0.020	0.478	0.503	0.489	0.471
CD at 5%	0.052	0.061	0.027	0.023	0.053	0.041	0.972	1.022	0.994	0.956

**Phosphorus uptake:** The microbial inoculants influenced on phosphorus uptake and ranged between 15.98-22.92, 18.21-24.65 and 34.19-47.58 kg ha<sup>-1</sup> for straw, grain and total respectively. The higher phosphorus uptake recorded in *Pseudomona striata* i.e. 22.92 kg ha<sup>-1</sup> for straw, 24.65 kg ha<sup>-1</sup> grain and total phosphorus uptake 47.58 kg ha<sup>-1</sup>. This was superior over other treatments. The lower phosphorus uptake was observed in uninoculated control treatment (15.98, 18.21 and 34.19 for straw, grain and total respectively). Graded levels of iron and zinc in the form of FeSO<sub>4</sub> and ZnSO<sub>4</sub> also influenced on phosphorus uptake in straw and grain upto 40 kg ha<sup>-1</sup>. The phosphorus uptake influenced by iron sulphate ranged between 12.86-27.25, 16.31-26.48 kg ha<sup>-1</sup> and 29.17- 53.74 kg ha<sup>-1</sup> for straw, grain and total phosphorus uptake respectively. The maximum phosphorus uptake was reported in 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> which was 27.25 kg ha<sup>-1</sup> for straw, 26.48 kg ha<sup>-1</sup> for grain uptake and total phosphorus uptake 53.74 kg ha<sup>-1</sup>. Moreover, levels of zinc sulphate influenced on phosphorus uptake and ranged between 15.32-23.76, 17.58-24.97 and 32.90-48.74 kg ha<sup>-1</sup> for straw, grain and total respectively and maximum phosphorus uptake noted in 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> i.e. 23.76, 24.97 and 48.74 kg ha<sup>-1</sup> for straw, grain and total respectively.

**Potassium uptake:** Microbial treatments influenced on potassium uptake of straw and grain which was ranged between 55.48-70.69 and 13.91-21.65 kg ha<sup>-1</sup> respectively. For total potassium uptake of wheat influenced by microbial inoculation and ranged between 69.39-92.33 kg ha<sup>-1</sup>. The higher potassium uptake influenced by *Pseudomona striata* i.e. 70.69, 21.65 and 92.33 kg ha<sup>-1</sup> for straw, grain and total respectively. The levels of iron sulphate and zinc sulphate also influenced on potassium uptake. The iron sulphate upto 40 kg ha<sup>-1</sup> influenced on potassium uptake of straw and grain which ranged 51.76-77.06 and 12.52-23.48 kg ha<sup>-1</sup> respectively. For total uptake of potassium, it was influenced by iron sulphate and ranged between 64.28-100.54 kg ha<sup>-1</sup>. The more potassium uptake was reported in 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (77.06, 23.48 and 100.54 kg ha<sup>-1</sup> for straw,

grain and total respectively). Similarly, levels of zinc sulphate upto 40 kg ha<sup>-1</sup> influenced on potassium uptake which was ranged between 55.18-71.50, 12.95-22.35 and 68.13-93.85 kg ha<sup>-1</sup> for straw, grain and total respectively. The more potassium uptake was noted in 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (71.50, 22.35 and 98.85 kg ha<sup>-1</sup> at straw, grain and total respectively).

**Iron uptake:** The data narrated in Table 4. about iron uptake is given. Iron uptake of straw and grain estimated separately and total iron uptake was calculated. The microbial inoculants influence on iron uptake and ranged between 1047.75-1170.26, 1340.26-1585.51 g ha<sup>-1</sup> for straw, grain and 2093.53- 2411.55 g ha<sup>-1</sup> for total respectively. The higher iron uptake recorded in *Pseudomona striata* i.e. 1170.26 g ha<sup>-1</sup> for straw, 1585.51 g ha<sup>-1</sup> grain and total iron uptake 2411.55 g ha<sup>-1</sup>. This was superior over other treatments. The lower iron uptake was observed in uninoculated control treatment (1047.75, 1340.26 and 2093.53 g ha<sup>-1</sup> for straw, grain and total respectively). Moreover, highest increase in total Fe uptake over control was reported in treatment M2 i.e. *Pseudomonas striata* (15.19 percent). Further, graded levels of iron and zinc in the form of FeSO<sub>4</sub> and ZnSO<sub>4</sub> also influenced on iron uptake in straw and grain upto 40 kg ha<sup>-1</sup>. The iron uptake was ranged from 1024.42-1222.50 g ha<sup>-1</sup> for straw, 1308.39-1592.75 g ha<sup>-1</sup> for grain and 2045.53-2469.48 g ha<sup>-1</sup> for total uptake. The highest iron uptake was noted in 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> which was (1222.50 g ha<sup>-1</sup>) for straw uptake (1592.75 g ha<sup>-1</sup>) for grain uptake and total iron uptake (2469.48 g ha<sup>-1</sup>). Furthermore, higher total Fe uptake over control reported @ 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (20.72 percent) followed by 20 kg FeSO<sub>4</sub> ha<sup>-1</sup> over control (8.30 percent). For levels of zinc sulphate iron uptake ranged between 1054.65-1185.19, 1314.89-1589.96 and 2081.08-2429.85 g ha<sup>-1</sup> for straw, grain and total uptake respectively and higher iron uptake noted in 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> which was straw (1185.19 g ha<sup>-1</sup>), grain (1589.96 g ha<sup>-1</sup>) and total uptake of iron (2429.85 g ha<sup>-1</sup>). Moreover, higher total Fe uptake over control reported @ 40

kg ZnSO<sub>4</sub> ha<sup>-1</sup> (16.75 percent) followed by 20 kg ZnSO<sub>4</sub> ha<sup>-1</sup> over control (6.65 percent).

**Zinc uptake:** The data narrated in Table 4. regarding zinc uptake is given. Zinc uptake of straw and grain estimated separately and total zinc uptake was also calculated. Microbial treatments influenced on zinc uptake of straw and grain which was ranged between 120.40-250.78 and 125.11-163.02 g ha<sup>-1</sup> respectively. For total zinc uptake of wheat influenced by microbial inoculation and ranged between 326.50-413.80 g ha<sup>-1</sup>. The maximum zinc uptake influenced by *Pseudomonas striata* i.e. 250.78, 163.02 and 413.80 g ha<sup>-1</sup> for straw, grain and total respectively. Moreover, highest increase in total Zn uptake over control was noted in treatment M2 i.e. *Pseudomonas striata* (26.73 percent) followed by *Pseudomonas fluorescens* and *Bacillus megaterium* (17.02 and 15.51 percent respectively). The levels of iron sulphate and zinc sulphate also influenced on zinc uptake. The iron sulphate upto 40 kg ha<sup>-1</sup> influenced on zinc uptake of straw and grain which ranged 203.74-266.11

and 124.19-164.46 g ha<sup>-1</sup> respectively. For total uptake of zinc, it was influenced by iron sulphate and ranged between 327.93-430.57 g ha<sup>-1</sup>. The higher zinc uptake was reported in 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (266.11, 164.46 and 430.57 g ha<sup>-1</sup> for straw, grain and total respectively). Further, higher total Zn uptake over control reported @ 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> (31.29 percent) followed by 20 kg FeSO<sub>4</sub> ha<sup>-1</sup> over control (11.65 percent). Similarly, levels of zinc sulphate upto 40 kg ha<sup>-1</sup> influenced on zinc uptake which was ranged between 207.59-260.53, 124.31-165.13 and 331.90-425.66 g ha<sup>-1</sup> for straw, grain and total respectively. The higher zinc uptake was noted in 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (260.53, 165.13 and 425.66 g ha<sup>-1</sup> at straw, grain and total respectively) which was superior over control treatment. The lower zinc uptake was recorded in control treatment (207.59, 124.31 and 331.90 g ha<sup>-1</sup> at straw, grain and total respectively). Furthermore, higher total Zn uptake over control reported @ 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> (28.24 percent) followed by 20 kg ZnSO<sub>4</sub> ha<sup>-1</sup> over control (10.60 percent).

**Table 3:** Effect of microbial inoculants and graded levels of iron sulphate and zinc sulphate on uptake of macronutrients (kg ha<sup>-1</sup>)

Treatments	Nitrogen uptake (kg ha <sup>-1</sup> )			Phosphorus uptake (kg ha <sup>-1</sup> )			Potassium uptake (kg ha <sup>-1</sup> )		
	Straw	Grain	Total	Straw	Grain	Total	Straw	Grain	Total
<b>Microbial inoculants (M)</b>									
M0 (Control)	52.62	68.24	120.87	15.98	18.21	34.19	55.48	13.91	69.39
M1 ( <i>Pseudomonas striata</i> )	68.00	92.67	160.67	22.92	24.65	47.58	70.69	21.65	92.33
M2 ( <i>Pseudomonas fluorescens</i> )	56.81	75.91	132.73	18.69	20.86	39.55	60.17	16.68	76.85
M3 ( <i>Bacillus megaterium</i> )	64.33	75.16	139.48	19.90	20.52	40.42	66.62	17.69	84.31
SE ±	1.819	1.410	2.535	0.831	0.520	1.254	1.834	0.583	2.341
CD at 5%	3.695	2.865	5.150	1.688	1.057	2.547	3.727	1.185	4.756
<b>Levels of FeSO<sub>4</sub> (Fe)</b>									
Fe0 (FeSO <sub>4</sub> 0 kg ha <sup>-1</sup> )	48.62	65.56	114.18	12.86	16.31	29.17	51.76	12.52	64.28
Fe1 (FeSO <sub>4</sub> 20 kg ha <sup>-1</sup> )	58.20	76.87	135.07	18.02	20.38	38.39	60.90	16.45	77.35
Fe2 (FeSO <sub>4</sub> 40 kg ha <sup>-1</sup> )	74.50	91.56	166.06	27.25	26.48	53.74	77.06	23.48	100.54
SE±	1.575	1.221	2.195	0.720	0.451	1.086	1.589	0.505	2.027
CD at 5%	3.200	2.481	4.460	1.462	0.915	2.206	3.227	1.026	4.119
<b>Levels of ZnSO<sub>4</sub> (Zn)</b>									
Zn0 (ZnSO <sub>4</sub> 0 kg ha <sup>-1</sup> )	52.20	65.56	117.76	15.32	17.58	32.90	55.18	12.95	68.13
Zn1 (ZnSO <sub>4</sub> 20 kg ha <sup>-1</sup> )	60.51	77.59	138.10	19.04	20.62	39.67	63.04	17.15	80.19
Zn2 (ZnSO <sub>4</sub> 40 kg ha <sup>-1</sup> )	68.62	90.83	159.45	23.76	24.97	48.74	71.50	22.35	93.85
SE ±	1.575	1.221	2.195	0.720	0.451	1.086	1.589	0.505	2.027
CD at 5%	3.200	2.481	4.460	1.462	0.915	2.206	3.227	1.026	4.119

**Table 4:** Effect of microbial inoculants and graded levels of iron sulphate and zinc sulphate on uptake of iron (g ha<sup>-1</sup>)

Treatments	Iron uptake (g ha <sup>-1</sup> )				Zinc uptake (g ha <sup>-1</sup> )			
	Straw	Grain	Total	% increase over control	Straw	Grain	Total	% increase over control
<b>Microbial inoculants (M)</b>								
M0 (Control)	1047.75	1340.26	2093.53	-	201.40	125.11	326.50	-
M1 ( <i>Pseudomonas striata</i> )	1170.26	1585.51	2411.55	15.19	250.78	163.02	413.80	26.73
M2 ( <i>Pseudomonas fluorescens</i> )	1101.27	1437.50	2225.75	6.31	235.41	146.67	382.08	17.02
M3 ( <i>Bacillus megaterium</i> )	1141.99	1409.49	2243.16	7.14	239.50	137.65	377.15	15.51
SE ±	12.985	18.468	20.559	-	4.412	2.612	6.186	-
CD at 5%	26.378	37.518	41.765	-	8.963	5.306	12.567	-
<b>Levels of FeSO<sub>4</sub> (Fe)</b>								
Fe0 (FeSO <sub>4</sub> 0 kg ha <sup>-1</sup> )	1024.42	1308.39	2045.53	-	203.74	124.19	327.93	-
Fe1 (FeSO <sub>4</sub> 20 kg ha <sup>-1</sup> )	1099.03	1428.43	2215.48	8.30	225.46	140.68	366.14	11.65
Fe2 (FeSO <sub>4</sub> 40 kg ha <sup>-1</sup> )	1222.50	1592.75	2469.48	20.72	266.11	164.46	430.57	31.29
SE±	11.245	15.994	17.804	-	3.821	2.262	5.357	-
CD at 5%	22.844	32.491	36.170	-	7.762	4.595	10.884	-
<b>Levels of ZnSO<sub>4</sub> (Zn)</b>								
Zn0 (ZnSO <sub>4</sub> 0 kg ha <sup>-1</sup> )	1054.65	1314.89	2081.08	-	207.59	124.31	331.90	-
Zn1 (ZnSO <sub>4</sub> 20 kg ha <sup>-1</sup> )	1106.11	1424.72	2219.56	6.65	227.19	139.90	367.09	10.60
Zn2 (ZnSO <sub>4</sub> 40 kg ha <sup>-1</sup> )	1185.19	1589.96	2429.85	16.75	260.53	165.13	425.66	28.24
SE ±	11.245	15.994	17.804	-	3.821	2.262	5.357	-
CD at 5%	1054.65	32.491	36.170	-	7.762	4.595	10.884	-

## Discussion

Availability of sufficient amount of nutrients by solubilization of native status of nutrient present in soil and increased uptake of nutrients and their utilization increased metabolism and synthesis of carbohydrates, better vegetative growth and subsequent partitioning and translocation from top to head and also release of energy rich compounds by the biofertilizers which ultimately increased auxin activities, growth and activity of microbial saprophytes and phosphates activity which ultimately affect the yield and yield attributes (Choudhary *et al.* 2014) <sup>[10]</sup>. Our results also correspond with the findings of Kandoliya *et al.* (2018) <sup>[19]</sup> reported significantly highest grain yield (4786 kg ha<sup>-1</sup>) was observed for the treatment T<sub>12</sub> (RDF + Soil application of ZnSO<sub>4</sub> @ 10 kg ha<sup>-1</sup> + FeSO<sub>4</sub> @ 20 kg ha<sup>-1</sup>) which was almost 38.2 percent higher as compared to control treatment (N-P-K: 120-60-60 kg ha<sup>-1</sup>). Cotton yield was significantly increased with application of RD + soil application of FeSO<sub>4</sub> @ 25 + ZnSO<sub>4</sub> @ 20 kg ha<sup>-1</sup> reported by Durgude *et al.* (2014) <sup>[11]</sup>.

N, P and K absorption by plants increased by PGPR supply which decreased rhizosphere soil pH due to increased concentrations of organic acids and antioxidative activity. As a result of root exudation mediated rhizosphere acidification, growth enhancement by PGPR supply was associated with increased plant antioxidation activity as well as nutrient uptake Israr *et al.* (2016) <sup>[17]</sup>. Kapadia *et al.* (2021) <sup>[21]</sup> stated that inoculation of microbial strains enhanced the more secondary roots, which store and transport more mineral nutrients to the various regions of the plant, resulting in increased biomass, nutrient contents, uptake and growth. Increase in the nutrient content in the plants showed that more bioavailability of available nutrients in soil solution and also supply of added fertilizers. Microbial isolates release the different organic acids make the nutrient available to crop plant. Similar findings reported by Hassan and Bano (2015) <sup>[16]</sup> reported that carrier-based biofertilizer enhanced the growth of wheat and also stated that the application of biofertilizer increased nutrients content and uptake of wheat over control treatment. The similar results confirmed with findings of Pawar *et al.* (2015) <sup>[25]</sup> concluded that soil application of 15 kg ZnSO<sub>4</sub> ha<sup>-1</sup> + 15 kg FeSO<sub>4</sub> ha<sup>-1</sup> with recommended dose of fertilizers recorded highest NPK concentration in grain and fodder of kharif sorghum.

The enhancement of micronutrient concentration by plants may be due to microbial inoculation with additional supplement of fertilizers may effect on initiation and development of effective roots system and nutrient uptake also increased in iron content of leaves and grain was might be due to increased iron availability in soil due to siderophore producing ability (Schwyn and Neilands 1987). *Pseudomonas fluorocens* (52.83%) CAS medium shows maximum siderophore production followed by *Pseudomonas striata* (48.33%) and *Bacillus megaterium* (46.67%) reported by Akshay *et al.* (2023) <sup>[2]</sup>. The maximum available Fe and Zn was noticed under RDF + 25 kg ha<sup>-1</sup> FeSO<sub>4</sub> + 25 kg ha<sup>-1</sup> ZnSO<sub>4</sub> reported by Waikar *et al.* (2021) <sup>[33]</sup>. Parmer *et al.* (2020) <sup>[24]</sup> concluded that higher content of Fe recorded with 25 kg ha<sup>-1</sup> ZnSO<sub>4</sub> + 50 kg ha<sup>-1</sup> FeSO<sub>4</sub>. Further, similar findings reported by Habib (2009) <sup>[14]</sup> and Kandoliya and Kunjadia (2018) <sup>[19]</sup>.

The increased nutrient uptake may be due to greater availability of nutrients through inorganic and biological

sources and well-developed root system. The increased uptake was also due to added supply of nutrients, balanced nutrient application and acid secretions by the microbial cultures might have acted on unavailable K in soil and made it more available for wheat crop resulting in better absorption of water and nutrients Sheng (2005) <sup>[29]</sup>. The maximum N, P and K uptake by pearl millet grain and stover was noticed in treatment consisting of RDF + 25 kg ha<sup>-1</sup> FeSO<sub>4</sub> + 25 kg ha<sup>-1</sup> ZnSO<sub>4</sub> reported by Waikar *et al.* (2021) <sup>[33]</sup>

Increase in Fe uptake might be due to siderophores produced by rhizosphere microorganisms are iron chelating ligands which can be beneficial to plants can supply iron to plants under iron stress or iron limiting conditions by increasing the solubility of ferric iron (Fe III), which otherwise is unavailable for plant nutrition. This element is assimilated by root cells in the reduced form (Fe II); however, especially in sufficiently aerated soils, the oxidized state (Fe III) is predominant and needs to be reduced to be taken up by plants. Plant roots have receptors or channels which can receive microbial siderophore, and plant ferric reductase helps in unloading of iron and converting it to the ferrous form (Altomare *et al.* 1999) <sup>[4]</sup>. Moreover, supply of additional iron through fertilizers also play important role in increasing more uptake by wheat crop. The research of this investigation are in consonance with the findings of Sable *et al.* (2016) <sup>[27]</sup> reported the content and uptake of Fe, Zn, Mn and Cu by groundnut was also found to be significantly highest with the inoculation of RDF + *Rhizobium* + *Pseudomonas striata*. Kiran *et al.* (2017) <sup>[22]</sup> reported highest increase in Fe uptake (4850 g ha<sup>-1</sup>) in treatment T<sub>7</sub> (GRDF + 20 kg ZnSO<sub>4</sub> ha<sup>-1</sup> + 25 kg FeSO<sub>4</sub> ha<sup>-1</sup> + seed inoculation of Fe and Zn solubilizers) over other treatments in wheat crop. Similarly, Parmer *et al.* (2020) <sup>[24]</sup> reported higher uptake of Fe noted with 25 kg ha<sup>-1</sup> ZnSO<sub>4</sub> + 50 kg ha<sup>-1</sup> FeSO<sub>4</sub>. The treatment RDF + 25 kg ha<sup>-1</sup> FeSO<sub>4</sub> + 25 kg ha<sup>-1</sup> ZnSO<sub>4</sub> recorded the significantly highest iron uptake reported by Waikar *et al.* (2021) <sup>[33]</sup> noted that highest iron uptake in treatment RDF + 25 kg ha<sup>-1</sup> FeSO<sub>4</sub> + 25 kg ha<sup>-1</sup> FeSO<sub>4</sub> and lowest iron uptake was observed in absolute control.

Enhanced Zn uptake might be due to solubilization of insoluble soil Zn by production of gluconic acids by the bacterial sp. Like *Pseudomonas striata*, *Pseudomonas fluorescens* and *Bacillus megaterium*. The bacterial inoculation increased Zn uptake at all ZnSO<sub>4</sub> application levels. Zn-solubilizers, owing to the organic acid production and proton extrusion (H<sup>+</sup>), lowers the rhizospheric pH, which sequesters zinc cations and improves uptake by plants. (Alexander 1997; Fasim *et al.* 2002; Wu *et al.* 2006; Anuradha *et al.* 2015) <sup>[3, 2, 35, 6]</sup>. The experimental findings are confirmed with Waikar *et al.* (2021) <sup>[33]</sup> reported that highest zinc uptake was noted with treatment RDF + 25 kg ha<sup>-1</sup> FeSO<sub>4</sub> + 25 kg ha<sup>-1</sup> ZnSO<sub>4</sub> and the lowest zinc uptake was observed in absolute control. Parmer *et al.* (2020) <sup>[24]</sup> reported higher uptake of Zn noted with 25 kg ha<sup>-1</sup> ZnSO<sub>4</sub> + 50 kg ha<sup>-1</sup> FeSO<sub>4</sub>. Similar findings reported by Kiran *et al.* (2017) <sup>[22]</sup> and Banafsheh *et al.* (2022) <sup>[7]</sup>.

## Conclusion

Seed inoculation with microbial inoculants (Iron and zinc solubilizer) + soil application of Fe and Zn in the form of FeSO<sub>4</sub> and ZnSO<sub>4</sub> along with RDF found beneficial in improving the yield, concentration and nutrient uptake of

wheat crop. Microbial inoculant particularly *Pseudomonas striata* and 40 kg FeSO<sub>4</sub> ha<sup>-1</sup> and 40 kg ZnSO<sub>4</sub> ha<sup>-1</sup> noted higher yield, nutrient concentration and uptake of nutrients by wheat.

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