International Journal of Plant & Soil Science

34(11): 23-32, 2022; Article no.IJPSS.83377
ISSN: 2320-7035

Impact of Inorganic Iron and Siderophore Producing Microbes on Growth and Content of Nutrient in Indian Sorrel Leaves (*Rumex vasicarias*)

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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/2022/v34i1130935

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/83377

Original Research Article

Received 25 December 2021
Accepted 28 February 2022
Published 26 March 2022

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 experimental results revealed that significant higher values of biometric observation in 45, 55 DAS and nutrient content a nd uptake in sorrel leaves at after harvest of crop were recorded. The maximum value regarding growth attributes as well as quality parameter was recorded under the treatment of 100% RDF + 60 mg $FeSO_4$ kg^{-1} soil + *Azospirillum lipoferum*. In which maximum plant height recorded with 23.14, and 29.44cm, leaves number (10.50 and 21.92) at 45 and 55 DAS respectively, chlorophyll 3.42 mg g-1, fresh leaf yield and dry matter yield (214.33, 30.87 g pot^{-1}) respectively which were superior among the rest of treatments. Moreover, regarding to quality attributes of sorrel leaves via. macro and micronutrient uptake significantly higher under the treatment of 100% RDF + 60 mg $FeSO_4$ kg^{-1} soil + *Azospirillum lipoferum* as compared to control (only RDF).

Keywords: Azospirillum lipoferum; Biofortification; iron; sorrel leaves (Rumex vasicarias); siderophore.

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1. INTRODUCTION

Leafy vegetables represents pillar of good nutritional value since, they provide important phytochemicals such as dietary fibre, vitamins, antioxidant like minerals and also, leafy vegetables are called as health capsule. The potential function of leafy vegetables is having additional physiological benefits beyond that of meeting basic nutritional and health properties. Leafy vegetable are the key factor in finding solution and initiating sustainable strategies to overcome present and upcoming health and nutrition challenges. After China India is the second largest producer of vegetables in the world.

Indian sorrel leaves (*Rumex vasicarias*) is a native of India. Whose botanical name is *Rumex vasicarias*. The sorrel leaves are considered to be poor people's nutritive vegetable. *Rumex vasicarias linn* (polyganaceas) is commonly called as Khatta Pala in Hindi, Chukka & Kura in Telgu and Indian sorrel leaves (Spinach) in English. The plant is herbaceous type, which can grow throughout seasons in India. Indian sorrel leaves containing Ca, carotene flavonoids oxalic acid, tannins, mucilage, salt minerals and vitamin-C, astringent and slightly purgative sorrel leaves are sour in taste and are considered to have medical value and are used in various treatments such as digestive flatulence constipation, asthma, bronchitis etc. It is also useful in analgesic and sometimes used to treatment of fevers, scurvy, itchy skin and ringworm. The fresh leaf or in the dried form, helps in cleaning the system by serving as a diuretic or laxative. Indian sorrel leaves are generally considered as field weed but it used as leafy vegetable in many parts of India. It may be boiled and used in dishes or raw in salads [1].

Iron is the second most abundant metal in nature and fourth most abundant element in the earth's crust; about 11 percent of Indian soil is in deficient supply of iron [2]. Iron is also known to rapidly convert into unavailable forms upon application to calcareous soils and poses poor mobility in phloem, soil or foliar iron [3]. Specially, in alkaline and calcareous soils, once applied by fertilization, iron rapidly becomes inaccessible to roots absorption, because of precipitation, absorption and oxidation circumstances [4]. Iron acts as co-factor for several enzymes performing basic function in human body. Insufficient supply of iron contributes to disability anaemia & stunted metal growth. Its malnutrition may be reduced by enhancing the bio-available iron content through iron supplementations and food fortification [5]. Major health concerns regarding iron deficiency are impairments of immune system cognitive function. In human health, the main function of iron is related to the synthesis of haemoglobin and myoglobin besides being essential to various metabolic processes such as oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport which is also required for energy production.

Siderophore are low molecular weight organic compound having high affinity for iron. Siderophore form very stable iron complexes and enhance the dissolution of iron bearing minerals of coordinating with iron atom at mineral surface, In addition act as a chelating agents produced by microorganism and higher plants to encourage take up of iron. PGPR synthesizes siderophore, which release those into surrounding environment, dissolute the iron by making iron siderophore complexes are taken up by the plant through transporter proteins that are located on plasma membrane of roots [6]. It is reported that heavy metals in the background siderophore are biosynthesised by microorganism [7].

Plant Growth Promoting Rhizobateria (PGPR) affects plant growth directly or indirectly by producing growth substances such as idole-acetic, gibberelic acid and cytokinins, fixing nitrogen from the atmosphere and providing the plant with this element and the being antagonistic toward phytopathogenic microorganisms [8]. PGPR have also been observed to fertility the iron contents in food crops apart from enhance the soil fertility and crop yield through siderophore production [9].In addition , these rhizobacteria improve plant growth by referring various beneficial effects such as increased nitrogen fixation, phosphorous solubilization, exhibiting antifungal activity production of volatile organic compound, induction of systemic resistance, promoting beneficial plant-microbe symbiosis, siderophore production, and increasing iron availability used for plant growth promotion, [10]. These rhizobacteria are impacts on plant development about 2-5% of rhizobacteria [11].

2. MATERIALS AND METHODS

2.1 Experiment Site

The experiment pots are placed in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vasantrao

Table 1. Siderophore production by microbial isolates on CAS agar plate

Sr. No	Microbial inoculation	CAS Agar test	% Siderophore
1	*Azotobacter chrococcum*	+	63
2	*Azospirillum lipoferum*	+	45
3	*Bacillus lichneformis*	+	32

Naik Marathwada Krishi Vidyapeeth, Parbhani MH. India (76046' East longitude and 190 16' North latitude) during 2021. This region has a semi-arid tropical climate, where the annual average temperature varies from 33.50°C in winter to 43.70°C in summer and means minimum temperature varies from 19.50°C to 27.70°C. The soils of the region are medium to deep black (Inceptisol/Vertisol). And the major portion of precipitation (75 per cent) is being received through South-West monsoon from July and August.

2.2 Experiment Design and Management

The experiment was performed from the beginning of March to May 2021, and induced three replication and 16 treatment, which treatment combinations includes with four graded levels of iron (0, 20, 40, and 60 mg $FeSO_4$ kg^{-1} soil) and four siderophore producing microorganisms (Uninoculated control, *Azotobacter chrococcum*, *Azospirillum lipoferum* and *Bacillus licheniformis*) in Factorial complete randomized design.

2.3 Detection of Siderophore

Siderophore production by plant growth promoting microorganisms was tested qualitatively by Chrome Azural S (CAS) liquid as well as plate assay. The stains were spread over CAS agar plate and incubated for 48 hrs at 28°C. After incubation a thin layer of CAS reagent in 0.7% agar was spread on the bacterial growth and plates were again incubated for 24 hrs at 28°C, formation of yellow orange colour zone around the colonies in plates assay and colour change from blue to orange in liquid assay, indicated the siderophore production [12].

2.4 Quantitative CAS Assay

The data presented in Table 1 revealed the assesement of siderophore production capacity of different promising microbial isolates by assay technique. All of three promising microbial isolates tested found to produce siderophore. These promising isolates produced the siderophore among that *Azospirilum lipoferum* showed maximum % siderophore (63) followed by *Bacillus lichneformis* (45%), While, the lowest value was recorded with *Azotobacter chrococcum* (32%). Among eight microbial isolates were evaluated for different PGPR traits including siderophore production, the following with good PGPR traits were used in the experiment.

3. RESULTS AND DISCUSSION

3.1 Growth Parameters

Plant height reading of sorrel leaves was taken at 45 and 55 days after sowing. The data showed in Table 2 that showed significant influence by graded levels of $FeSO_4.5H_2O$ fertilizer and bio-inoculant application. The plant height ranges from 19.84-22.02cm and 26.80-28.96cm at the stage of 45, 55 DAS respectively. In graded levels of iron (factor-1) the highest plant height was recorded was 22.11 cm and 28.96 cm at 45, 55 DAS respectively, under the treatment of 100% RDF + 60 mg $FeSO_4$ kg^{-1} soil, in graded levels of iron, while in bio-inoculant (factor-II) the maximum height was recorded under the treatment of 100% RDF + *Azospirillum lipoferum* treated pot with 22.02 and 28.58 cm at 45 and 55DAS respectively and it was followed by 100% RDF+ *Azotobacter chrococcum* with 28 cm height (at 55DAS).

Moreover significant lower value was recorded in control. The improvement of plant growth might be due to application of iron fertilizer along with nitrogen. Both nutrients has play role in related to basic biological process such as photosynthesis, respiration, protein synthesis and nitrogen fixation [13] and also responsible phosphorous application for height improvement because P play key role in division and development of new tissue. The growth promotion in Indian sorrel leaves might be due to greater availability of nutrient through inorganic and biological source. The improvement in plant growth might be due to application of siderophore producing microbes which resulting enhancing nutrient solubilization and developed various plant growth promoters via. auxins, gibberellins, cytokinins, and siderophore production [14]. These results are in agreement with the finding of Khalid et al. [15] who reported that selected bacterial isolates shows significantly higher in plant plants height as compared to uninoculated (Control) plant in presence of iron.

3.2 Number of Leaves

Number of leaf reading of sorrel leaf was taken at 45 and 55 days after sowing. The data showed in Table 2 showed significantly influenced by graded levels of $FeSO_45.H_2O$ application and bio- inoculant application. The number of leaves varies from 7.75-9.63 and 18.76-21.41 at the stage of 45, 55 DAS respectively. The significant highest number of leaves in graded levels of iron (Factor -I) were recorded with 9.28 and 21.41 at 45, 55 DAS respectively, under the treatment of 100% RDF + 60 mg FeSO4 kg^{-1} soil, and it was found to be at par with 9.10 and 21.01(100% RDF + 40 mg $FeSO_4$ kg^{-1} soil). While in bio-inoculant (Factor-II) the maximum number of leaves plant-1 were recorded under the treatment of 100% RDF + *Azospirillum lipoferum* treated pots with 9.63 and 21 at 45 and 55 DAS respectively and while significant lower value was recorded in control (Only RDF).

The increasing leaf number might be due to positive effect of RDF of NPK along with iron fertilizer. Because of iron increase in rate of metabolic process such as photosynthesis, respiration which resultant higher food material production translocation of food material and additional nitrogen application improve protein synthesis which ultimately caused higher plant growth as well as increasing in number of leaves. Application of RDF along with micronutrients on vegetables growth and accumulation of metabolic material have been reported by Suryavanshi et al. [16]. Moreover, Kaur et al. [17] also reported that the improvement in vegetative plant growth might be due to application of PGPR which resulting enhancing nutrient solubilization and developed various plant growth promoters via auxins, gibberellins, cytokinins, and siderophore. These results are in agreement with the finding of Khalid et al. [15].

3.3 Chlorophyll Content

Data presented in Table 3 indicates significant variation in chlorophyll content in sorrel leave. In case of graded levels iron fertilizer (Factor-I) the maximum leaf chlorophyll content was recorded 3.28 mg g^{-1} by the treatment of 100% RDF + 60 mg $FeSO_4$ kg^{-1} soil and followed by 3.10 g $plant^{-1}$ under the treatment of 100% RDF + 40 mg $FeSO_4$ kg^{-1} soil. In bio-inoculant (Factor-II) the highest chlorophyll content was recorded by the treatment of 100% + *Azospirillum lipoferum* with 3.11 mg g^{-1} which was significantly superior among the bio-inoculant as compare to control one.

Table 2. Effect of levels of iron and siderophore producing microorganisms on height of Indian sorrel leaf

Treatment	Plant height (cm)		Number of leaves $plant^{-1}$	
	45 DAS	55 DAS	45 DAS	55 DAS
Levels of iron ($FeSO_4.5H_2O$) (Fe)				
Fe0: 0 mg $FeSO_4$ kg^{-1} soil (only RDF)	20.27	27.16	7.75	18.76
Fe1:20 mg $FeSO_4$ kg^{-1} soil	21.14	27.21	8.55	20.50
Fe2:40 mg $FeSO_4$ kg^{-1} soil	21.20	28.37	9.10	21.01
Fe3:60 mg $FeSO_4$ kg^{-1} soil	22.11	28.96	9.28	21.41
S.E±	0.137	0.196	0.102	0.163
C.D. at 5%	0.396	0.566	0.295	0.474
Siderophore producing microbes (S)				
S0: Uninoculated(Control)	19.84	26.80	7.75	19.22
S1: *Azotobacter chrococcum*	21.48	28.26	8.75	20.65
S2: *Azospirillum Lipoferum*	22.02	28.58	9.63	21.24
S3: *Bacillus lichneformis*	21.39	28.05	8.55	20.58
S.E±	0.137	0.196	0.102	0.163
C.D. at 5%	0.396	0.566	0.295	0.474
Interaction(Fe x S)				
S.E±	0.274	0.392	0.20	0.326
C.D. at 5%	0.792	1.133	0.58	0.941

Improvement in chlorophyll content might be due to application of iron and nitrogen fertilizer. The increases in chlorophyll at the stage of 45 DAS might be due to increased uptake of nitrogen and iron in early stage of plant growth which was supplied by RDF and ferrous sulphate. This iron is not an integral part of chlorophyll and the maintenance of chloroplast. Nitrogen- chlorophyll synthesis also depends on mineral nutrition such as nitrogen because it promotes the formation of photosynthetic pigments by enhancing the amount of stromal and thylakoid proteins in leaves of plants [18]. Dawwam et al. [19] reported that effect of inoculation with PGPR on photosynthetic pigments showed significant. The results recorded P35 and P18 isolates showed significant increase in chlorophyll a and chlorophyll b about 36.8% and 46.01%, respectively, over control, Furthermore Eleiwa et al. [20] found that inoculation of wheat grains with bio-inoculants, *Bacillus polymyxa*, or *Azospirillum brasilinseas* that produced auxin significantly increased the chlorophyll a, chlorophyll b, and carotenoids as compared with uninoculated treatment.

3.4 Yield

3.4.1 Fresh weight of leaves (Fresh leaf yield)

The data presented in Table 3 indicates that the fresh leaf yield was ranged from 176-205 g pot^{-1}. The graded levels iron fertilizer (Factor-I) showed highest fresh leaf yield (205 g pot^{-1}) under the treatment of 100% RDF + 60 mg $FeSO_4$ kg^{-1} soil. While in case of bio-inoculant (Factor-II) the highest fresh yield was recorded (188.92 g pot^{-1}) under the treatment of 100% RDF + *Azospirillum lipoferum* followed by (188.42 g pot^{-1}) by the treatment of 100% RDF + *Bacillus licheniformis* and the lowest value was recorded in control (only RDF).

Increasing in fresh yield may be due to RDF and micronutrient are reported to enhance the absorption of native as well as added major nutrient such as N and P which might have been attributed to improvement in fresh yield. Similar findings were also observed by Suryavanshi et al. [16].

Table 3. Effect of levels of iron and siderophore producing microorganisms on chlorophyll content, fresh leaf yield and dry matter yield of Indian sorrel leaf

Treatments	Chlorophyll content (mg g^{-1}) 45 DAS	Fresh leaf yield (g pot^{-1})	Dry matter yield (g pot^{-1})
Levels of iron ($FeSO_4.5H_2O$) (Fe)			
Fe0: 0 mg $FeSO_4$ kg^{-1} soil (Only RDF)	2.59	176.00	25.34
Fe1: 20 mg $FeSO_4$ kg^{-1} soil	2.84	176.67	25.77
Fe2: 40 mg $FeSO_4$ kg^{-1} soil.	3.10	181.50	26.07
Fe3: 60 mg $FeSO_4$ kg^{-1} soil.	3.28	205.42	29.51
S.E±	0.08	0.430	0.089
C.D. at 5%	0.23	1.230	0.257
Siderophore producing microbes (S)			
S0: Uninoculated (control)	2.72	177.50	25.56
S1: *Azotobactor chrococcum*	2.92	184.75	26.95
S2: *Azospirillum Lipoferum*	3.11	188.92	27.45
S3: *Bacillus lichneformis*	3.06	188.42	26.90
S.E±	0.08	0.430	0.089
C.D. at 5%	0.23	1.230	0.257
Interaction(Fe x S)			
S.E±	0.16	0.850	0.188
C.D. at 5%	NS	2.460	0.514

3.4.2 Dry matter yield

Dry weight of sorrel leaf was ranged from 25.34 to 29.51 g pot^{-1}. In iron levels (Factor-I) the highest dry matter was recorded with ranges from 25.34 to 29.51 g pot^{-1}. In graded levels of iron (Factor- I) the highest dry matter was recorded with 29.51 g pot-1 under the treatment of 100% RDF + 60 mg $FeSO_4$ kg^{-1} soil, Further, in bio-inoculant (Factor-II) application the highest dry matter was recorded with 27.45 g pot^{-1} under the treatment of 100% RDF + *Azospirillum lipoferum* and the lowest value was recorded in control (only RDF).

This increasing in fresh yield may be due to RDF and micronutrient such as iron are reported to enhance nitrogen deprivation in leaves increases sugar and dietary fibres accumulation, which leads to an increase in leaf dry matter content. Nitrogen application rate not only control LDMC but also regulates dry matter allocation between roots and shoot of plants [21].

3.5 Phosphorous Content in Sorrel Leaf

There is data showed in Table 4, as data seems significant effect of graded levels of iron and siderophore producing microbes on phosphorous content of sorrel leaf. The effect of iron levels showed significant on phosphorous content by sorrel leaf. The highest P content was recorded (0.96%) by the treatment of 100% + 60 mg $FeSO_4$ kg^{-1} soil. The highest P content in plant found in pot inoculated with 100%RDF + *Azospirillum lipoferum* (0.62%). The lowest P content recorded in control (Uninoculated) pots.

Bairwa et al. [22] revealed that the combined use of organic manures, bio-inoculants and chemical fertilizers showed the remarkable improvement in N, P and K content of plant leaves of okra plant under the treatment of neem cake (6 q ha^{-1}) + vermicompost (10 q ha^{-1}) + *Azotobacter* + PSB + 60% recommended dose of NPK through inorganic fertilizer(T15) with 2.275 %, 1.060% 1.443% of N,P and K nutrient respectively influence of combined application of nutrient through integrated nutrient management as compared to control. Kumawat [23], reported that application of organic manures with bio-inoculants at different combination levels show significantly increased N, P and K content of seed and straw of N, P and K. The maximum content of N, P and K were recorded under the treatment of FYM 5t ha^{-1}+Vermicompost 2.5t ha^{-1} +*Rhizobium* + PSB treatment with N, P and K content were 1.076%, 0.180-0.259% and 1.41% in straw of N,P and K respectively which were significantly highest as compare to control.

Table 4. Effect of graded iron level and siderophore producing microorganisms on potassium and iron content and uptake by sorrel leaf

Treatments	Phosphorous (P) content in plant (%)	Potassium (K) content in plant (%)	Iron (Fe) content In plant (mg kg^{-1})
Levels of iron ($FeSO_4.5H_2O$) (Fe)			
Fe0: 0 mg $FeSO_4$ kg^{-1} soil (only RDF)	0.54	1.21	313.97
Fe1: 20 mg $FeSO_4$ kg^{-1} soil.	0.54	1.24	313.46
Fe2: 40 mg $FeSO_4$ kg^{-1} soil	0.61	1.25	333.61
Fe3: 60 mg $FeSO_4$ kg^{-1} soil	0.69	1.30	335.38
S.E±	0.008	0.009	4.1610
C.D. at 5%	0.022	0.025	12.032
Siderophore producing microbes (S)			
S0: (Uninoculated)	0.56	1.23	277.95
S1: *Azotobactor chrococcum*	0.59	1.24	340.49
S2: *Azospirillum Lipoferum*	0.62	1.28	340.18
S3: *Bacillus lichneformis*	0.60	1.25	337.79
S.E±	0.008	0.009	4.1610
C.D. at 5%	0.022	0.025	12.032
Interaction(Fe x S)			
S.E±	0.015	0.018	8.330
C.D. at 5%	0.44	0.049	24.06

3.6 Potassium Content in Sorrel Leaves

The K content in plant has influenced by both $FeSO_4$ and bio-inoculants. The data related to potassium content in sorrel leaves showed in Table 4. In which, Factor-I, application of RDF chemical fertilizer along with iron levels exhibited direct effect on K concentration. The highest being (1.30% and 0.38 g pot^{-1}K content and uptake respectively was recorded with 100% RDF + 60 mg $FeSO_4$ kg^{-1} soil. While, in bio-inoculant (Factor-II) application the maximum K content and uptake was recorded 1.28%, 0.38 g pot^{-1} under the treatment of 100% RDF + *Azospirillum lipoferum*. The lowest K content and uptake recorded in control (only RDF).

Increased potassium content was might be due to increased availability from added supply of inorganic nutrient fertilizer. And this added potassium reduction of potassium fixation in soil and make increase in soil for uptake by plants also these potassium also regulates nutrient transports and inoculation of siderophore producing microorganisms, these siderophore help in mineralization, solubilization and greater absorption by root surface area. Moreover, Ribeiro and Cardoso, [24] found that the seed inoculation with different bacterial isolates which improve significantly in nutrient content as well as uptake.

3.7 Iron Content in Sorrel Leaf

The data presented in Table 5 showed significant effect of biofortification of iron in sorrel leaf with iron levels and siderophore producing microbes on iron content of sorrel leaf. The effect of iron levels showed significant on iron content by sorrel leaf. The highest iron content (335.38 mg kg^{-1}) and uptake (9.92 mg pot^{-1}) was recorded under the treatment of 100% + 60 $mgkg^{-1}$ soil $FeSO_4$. Followed by, the 100% RDF+ 40 mg $FeSO_4$ kg^{-1} soil (333.61mg kg^{-1}) content. The highest Fe content (340.49 mg kg^{-1}) in plant found in pot inoculated with 100% RDF + *Azotobacter chrococcum* and RDF+ *Azospirillum lipoferum* for respectively and at par with treatment of 100% RDF+ *Azospirillum lipoferum* with content (340.18 g kg^{-1}). The lowest iron content recorded in control pots.

Iron rich biofortified sorrel leaf crop was successfully obtained through the addition of iron via foliar application of ferrous sulphate, foliar application proved to be efficient for iron absorption as the absorption mainly occurs mainly through the stomata and iron involved in activation of several enzymes and hormones. It is possible to be triggered by phloem signal generating in the shoots that interact with hormones to balance the uptake of mineral nutrients from soil [25]. And on the other hand siderophore producing microbes also makes available iron, acidification of the rhizosphere, which ultimately increase iron content, and uptake, this facilitate iron uptake by both enhancement of the reductase activity and solubilization of iron in the rhizosphere. In the experiment increasing in iron content and uptake might be due to additional application of iron fertilizer along with siderophore. These siderophore make more available in soil for plant through solubilization of insoluble soil iron. Experiment result shows that, *Azospirillum lipoferum* and *Azotobacter chrococcum* also produce the high % of siderophore and also increase the nutrient content and uptake of iron.

3.8 Zinc Content in Sorrel Leaf

The data showed in Table 5 seems significant effect of graded levels of iron and siderophore producing microbes on zinc content and uptake of sorrel leaf. The effect of iron levels showed significant on zinc content by sorrel leaf. The highest zinc content (45.74 mg kg^{-1}) and uptake (1.35 mg pot^{-1}) was recorded (0.96%) under the treatment of 100% + 60 mg $FeSO_4$ kg^{-1} soil and at par with (43.98 mg kg^{-1} zinc content) 100%RDF +40 mg $FeSO_4$ kg^{-1} soil. The highest zinc content (44.84 g kg^{-1}) and uptake (1.30g pot^{-1}) in plant found in pot inoculated with 100%RDF + *Azospirillum lipoferum* and followed by *Azotobacter chrococcum*, *Bacillus licheniformis*. The lowest zinc content and uptake recorded in control pots.

Increases zinc content and uptake by sorrel leaf might be due to application of inorganic fertilizer such as urea because nitrogen and zinc are positively correlated to each other. And also iron involved in activation of several enzymes and hormones it's possible to triggered by phloem signal generating in the shoots that interact with hormones to balance the uptake of minerals nutrient from soil [25]. And also its possible might be due to inoculation of siderophore microbes increased in Zn content and uptake by plant. They help in accumulation of more nutrients in leaves with metabolization and root assimilation of nutrients at rhizosphere.

Table 5. Effect of graded level iron and siderophore producing micro-organisms on Zn and Cu content by sorrel leaf

Treatments	Zinc Content (mg kg^{-1})	Copper Content Cu (mg kg^{-1})
Levels of iron ($FeSO_4.5H_2O$) (Fe)		
Fe0: 0 mg $FeSO_4$ kg^{-1} soil (only control)	40.23	19.97
Fe1: 40 mg $FeSO_4$ kg-^{1} soil	41.02	21.35
Fe2: 40 mg $FeSO_4$ kg^{-1} soil	43.98	21.40
Fe3: 60 mg $FeSO_4$ kg^{-1} soil	45.74	23.12
S.E±	0.616	0.255
C.D. at 5%	1.780	1.736
Siderophore producing microbes (S)		
S0: Uninoculated(Control)	37.39	20.78
S1: *Azotobactor chrococcum*	44.18	21.68
S2: Azospirillum lipoferum	44.84	21.78
S3: *Bacillus lichneformis*	44.55	21.59
S.E±	0.616	0.255
C.D. at 5%	1.780	1.736
Interaction(Fe x S)		
S.E±	1.233	0.51
C.D. at 5%	3.56	NS

3.9 Copper Content and Uptake in Sorrel Leaf

There was significant effect of graded levels of copper and siderophore producing microbes on copper content and uptake of sorrel leaf. As Table 5, the effect of iron levels showed significant on copper content and uptake by sorrel leaf. The highest copper content (23.12 mg kg^{-1}) and uptake (0.68 g pot^{-1}) was recorded under the treatment of 100% + 60 mg kg^{-1} soil $FeSO_4$. The highest copper content (21.78 mg kg^{-1}) and uptake (0.60g pot^{-1}) in plant found in pot inoculated with 100%RDF + *Azospirillum lipoferum* and followed by 100%RDF + *Azotobacter chrococcum* (21.68 mg kg^{-1}, 0.59 g pot^{-1}), 100% RDF + *Bacillus licheniformis* (21.59 mg kg^{-1}). The lowest copper content and uptake recorded in control pots.

Iron involved in activation of several enzymes and hormones it's possible to trigger by phloem signal generating in the shoots that interact with hormones to balance the uptake of minerals nutrient from soil [25]. And also its possible might be due to inoculation of siderophore microbes. They help in accumulation of more nutrients in leaves with metabolization and root assimilation of nutrients at rhizosphere.

4. CONCLUSION

Significant effect of graded levels of iron up to 60 mg FeSO4 kg^{-1} soil was observed and siderophore producing microbe's culture particularly *Azospirillum lipoferum* was noted on all growth attributes such as plant height, leaves number, fresh leaves yield and dry matter yield over the other treatments. The content and uptake of potassium and micronutrients (Fe, Zn, and Cu) were found increasing with gradually increasing levels of $FeSO_4$ kg^{-1} soil and siderophore producing microbes particularly *Azospirillum lipoferum, Azotobacter chrococcum* treated pots as compared to control (only RDF).

ACKNOWLEDGEMENT

We feel grateful to all Indian Network Project on Soil Biodiversity-Biofertilizers, VNMKV Parbhani and National Collection of Industrial Microorganisms (NCIM) Pune for providing the microbial strain for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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