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Mineral biofortification of Indian sorrel leaves as influenced by levels of iron and microbial cultures on physico-chemical, enzymatic activities and microbial population in soil

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Abstract
A pot culture experiment entitled "Biofortification of Iron in Indian Sorel Leaves using Graded Levels of Iron and Siderophore Producing Microbial Cultures" was planned and conducted during summer season of 2021 at Department of soil science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The pre-evaluated siderophore producing microbes *i.e. Azotobacter chrococcum, Azospirillum lipoferum* and *Bacillus licheniformis* laboratory were used with RDF in factorial complete randomized design. Result emerged out indicated that the soil physiochemical properties such as pH, EC, OC, enzymatic activities and microbial population were highly influenced by co-inoculation over uninoculated without iron (only RDF). The all properties are showed significant response with 100% + 60 mg $FeSO_4$ kg^{-1} soil + *Azospirillum lipoferum,* this combination found more potential than the rest of combination.

Keywords: Biofortification, biological properties, enzymatic activity, microbial count, iron, siderophore

Introduction
Minerals are considered essential nutrient. They are not synthesized by human and must be obtained from the diet. The potential of function leaf vegetables with addition physiological benefits beyond that of meeting basic nutritional and health properties of leafy vegetable are the key of factor in finding solution and initiating sustainable strategies to overcome present and upcoming health and nutrition challenges.

Biofortification is a process to produce micronutrient enriched staple food through mineral fertilizer, conventional breeding and transgenic approaches, Biofortification, the delivery of micronutrient *via*. micronutrient-dense crops, offers a cost effective and sustainable approach, complementing these efforts by reaching rural populations (Murgia *et al*, 2012) [11]. Vegetable crops are mostly grown in agro-systems distinguish by a high degree of boosting of the production processes and therefore the supply of nutrients are mostly based on application of fertigation, soilless cultivation, and foliar fertilization *etc*. the latter can be done either through conventional breeding or transgenic methods (White and Broadly, 2009) [22]. The biofortification strategy seeks to take advantage of the consistent daily consumption of large amounts of food staples by all family members, including women and children as they are most at risk for micronutrient malnutrition. So that agronomic biofortification with Iron fertilizer particularly foliar application performs well and provide edible parts of crop plant with sufficient nutrients to combat the global Fe malnutrition problem. Therefore biofortification in staple crops seem to be an effective method to reach people needs. Therefore iron biofortification an important and high priority challenge in research area to improve iron concentration as well as its bioavailability leafy vegetable crops.

Materials and Methods
The serial dilution technique described by Dhingra and Sinclair (1993) [5] was used for particular group of microbes. Enzymes *i.e*. dehydrogenase, acid phosphatase and alkaline phosphates which play important role in soil microbial respiration and phosphorous mobilization respectively were analyzed by using standard procedures described by Tabatabai and Bremner (1969) [18].

Statistical analysis

The results obtained were statistically analyzed and appropriately interpreted as per the methods described by Panse and Sukhatme (1985) [14]. Appropriate standard error (S.E.) and critical differences (C.D.) at 5% levels were worked out for interpretation of result.

Results and Discussion

Effect on soil pH

The interaction effect of iron levels and siderophore producing microorganisms are found in present investigation showed significant. The gradually pH decline ranges from 8.15-7.97. And lowest value was recorded (7.97) in pot with application of 100% RDF + 60 mg $FeSO_4$ kg-1 soil + Azospirillum lipoferum, which showed significant decline in pH over the rest of treatment.

The decline in pH might be due to application of iron which cause acidic in soil and also microorganism the produce many organic acid at rhizosphere.

The decrease in soil pH due to application of urea and phosphatic fertilizer both are cause slightly acidification in soil. And microbes also help to lower the pH during crop development soil microbes produced several organic acids which combine with minerals present in surface soil particle and enclosed the cation, which cause lower the surface pH minute. Also, observed lowest pH in the soil rhizospheric area when crops inoculated with PSB. This might be due to the buffering capacity of soil coupled with the inability of bacteria to secrete high concentration of organic acids which help to lower the pH. The results are conformity with Walpola and Yoon (2013) [21] revealed that inoculation with P. agglomerans or *B. anthina* or co-inoculation of isolates showed significantly decline soil pH. In accordance with our results, Hariprasa and Niranjana (2009) [6].

Table 1: Interaction effect of graded levels iron and siderophore producing microorganisms on pH EC and organic carbon under of sorrel leaf soil

Treatment	pH	EC (dS m^{-1})	Organic Carbon (OC) (g kg^{-1})
Fe_0S_0	8.15	0.22	4.56
Fe_0S_1	8.13	0.23	4.72
Fe_0S_2	8.12	0.21	4.75
Fe_0S_3	8.13	0.22	4.70
Fe_1S_0	8.12	0.22	4.68
Fe_1S_1	8.07	0.23	4.70
Fe_1S_2	8.06	0.22	4.84
Fe_1S_3	8.07	0.22	4.55
Fe_2S_0	8.07	0.23	4.73
Fe_2S_1	8.06	0.22	4.90
Fe_2S_2	8.05	0.23	5.02
Fe_2S_3	8.06	0.23	4.89
Fe_3S_0	8.07	0.22	4.55
Fe_3S_1	7.97	0.22	4.67
Fe_3S_2	7.99	0.21	5.30
Fe_3S_3	8.07	0.21	4.68
S.E±	0.02	0.00	0.08
C.D. at 5%	0.04	NS	0.22

Where Fe0 = 0 mg $FeSO_4$ kg^{-1} soil (Control), Fe1 = 20 mg $FeSO_4$ kg^{-1} soil, Fe2 = 40 mg $FeSO_4$ kg^{-1} soil, Fe3 = 60 mg $FeSO_4$ kg^{-1} soil, S0 = Uninoculated (control), S1 = *Azotobacter chrococcum*, S2 = *Azospirillum lipoferum*, S3 = *Bacillus licheniformis*.

Effect on EC

Electrical conductivity ranges from 0.22-0.21 dSm^{-1}. The interaction effect of iron levels and siderophore producing microorganisms found the non-significant. Results are similar with Chetan (2019) [4] he found that relation between DTPA-extractable iron and soil EC showed positive correlation with non-significant effect. Kumawat *et al*, (2009) [9] reported that ECe and pH of soil at harvest of fenugreek crop were influenced significantly by the application of organic manures alone or with bio-inoculants. The ECe and pH of soil at the time of harvest crop was showed significantly decline with increasing levels of organic manures with bio-inoculants and the lowest ECe 1.16 dS m^{-1} and pH 7.69 were recorded with the treatment of F5V2.5 R+P (FYM @ 5 t ha^{-1} + vermicompost @ 2.5 t ha^{-1} + *Rhizobium* + PSB. The lower % decline in ECe and pH were observed that as 9.48 and 8.84% lower, respectively over control.

Soil Organic carbon

Interaction effects of graded levels of iron and siderophore producing microbes found significant for soil organic carbon of sorrel leaf. The soil organic carbon ranges from 4.55-5.30 g kg^{-1}. The highest soil organic carbon (5.30 g kg^{-1}) was recorded in pots treated with 100% RDF +60 mg $FeSO_4$ kg^{-1} soil + *Azospirillum lipoferum*, which was significantly superior among rest of treatments.

Similar finding noted by Ahirwar *et al*, (2015) [1], the tomato (*Lycopersicon esculentum Mill.*) plants by inoculation with *Pseudomonas fluorescence* (SS5) treatments at post harvest soil fertility showed significant differences as compared to un-inoculated. Soil pH (7.9), EC (2.3 dSm^{-1}), and OC was found improved over control (pH-7.6, EC -2.2 dS m^{-1}, OC-0.48%, N- 70.93, P- 260.33 and K- 12.77 mg kg^{-1} soil). Similar results were reported by Chetan (2019) [4], he found that DTPA-extractable iron and organic carbon showed positive correlation with soil organic carbon.

Relation between iron fertilizer and organic carbon seems positive and significant effect. Pawar and Ismail (2016) [15] also reported that the soil organic carbon increased under the application of RDF of fertilizer along with bio-inoculants.

Soil Enzyme activities

Dehydrogenase enzymes

In interaction effects of graded levels of iron and siderophore producing microbes found significant for soil dehydrogenase enzyme activities in soil of sorrel leaf crop. The soil dehydrogenase enzyme activities content ranges from (29.33-38.33 µg g^{-1} soil). The highest dehydrogenase enzyme activities in soil (38.33 µg g^{-1} soil) was recorded in pots treated with 100% RDF+ 60 mg $FeSO_4$ kg^{-1} soil + *Azospirillum lipoferum* and lowest value recorded in control (only RDF).

Higher soil enzymes activity might be due to mineral fertilizer of NPK with normal does because dehydrogenase enzyme and nitrogen with medium level influence positive action (Koper *et al*. 1999) [8]. The experimental findings was similar with Rana *et al*. (2012) [17] who concluded that the maximum dehydrogenase enzyme activity in the soil rhizosphere area by the inoculation might be due to the availability of a higher quantity of biodegradable matter which help in improvement of their microbial activities. Further, Baghmare (2019) [2] noted that highest dehydrogenase enzyme activity significantly increased in RDF + *Rhizobium phaseoli* + *Pseudomonas flurescens* treated plots. Supply of iron has an additive effect on soil dehydrogenase enzyme activities in soil.

Acid Phosphatase

In interaction effects of graded levels of iron and siderophore producing microbes found significant for soil acid phosphatase enzymes activities in sorrel leaf crop. The soil acid phosphatase enzymes activities content ranges from (29.33-44.67 µg g^{-1} soil). The highest available acid phosphatase enzymes activities in soil (44.67 µg g^{-1} soil) was recorded in pots treated with 100% RDF+ 60 mg $FeSO_4$ kg^{-1} soil + *Azospirillum lipoferum* and however, lowest value recorded in control (only RDF).

The increase in the acid phosphates enzyme activity in soil under the iron siderophore producing microorganisms might be due to various organic acids were produced during the solubilisation of nutrients which leads to reduce soil reaction and slightly enhance in acid phosphatase enhance the enzyme activities in soil. Further, Vandana *et al*. (2012) [20] reported that the acid phosphatase value for spinach rages from 14.89 to 45.31. For all the plants species grown, the acid phosphatase activity in soil was recorded significantly enhanced with different growth stage of the crop. While for the growth period from 0 to 120 DAS, the acid phosphatase activity was recorded to be significantly higher than their corresponding controls for all crops. The enzymes activity increased drastically up to 60 days after transplanting and there after decreasing gradually to 0 to 120 day level for all the cropped plants. Acid and alkaline phosphatase enzyme was significantly activated to different degrees in cropped sites than control.

Table 2: Interaction effect of graded levels iron and siderophore producing microorganisms on soil enzyme activities under sorrel leaf

Sr. No.	Treatment	Soil Dehydrogenase (µg g^{-1} soil)	Acid phosphatase (µg g^{-1} soil)	Alkaline Phosphatase (µg g^{-1} soil)
T_1	Fe_0S_0	29	29	53
T_2	Fe_0S_1	35	34	66
T_3	Fe_0S_2	31	40	63
T_4	Fe_0S_3	31	35	64
T_5	Fe_1S_0	30	30	54
T_6	Fe_1S_1	36	34	67
T_7	Fe_1S_2	34	41	64
T_8	Fe_1S_3	33	40	67
T_9	Fe_2S_0	33	29	55
T_{10}	Fe_2S_1	36	38	69
T_{11}	Fe_2S_2	34	43	66
T_{12}	Fe_2S_3	34	41	69
T_{13}	Fe_3S_0	33	29	57
T_{14}	Fe_3S_1	37	38	70
T_{15}	Fe_3S_2	38	44	67
T_{16}	Fe_3S_3	36	42	69
	S.E±	0.3	0.3	0.3
	C.D. at 5%	0.9	0.9	0.8

Where Fe0 = 0 mg $FeSO_4$ kg^{-1} soil (Control), Fe1 = 20 mg $FeSO_4$ kg^{-1} soil, Fe2 = 40 mg $FeSO_4$ kg^{-1} soil, Fe3 = 60 mg $FeSO_4$ kg^{-1} soil, S0 = Uninoculated (control), S1 = *Azotobacter chrococcum,* S2 = *Azospirillum lipoferum,* S3 = *Bacillus licheniformis.*

Alkaline phosphate

In interaction effects of graded levels of iron and siderophore producing microbes found significant for soil alkaline phosphatase enzymes activities content in soil of sorrel leaf crop. The soil alkaline phosphatase enzymes activities content ranges from (353.67-70.00 µg g^{-1} soil). The highest alkaline phosphatase enzymes activities in soil (70.00 µg g^{-1} soil) were recorded in pots treated with 100% RDF+ 60 mg $FeSO_4$ kg^{-1} soil + *Azotobacter chrococcum*. However, lowest value recorded in control (only RDF).

Increased soil enzymes might be due to mineral fertilizer of NPK with normal does because phosphatase enzyme and nitrogen positive correlated (Koper *et al*. 1999) [8]. The alkaline phosphatase activity and increase in soil is depends on plant species such as *Allium cepa*, *Triticum aestivum* and *Trifolium alexandrium* rhizoplanes, (Tarafdar and Jungk, 1987) [19]. Our results are confirmed with the findings of Manna *et al*. (2007) [10] revealed that the activity of alkaline phosphatase enzyme activity in soil was showed significantly increased with increase in FYM levels along with PSM isolates. Similar finding are found by Jadhav (2020) [7].

Microbial Population

Bacterial Count

In interaction effects of graded levels of iron and siderophore producing microbes found significant for soil bacterial population in soil of sorrel leaf crop. The soil bacterial population ranges from (23.00-40.33 CFU x 10^{-7}). The highest bacterial population in soil (40.33 CFU x 10^{-7}) was recorded in pots treated with 100% RDF+ 60 mg $FeSO_4$ kg^{-1} soil + *Bacillus licheniformis* and at par with 60 mg $FeSO_4$ kg^{-1} soil + *Azotobacter chrococcum* (38.33 CFU x 10^{-7}). However, lowest population recorded in control (only RDF).

Increased microbial population may be application of mineral fertilizer such as NPK. These minerals and growth stages of crops positively correlated and great influence on the quantity of microbial count and soil enzymes activity and also higher concentration of root exudates in rhizosphere which secreted by crops root may also help in growth of microbial population (Koper *et al*. 1999) [8]. Moreover, Chalwade *et al*. (2005) [3] he reported that microbial population highest received from treatment T_{11} (RDF+ FYM+ Micronutrients) which were 290.8 CFU x 10^{-7} bacteria, and CFU x 10^{-4} actinomycetes and 45 CFU x 10^{-5} fungi with RDF + FYM treatment**s.**

Actinomycetes Count

In interaction effects of graded levels of iron and siderophore producing microbes found significant for soil actinomycets count content in soil of sorrel leaf crop. The soil actinomycets count ranges from (21.33-32.99 CFU x 10^{-5}). The highest actinomycets count in soil (32.99 CFU x 10^{-5}) was recorded in pots treated with 100% RDF+ 60 mg $FeSO_4$ kg^{-1} soil + *Azospirillum lipoferum* which was showed significantly superior among the treatments. However, lowest actinomycets count recorded in control (only RDF).

Increased in soil actinomycets may be due to application of mineral nitrogen fertilizer, However microbial population mainly actinomycets and fungi due to added supply nitrogen in soil and change of its physiochemical characteristics (Natyawa *et al*. 2010) [13].

Ramalakshmi *et al.* (2008) [16] reveal that total actinomycets population was showed significantly increased in PGPR inoculated plots. The highest actinomycets population was reported with inorganic fertilizer along with *PSB* inoculation. Similarly, Baghmare (2019) [2] reported that the total Actinomycetes population was increased significantly due to application of RDF along with siderophore producing microorganisms. The highest actinomycets population recorded with application of RDF + *Rhizobium phaseoli* + *Pseudomonas phaseoli* over control.

Fungal Count

In interaction effects of graded levels of iron and siderophore producing microbes found significant better for soil fungi population in soil of sorrel leaf crop. The soil fungi count ranges from (3.33-5.33 CFU x 10^{-4}). The highest fungi count in soil (5.33 CFU x 10^{-4}) was recorded in pots treated with 100% RDF+ 60 mg $FeSO_4$ kg^{-1} soil + *Azotobacter chrococcum* and at par with 60 mg $FeSO_4$ kg^{-1} soil +*Bacillus licheniformis* (5.00 CFU x 10^{-4}), 40 mg $FeSO_4$ kg^{-1} soil + *Azospirillum lipoferum* (5.00 CFU x 10^{-4}), 40 mg $FeSO_4$ kg^{-1} soil +*Azotobacter chrococcum*. However, lowest fungi population recorded in control pots.

Table 3: Interaction effect of graded levels iron and siderophore producing microorganisms on soil microbial count under Indian sorrel leaf

Sr. No.	Treatment	Bacteria (CFUx10^{-7})	Actinomycetes CFU x 10^{-5})	Fungi CFU x 10^{-4})
T_1	Fe0S0	23	21	3.00
T_2	Fe0S1	27	23	4.00
T_3	Fe0S2	27	24	3.67
T_4	Fe0S3	28	23	3.33
T_5	Fe1S0	28	24	3.33
T_6	Fe1S1	28	25	4.33
T_7	Fe1S2	28	27	4.67
T_8	Fe1S3	29	25	3.67
T_9	Fe2S0	39	25	4.00
T_{10}	Fe2S1	31	27	5.00
T_{11}	Fe2S2	30	29	5.00
T_{12}	Fe2S3	31	28	4.00
T_{13}	Fe3S0	33	27	3.33
T_{14}	Fe3S1	38	30	5.33
T_{15}	Fe3S2	37	32	4.00
T_{16}	Fe3S3	40	30	5.00
	S.E±	0.7	0.3	0.28
	C.D. at 5%	2.1	0.9	0.79

Where Fe0 = 0 mg $FeSO_4$ kg^{-1} soil (Control), Fe1 = 20 mg $FeSO_4$ kg^{-1} soil, Fe2 = 40 mg $FeSO_4$ kg^{-1} soil, Fe3 = 60 mg $FeSO_4$ kg^{-1} soil, S0 = Uninoculated (control), S1 = *Azotobacter chrococcum*, S2 = *Azospirillum lipoferum*, S3 = *Bacillus licheniformis.*

The increased in soil actinomycets may be due to application of mineral nitrogen fertilizer, However microbial population mainly actinomycets and fungi due to added supply nitrogen in soil and change of its physiochemical characteristics and crops root exudates are also excellent source of nutrients for microbes mainly those microbes which living in the rhizosphere (Natyawa *et al*., 2010) [13]. Moreover, Baghmare (2019) [2] who revealed that fungal count in rhizosphere soil area were reported significantly increased under the treatment of siderophore producing microorganisms along with *Rhizobium phaseoli*. The highest fungal population recorded with application of RDF+ *Rhizobium phaseoli* + *Pseudomonas flurescens.*

Summary

The interaction effect of graded levels of iron and siderophore producing microbe’s inoculants was also observed significant influencing soil pH. The soil pH was slightly low recorded with treatment of RDF + 60 mg $FeSO_4$ kg^{-1} soil + *Azotobacter chrococcum*. And high organic carbon received from RDF + 60 mg $FeSO_4$ kg^{-1} soil + *Azospirilum lipoferum*, except EC which was found statistically non-significant in soil. Further, significantly higher soil dehydrogenase enzymes, acid phosphatase activities recorded from RDF + 60 mg $FeSO_4$ kg^{-1} soil + *Azospirillum lipoferum* as compared to other treatments. However, alkaline phosphatase enzymes highest recorded in treatment of RDF + 60 mg $FeSO_4$ kg^{-1} soil + *Azotobacter chrococcum*. Moreover, bacteria population was significantly increased with RDF + 60 mg $FeSO_4$ kg^{-1} soil + *licheniformis*. While actinomycetes population was found highest in the treatment of RDF + 60 mg $FeSO_4$ kg^{-1} soil + *Azospirilum lipoferum* and maximum fungi population was found in RDF + 60 mg $FeSO_4$ kg^{-1} soil+ *Azotobacter chrococcum* in soil over the control.

Conclusion

The interaction effect of graded levels of iron and siderophore producing microbe’s inoculants was also observed significant influencing soil pH. The soil pH was slightly low recorded with treatment of RDF + 60 mg $FeSO_4$ kg^{-1} soil + *Azotobacter chrococcum*. And high organic carbon received from RDF + 60 mg $FeSO_4$ kg^{-1} soil + *Azospirilum lipoferum*, except EC which was found statistically non-significant in soil. The soil physiochemical and biological properties, such as organic carbon, nutrient availability, microbial population and soil enzymatic activities were found significantly positive with 60 mg $FeSO_4$ kg^{-1} soil x *Azospirillum lipoferum*, and also positive effect was recorded. Alkaline phosphatase and fungal population was recorded maximum in 60 mg $FeSO_4$ kg^{-1} soil x *Azotobacter chrococcum* treated pots. Moreover, soil bacterial population was observed significantly higher in 60 mg $FeSO_4$ kg^{-1} soil x *Bacillus licheniformis*.

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