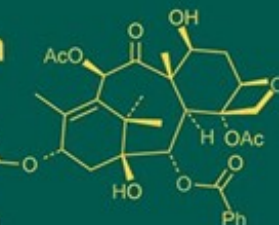
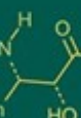
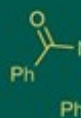
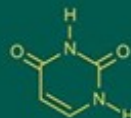


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## Effect of iron biofortification on growth, yield and soil nutrient availability of spinach (*Spinacia Oleracea* L.) grown in calcareous soil

Chaudhari Payal<sup>1</sup>, SL Waikar<sup>2</sup>, HK Kausadikar<sup>3</sup>, SP Zade<sup>4</sup>, GS Pawar<sup>5</sup>, PH vaidya<sup>6</sup>, AL Dhamak<sup>7</sup>

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### ABSTRACT

A field experiment was conducted during Rabi 2025–26 at the Research Farm, Department of Soil Science, Vasantao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, to study the effect of iron biofortification on growth, yield, soil physico-chemical properties and nutrient availability in spinach (*Spinacia oleracea* L.) grown in calcareous soil. The experiment was conducted in a Randomized Block Design with three replications and eight treatments comprising soil and foliar application of FeSO<sub>4</sub>, Fe-EDTA and iron-solubilizing bacteria along with recommended dose of fertilizers (RDF). The initial soil was alkaline (pH 7.71) with 51.67 g kg<sup>-1</sup> CaCO<sub>3</sub>. Treatment T<sub>7</sub> (RDF + soil Fe-EDTA @ 2.5 kg ha<sup>-1</sup> + foliar Fe-EDTA @ 0.25%) recorded the highest plant height (26.51 cm), number of leaves (6.27 plant<sup>-1</sup>), leaf area (376 cm<sup>2</sup>), fresh yield (186.00 q ha<sup>-1</sup>) and dry matter yield (33.78 q ha<sup>-1</sup>). Soil pH, EC, organic carbon and CaCO<sub>3</sub> were not significantly influenced by treatments. The highest available N (206.97 kg ha<sup>-1</sup>), P (14.11 kg ha<sup>-1</sup>), K (691.71 kg ha<sup>-1</sup>), DTPA-Fe (4.58 mg kg<sup>-1</sup>), Mn (4.58 mg kg<sup>-1</sup>), Zn (0.71 mg kg<sup>-1</sup>) and Cu (2.51 mg kg<sup>-1</sup>) were recorded under T<sub>7</sub>. Thus, combined soil and foliar application of Fe-EDTA improved spinach growth, yield and nutrient availability in calcareous soil.

**Keywords:** Spinach, Iron biofortification, Fe-EDTA, Calcareous soil, Soil fertility, Nutrient availability, Growth, Yield

### 1) Introduction

Green leafy vegetables are important components of the human diet due to their nutritional value and short growing period. Among them, spinach (*Spinacia oleracea* L.) is widely cultivated and consumed for its high biomass production and mineral-rich leaves. It is a good source of iron, calcium, vitamins and other essential nutrients. However, alkaline and calcareous soil conditions can restrict the availability of micronutrients, particularly iron. Iron is an essential micronutrient involved in chlorophyll formation, photosynthesis, respiration, electron transport and enzyme activity, thereby contributing to plant growth and yield. In calcareous soils, high pH and calcium carbonate reduce Fe solubility and availability to plants. Conventional Fe fertilizers may also become immobilized through precipitation, adsorption and oxidation. Chelated Fe fertilizers such as Fe-EDTA can maintain Fe in a more available form and may be effective under such conditions. Agronomic biofortification through soil and foliar application of micronutrients can improve nutrient availability and crop performance. Previous studies have reported beneficial effects of Fe application on growth, yield and nutrient availability in spinach and other leafy vegetables (Rengel *et al.*, 1999; Patel *et al.*, 2021; Das *et al.*, 2020) [2, 3, 1]. Priyadarshini *et al.* (2019) [4] also reported improved Fe availability with Fe-EDTA application in calcareous soils. Therefore, the present investigation was undertaken to evaluate the effect of different sources and methods of iron application on growth and yield of spinach and selected soil physico-chemical properties and nutrient availability under calcareous soil conditions.

### 2. Materials and Methods

#### 2.1 Experimental site

The field experiment was conducted during Rabi season 2025–26 at the Research Farm of the Department of Soil Science, Vasantao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra. The experiment was conducted on soil belonging to the Parbhani series and classified as Typic Haplustert. The experimental soil was alkaline and calcareous in nature.

**Corresponding Author:**  
Chaudhari Payal  
Department of Soil Science,  
Vasantao Naik  
Marathwada Krishi  
Vidyapeeth, Parbhani  
(Maharashtra), India

## 2.2 Initial soil characteristics

The initial soil had pH 7.71, EC 0.19 dS m<sup>-1</sup>, organic carbon 3.20 g kg<sup>-1</sup> and CaCO<sub>3</sub> 51.67 g kg<sup>-1</sup>. Available N, P and K were 173.09, 7.79 and 560.69 kg ha<sup>-1</sup>, respectively. DTPA-extractable Fe, Zn, Mn and Cu were 1.36, 0.42, 3.20 and 1.83 mg kg<sup>-1</sup>, respectively.

## 2.3 Experimental design and treatments

The experiment was conducted using spinach cv. *Pusa All Green* in a Randomized Block Design with three replications. The recommended dose of fertilizer was 80:40:40 kg N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O ha<sup>-1</sup>. The plot size was 1.5 × 1.2 m<sup>2</sup> and crop spacing was 15 × 10 cm. Sowing was carried out on 18 December 2025 and harvesting was done at 45 DAS. The treatments consisted of T<sub>1</sub>: control; T<sub>2</sub>: RDF + soil application of FeSO<sub>4</sub> @ 25 kg ha<sup>-1</sup>; T<sub>3</sub>: RDF + foliar spray of FeSO<sub>4</sub> @ 0.5%; T<sub>4</sub>: RDF + soil application of FeSO<sub>4</sub> @ 25 kg ha<sup>-1</sup> + foliar spray of FeSO<sub>4</sub> @ 0.5%; T<sub>5</sub>: RDF + soil application of Fe-EDTA @ 2.5 kg ha<sup>-1</sup>; T<sub>6</sub>: RDF + foliar spray of Fe-EDTA @ 0.25%; T<sub>7</sub>: RDF + soil application of Fe-EDTA @ 2.5 kg ha<sup>-1</sup> + foliar spray of Fe-EDTA @ 0.25%; and T<sub>8</sub>: RDF + soil application of iron-solubilizing bacteria @ 5 L ha<sup>-1</sup>.

## 2.4 Growth and yield observations

Plant height and number of leaves were recorded at 15, 30 and 45 DAS. Leaf area was recorded at harvest. Fresh yield was determined from harvested biomass and expressed as q ha<sup>-1</sup>, while dry matter yield was expressed on a hectare basis. Five plants were selected randomly from each plot for biometric observations.

## 2.5 Soil analysis

Soil samples were collected from 0–15 cm depth before sowing and after harvest. Samples were air-dried, gently ground and passed through a 2-mm sieve. Soil pH and EC were determined from 1:2.5 soil-water suspension. Organic carbon was determined by the Walkley and Black method, while CaCO<sub>3</sub> was estimated by the rapid titration method. Available N was estimated by the alkaline KMnO<sub>4</sub> method, available P by Olsen's method and DTPA-extractable Fe, Mn, Zn and Cu according to Lindsay *et al.* (1978) [5].

The data were statistically analysed according to the procedures described by Panse *et al.* (1985) [6], and SEM± and CD at 5% significance level were calculated.

## 3. Results and Discussion

### 3.1 Growth parameters

#### Plant height

Iron biofortification significantly improved plant height of spinach at different stages of crop growth. At 45 DAS, plant height ranged from 13.63 to 26.51 cm. Treatment T<sub>7</sub> recorded the maximum plant height

of 26.51 cm, followed by T<sub>4</sub> (23.70 cm) and T<sub>5</sub> (22.07 cm), whereas the control recorded the minimum plant height of 13.63 cm. At 15 DAS, plant height under T<sub>7</sub> was 4.40 cm compared with 2.85 cm under control, while at 30 DAS it increased to 12.13 cm compared with 6.10 cm in T<sub>1</sub>. The superior growth under T<sub>7</sub> may be associated with the combined soil and foliar supply of chelated iron. Fe-EDTA can remain relatively available under alkaline soil conditions and thereby support Fe nutrition during crop growth. Adequate Fe supply is associated with chlorophyll formation, photosynthetic activity and several metabolic processes. Similar improvement in spinach growth following iron application has been reported by Patel *et al.* (2021) [3] and Das *et al.* (2020) [1].

#### Number of leaves

The number of leaves increased with crop age. At 45 DAS, the number of leaves ranged from 4.99 to 6.27 plant<sup>-1</sup>. The maximum number of leaves was recorded under T<sub>7</sub> (6.27), followed by T<sub>4</sub> (5.80) and T<sub>5</sub> (5.73), whereas T<sub>1</sub> recorded the minimum value (4.99). At 30 DAS, T<sub>7</sub> recorded 4.87 leaves plant<sup>-1</sup> compared with 3.94 under control. The greater leaf production under combined soil and foliar Fe-EDTA application may be related to improved Fe availability and sustained vegetative growth. Adequate Fe nutrition supports chlorophyll formation and physiological activity, which can favour leaf development. Similar responses to adequate Fe nutrition have been reported by Cakmak and Marschner.

#### Leaf area

Leaf area at harvest ranged from 258 to 376 cm<sup>2</sup>. Treatment T<sub>7</sub> recorded the maximum leaf area (376 cm<sup>2</sup>), followed by T<sub>4</sub> (361 cm<sup>2</sup>), T<sub>5</sub> (329 cm<sup>2</sup>) and T<sub>2</sub> (328 cm<sup>2</sup>). The minimum leaf area was recorded in T<sub>1</sub> (258 cm<sup>2</sup>). The treatment differences were significant.

The increase in leaf area under T<sub>7</sub> could be attributed to better Fe availability from combined soil and foliar Fe-EDTA application. Enhanced Fe nutrition favours chlorophyll formation and photosynthetic activity, which can support leaf expansion and biomass accumulation. Similar improvement in vegetative growth following iron supplementation has been reported by Patel *et al.* (2021) [3].

### 3.2 Yield

Fresh yield was significantly affected by iron biofortification. Fresh yield ranged from 96.66 to 186.00 q ha<sup>-1</sup>. Treatment T<sub>7</sub> recorded the highest fresh yield (186.00 q ha<sup>-1</sup>), followed by T<sub>4</sub> (174.00 q ha<sup>-1</sup>), T<sub>5</sub> (172.00 q ha<sup>-1</sup>) and T<sub>2</sub> (166.00 q ha<sup>-1</sup>). The control recorded the lowest fresh yield of 96.66 q ha<sup>-1</sup>.

Dry matter yield ranged from 19.45 to 33.78 q ha<sup>-1</sup>. The highest dry matter yield was recorded under T<sub>7</sub> (33.78 q ha<sup>-1</sup>), followed by T<sub>4</sub> (32.23 q ha<sup>-1</sup>) and T<sub>5</sub> (31.97 q ha<sup>-1</sup>), while the control recorded 19.45 q ha<sup>-1</sup>.

**Table 1.** Effect of iron biofortification on plant height, number of leaves and leaf area

| Treatments                                                                                               | Plant height (cm) |        |        | Number of leaves Plant <sup>-1</sup> |        |        | Leaf area (cm <sup>2</sup> )<br>At harvest |
|----------------------------------------------------------------------------------------------------------|-------------------|--------|--------|--------------------------------------|--------|--------|--------------------------------------------|
|                                                                                                          | 15 DAS            | 30 DAS | 45 DAS | 15 DAS                               | 30 DAS | 45 DAS |                                            |
| T <sub>1</sub> – Control                                                                                 | 2.85              | 6.10   | 13.63  | 2.14                                 | 3.94   | 4.99   | 258                                        |
| T <sub>2</sub> – RDF + soil FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup>                                   | 3.60              | 11.15  | 20.27  | 3.89                                 | 4.20   | 5.67   | 328                                        |
| T <sub>3</sub> – RDF + foliar FeSO <sub>4</sub> @ 0.5%                                                   | 2.96              | 9.90   | 16.45  | 2.98                                 | 4.09   | 5.27   | 280                                        |
| T <sub>4</sub> – RDF + soil FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup> + foliar FeSO <sub>4</sub> @ 0.5% | 4.11              | 11.49  | 23.70  | 4.00                                 | 4.73   | 5.80   | 361                                        |
| T <sub>5</sub> – RDF + soil Fe-EDTA @ 2.5 kg ha <sup>-1</sup>                                            | 3.93              | 11.25  | 22.07  | 3.96                                 | 4.25   | 5.73   | 329                                        |
| T <sub>6</sub> – RDF + foliar Fe-EDTA @ 0.25%                                                            | 3.20              | 11.07  | 18.76  | 3.50                                 | 4.15   | 5.53   | 317                                        |
| T <sub>7</sub> – RDF + soil Fe-EDTA @ 2.5 kg ha <sup>-1</sup> + foliar Fe-EDTA @ 0.25%                   | 4.40              | 12.13  | 26.51  | 4.60                                 | 4.87   | 6.27   | 376                                        |
| T <sub>8</sub> – RDF + iron-solubilizing bacteria @ 5 L ha <sup>-1</sup>                                 | 2.90              | 10.93  | 17.05  | 3.15                                 | 4.15   | 5.10   | 305                                        |

(continued on next page)

| Treatments | Plant height (cm) |        |           | Number of leaves<br>Plant <sup>-1</sup> |           |           | Leaf area<br>(cm <sup>2</sup> ) |
|------------|-------------------|--------|-----------|-----------------------------------------|-----------|-----------|---------------------------------|
|            | 15 DAS            | 30 DAS | 45<br>DAS | 15<br>DAS                               | 30<br>DAS | 45<br>DAS | At harvest                      |
| Grand mean | 3.451             | 10.65  | 19.805    | 4.37                                    | 4.52      | 5.545     | 319.25                          |
| SEm (±)    | 0.09              | 0.22   | 0.66      | 0.20                                    | 0.19      | 0.20      | 4.86                            |
| CD at 5%   | 0.29              | 0.66   | 2.00      | NS                                      | 0.59      | 0.61      | 14.27                           |

The higher yield under T<sub>7</sub> can be related to the improved growth observed in terms of plant height, leaf production and leaf area. Greater vegetative development would provide a larger photosynthetic surface and greater biomass accumulation. The combined application of soil and foliar Fe-EDTA may have maintained Fe availability under calcareous conditions and supported physiological activity throughout the crop period. Patel *et al.* (2021) [3] similarly reported improved spinach growth and yield with suitable Fe sources and application methods.

**Table 2.** Effect of iron biofortification on yield of spinach

| Treatments                                                                                                        | Fresh Yield<br>(qt ha <sup>-1</sup> ) | Dry matter yield (qt ha <sup>-1</sup> ) |
|-------------------------------------------------------------------------------------------------------------------|---------------------------------------|-----------------------------------------|
| T <sub>1</sub> – Control                                                                                          | 96.66                                 | 19.45                                   |
| T <sub>2</sub> – RDF + soil<br>FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup>                                         | 166.00                                | 31.24                                   |
| T <sub>3</sub> – RDF + foliar<br>FeSO <sub>4</sub> @ 0.5%                                                         | 134.00                                | 22.65                                   |
| T <sub>4</sub> – RDF + soil<br>FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup><br>+ foliar FeSO <sub>4</sub> @<br>0.5% | 174.00                                | 32.23                                   |
| T <sub>5</sub> – RDF + soil Fe-<br>EDTA @ 2.5 kg ha <sup>-1</sup>                                                 | 172.00                                | 31.97                                   |
| T <sub>6</sub> – RDF + foliar Fe-<br>EDTA @ 0.25%                                                                 | 145.00                                | 27.06                                   |
| T <sub>7</sub> – RDF + soil Fe-<br>EDTA @ 2.5 kg ha <sup>-1</sup><br>+ foliar Fe-EDTA @<br>0.25%                  | 186.00                                | 33.78                                   |
| T <sub>8</sub> – RDF + iron-sol-<br>ubilizing bacteria @ 5<br>L ha <sup>-1</sup>                                  | 136.00                                | 20.64                                   |
| Grand mean                                                                                                        | 151.291                               | 27.37                                   |
| SEm(±)                                                                                                            | 4.35                                  | 1.34                                    |
| CD at 5 %                                                                                                         | 12.75                                 | 4.06                                    |

### 3.3 Physico-chemical properties of soil

#### Soil pH

The initial soil pH was 7.71. After harvest, soil pH ranged from 7.23 to 7.65. The lowest pH was recorded under T<sub>7</sub> (7.23), while the highest value was observed under T<sub>1</sub> (7.65). However, the differences among treatments were statistically non-significant. The relatively small changes in pH indicate that the Fe treatments did not markedly alter the soil reaction during the short crop period. This is important in calcareous soils where the buffering effect of carbonate limits rapid changes in soil pH.

#### Electrical conductivity

The initial EC was 0.19 dS m<sup>-1</sup> and values after harvest ranged from 0.21 to 0.26 dS m<sup>-1</sup>. Treatment effects were statistically non-significant. The highest EC was recorded in T<sub>1</sub> (0.26 dS m<sup>-1</sup>), while T<sub>4</sub> and T<sub>7</sub> recorded 0.21 dS m<sup>-1</sup>.

The limited variation in EC indicates that the iron treatments did not cause substantial changes in soluble salt concentration during the experimental period.

#### Organic carbon

Organic carbon increased numerically from the initial value of 3.20 g kg<sup>-1</sup> to 3.40–4.20 g kg<sup>-1</sup> after harvest. The highest value was recorded under T<sub>7</sub> (4.20 g kg<sup>-1</sup>), followed by T<sub>4</sub> (4.01 g kg<sup>-1</sup>) and T<sub>5</sub> (3.90 g kg<sup>-1</sup>). However, the differences were statistically non-significant.

The numerical increase may be associated with crop root residues and rhizosphere inputs during crop growth, although the short experimental period and absence of organic amendments resulted in no significant treatment effect.

#### Calcium carbonate

Initial CaCO<sub>3</sub> content was 51.67 g kg<sup>-1</sup> and increased slightly after harvest, ranging from 51.87 to 54.81 g kg<sup>-1</sup>. The highest value was recorded under T<sub>7</sub>, whereas the lowest was observed under T<sub>1</sub>. The treatment effect was statistically non-significant.

The minor changes in CaCO<sub>3</sub> indicate that the applied Fe treatments did not substantially modify the carbonate status of the soil during the experimental period.

### 3.4 Available macronutrients in soil

#### Available nitrogen

Available N increased from an initial value of 173.09 kg ha<sup>-1</sup> to 179.37–206.97 kg ha<sup>-1</sup> after harvest. T<sub>7</sub> recorded the highest available N (206.97 kg ha<sup>-1</sup>), followed by T<sub>4</sub> (204.46 kg ha<sup>-1</sup>) and T<sub>5</sub> (200.70 kg ha<sup>-1</sup>), whereas T<sub>1</sub> recorded the lowest value (179.37 kg ha<sup>-1</sup>). T<sub>7</sub> was statistically at par with T<sub>4</sub> and T<sub>5</sub>.

The higher available N under T<sub>7</sub> may be associated with better root growth and rhizosphere activity under improved Fe nutrition. Better root development can influence nutrient acquisition and nutrient cycling. Similar effects of micronutrient application on soil fertility and crop performance have been reported by Meena *et al.* (2013) [7] and Shillode *et al.* (2016) [8].

#### Available phosphorus

Available P increased from 7.79 kg ha<sup>-1</sup> initially to 9.41–14.11 kg ha<sup>-1</sup> after harvest. T<sub>7</sub> recorded the highest available P (14.11 kg ha<sup>-1</sup>), followed by T<sub>4</sub> (13.44 kg ha<sup>-1</sup>) and T<sub>5</sub> (12.99 kg ha<sup>-1</sup>). The lowest value was recorded under control (9.41 kg ha<sup>-1</sup>). T<sub>7</sub> was statistically at par with T<sub>4</sub> and T<sub>5</sub>.

**Table 3.** Effect of iron biofortification on physico-chemical properties

| Treatments                                                             | pH   | EC   | Organic carbon | CaCO <sub>3</sub> |
|------------------------------------------------------------------------|------|------|----------------|-------------------|
| T <sub>1</sub> – Control                                               | 7.65 | 0.26 | 3.40           | 51.87             |
| T <sub>2</sub> – RDF + soil FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup> | 7.61 | 0.23 | 3.85           | 53.70             |

(continued on next page)

| Treatments                                                                                               | pH   | EC    | Organic carbon | CaCO <sub>3</sub> |
|----------------------------------------------------------------------------------------------------------|------|-------|----------------|-------------------|
| T <sub>3</sub> – RDF + foliar FeSO <sub>4</sub> @ 0.5%                                                   | 7.43 | 0.24  | 3.50           | 52.50             |
| T <sub>4</sub> – RDF + soil FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup> + foliar FeSO <sub>4</sub> @ 0.5% | 7.35 | 0.21  | 4.01           | 54.67             |
| T <sub>5</sub> – RDF + soil Fe-EDTA @ 2.5 kg ha <sup>-1</sup>                                            | 7.51 | 0.22  | 3.90           | 54.48             |
| T <sub>6</sub> – RDF + foliar Fe-EDTA @ 0.25%                                                            | 7.63 | 0.23  | 3.54           | 53.31             |
| T <sub>7</sub> – RDF + soil Fe-EDTA @ 2.5 kg ha <sup>-1</sup> + foliar Fe-EDTA @ 0.25%                   | 7.23 | 0.21  | 4.20           | 54.81             |
| T <sub>8</sub> – RDF + iron-solubilizing bacteria @ 5 L ha <sup>-1</sup>                                 | 7.64 | 0.25  | 3.67           | 53.00             |
| Grand mean                                                                                               | 7.54 | 0.367 | 3.75           | 53.54             |
| SEm(±)                                                                                                   | 0.02 | 0.01  | 0.20           | 0.09              |
| CD at 5%                                                                                                 | NS   | NS    | NS             | NS                |
| Initial                                                                                                  | 7.71 | 0.19  | 3.20           | 51.67             |

Improved root activity and rhizosphere processes under adequate Fe nutrition may have contributed to greater P availability. Balanced nutrient management can influence nutrient mobilization and transformation in the soil (Marschner, 2012) [9].

### Available potassium

Available K increased from 560.69 kg ha<sup>-1</sup> initially to 565.71–691.71 kg ha<sup>-1</sup> after harvest. The highest available K was recorded under T<sub>7</sub> (691.71 kg ha<sup>-1</sup>), followed by T<sub>4</sub> (668.08 kg ha<sup>-1</sup>) and T<sub>5</sub> (646.80 kg ha<sup>-1</sup>). The lowest value was recorded under T<sub>1</sub> (565.71 kg ha<sup>-1</sup>).

The higher K availability under T<sub>7</sub> may be related to better root development and nutrient cycling under improved crop growth. Similar effects of integrated nutrient management on nutrient availability have been reported by Choudhary *et al.* (2015) [10].

### 3.5 DTPA-extractable micronutrients in soil

#### DTPA-extractable iron

Initial DTPA-extractable Fe was 1.36 mg kg<sup>-1</sup>. After harvest, it increased to 3.71–4.58 mg kg<sup>-1</sup>. The highest Fe availability was recorded under T<sub>7</sub> (4.58 mg kg<sup>-1</sup>), followed by T<sub>4</sub> (4.47 mg kg<sup>-1</sup>), T<sub>5</sub> (4.34 mg kg<sup>-1</sup>) and T<sub>2</sub> (4.26 mg kg<sup>-1</sup>). The control recorded the lowest value (3.71 mg kg<sup>-1</sup>). T<sub>7</sub> was statistically at par with T<sub>4</sub>, T<sub>5</sub>, T<sub>2</sub> and T<sub>6</sub>.

The increased DTPA-extractable Fe under Fe-treated plots demonstrates the direct effect of Fe fertilization on the available Fe pool. The relatively higher value under Fe-EDTA treatments can be associated with the ability of chelating agents to maintain Fe in a more available form under alkaline conditions. Priyadarshini *et al.* (2019) [4] reported improved Fe availability with Fe-EDTA application in calcareous soils.

#### DTPA-extractable manganese

Initial DTPA-extractable Mn was 3.20 mg kg<sup>-1</sup> and increased to 3.25–4.58 mg kg<sup>-1</sup> after harvest. T<sub>7</sub> recorded the highest value (4.58 mg kg<sup>-1</sup>), followed by T<sub>4</sub> (4.44 mg kg<sup>-1</sup>) and T<sub>5</sub> (4.27 mg kg<sup>-1</sup>), while the control recorded 3.25 mg kg<sup>-1</sup>.

The increase in available Mn may be associated with improved root development and rhizosphere activity under balanced nutrient conditions. Iron application may indirectly influence the availability of other micronutrients through changes in nutrient cycling and rhizosphere processes.

#### DTPA-extractable zinc

Initial available Zn was 0.42 mg kg<sup>-1</sup>. After harvest, DTPA-extractable Zn ranged from 0.48 to 0.71 mg kg<sup>-1</sup>. The highest Zn availability was recorded under T<sub>7</sub> (0.71 mg kg<sup>-1</sup>), followed by T<sub>4</sub> (0.68 mg kg<sup>-1</sup>), T<sub>5</sub> (0.64 mg kg<sup>-1</sup>) and T<sub>2</sub> (0.62 mg kg<sup>-1</sup>). The control recorded the lowest value (0.48 mg kg<sup>-1</sup>).

The improved Zn availability may be related to enhanced root activity and favourable rhizosphere conditions under improved crop growth. Balanced micronutrient management can influence micronutrient transformations and availability in soil (Alloway, 2008; Marschner, 2012) [11, 9].

#### DTPA-extractable copper

Initial DTPA-extractable Cu was 1.83 mg kg<sup>-1</sup>. After harvest, Cu ranged from 1.88 to 2.51 mg kg<sup>-1</sup>. T<sub>7</sub> recorded the highest Cu availability (2.51 mg kg<sup>-1</sup>), followed by T<sub>4</sub> (2.46 mg kg<sup>-1</sup>), T<sub>5</sub> (2.38 mg kg<sup>-1</sup>) and T<sub>2</sub> (2.30 mg kg<sup>-1</sup>). The control recorded the lowest value (1.88 mg kg<sup>-1</sup>).

The increase in DTPA-extractable Cu under Fe-treated plots may be related to improved rhizosphere activity and nutrient cycling associated with better crop growth. Similar principles regarding micronutrient availability under balanced nutrient management are discussed by Brady *et al.* (2017) [12] and Marschner (2012) [9].

**Table 4.** Effect of iron biofortification on Nutrient availability in soil

| Treatments                                                                                               | Available N<br>(kg ha <sup>-1</sup> ) | Available P<br>(kg ha <sup>-1</sup> ) | Available K<br>(kg ha <sup>-1</sup> ) | DTPA-Fe<br>(mg kg <sup>-1</sup> ) | DTPA-Mn<br>(mg kg <sup>-1</sup> ) | DTPA-Zn<br>(mg kg <sup>-1</sup> ) | DTPA-Cu<br>(mg kg <sup>-1</sup> ) |
|----------------------------------------------------------------------------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| T <sub>1</sub> – Control                                                                                 | 179.37                                | 9.408                                 | 565.71                                | 3.71                              | 3.25                              | 0.48                              | 1.88                              |
| T <sub>2</sub> – RDF + soil FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup>                                   | 194.43                                | 12.32                                 | 632.57                                | 4.26                              | 3.93                              | 0.62                              | 2.30                              |
| T <sub>3</sub> – RDF + foliar FeSO <sub>4</sub> @ 0.5%                                                   | 181.88                                | 10.08                                 | 592.48                                | 3.89                              | 3.40                              | 0.50                              | 1.99                              |
| T <sub>4</sub> – RDF + soil FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup> + foliar FeSO <sub>4</sub> @ 0.5% | 204.46                                | 13.44                                 | 668.08                                | 4.47                              | 4.44                              | 0.68                              | 2.46                              |
| T <sub>5</sub> – RDF + soil Fe-EDTA @ 2.5 kg ha <sup>-1</sup>                                            | 200.70                                | 12.99                                 | 646.80                                | 4.34                              | 4.27                              | 0.64                              | 2.38                              |

(continued on next page)

| Treatments                                                                             | Available N<br>(kg ha <sup>-1</sup> ) | Available P<br>(kg ha <sup>-1</sup> ) | Available K<br>(kg ha <sup>-1</sup> ) | DTPA-Fe<br>(mg kg <sup>-1</sup> ) | DTPA-Mn<br>(mg kg <sup>-1</sup> ) | DTPA-Zn<br>(mg kg <sup>-1</sup> ) | DTPA-Cu<br>(mg kg <sup>-1</sup> ) |
|----------------------------------------------------------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| T <sub>6</sub> – RDF + foliar Fe-EDTA @ 0.25%                                          | 188.16                                | 11.20                                 | 625.85                                | 4.08                              | 3.78                              | 0.58                              | 2.25                              |
| T <sub>7</sub> – RDF + soil Fe-EDTA @ 2.5 kg ha <sup>-1</sup> + foliar Fe-EDTA @ 0.25% | 206.97                                | 14.11                                 | 691.71                                | 4.58                              | 4.58                              | 0.71                              | 2.51                              |
| T <sub>8</sub> – RDF + iron-solubilizing bacteria @ 5 L ha <sup>-1</sup>               | 185.65                                | 10.53                                 | 613.53                                | 3.98                              | 3.48                              | 0.55                              | 2.19                              |
| Grand mean                                                                             | 192.70                                | 11.54                                 | 629.56                                | 4.20                              | 3.89                              | 0.595                             | 2.245                             |
| SEm (±)                                                                                | 5.89                                  | 0.67                                  | 13.85                                 | 0.25                              | 0.20                              | 0.02                              | 0.13                              |
| CD at 5%                                                                               | 17.8                                  | 2.02                                  | 41.82                                 | 0.76                              | 0.62                              | 0.06                              | 0.40                              |
| Initial                                                                                | 173.09                                | 7.79                                  | 560.69                                | 1.36                              | 3.20                              | 0.42                              | 1.83                              |

#### 4. Overall Discussion

The results indicate that iron biofortification influenced spinach growth, yield and soil nutrient availability, while the basic physico-chemical properties of the soil remained relatively stable. The combined application of Fe-EDTA through soil and foliar routes (T<sub>7</sub>) consistently produced higher values of plant height, number of leaves, leaf area and yield. The same treatment also recorded the highest available N, P, K and DTPA-extractable Fe, Mn, Zn and Cu.

The better performance of T<sub>7</sub> can be related to the complementary role of soil and foliar Fe application. Soil-applied Fe-EDTA could contribute to Fe availability in the root zone, whereas foliar Fe could provide an additional source directly to the plant foliage. This combination is particularly relevant under calcareous conditions, where high pH and CaCO<sub>3</sub> can restrict Fe availability. The thesis results also show that the Fe-EDTA treatment increased DTPA-extractable Fe substantially over the initial soil status.

The improvement in N, P and K availability under T<sub>7</sub> may not be interpreted as a direct addition of these nutrients through the Fe fertilizer because the experiment also received RDF. Rather, the higher post-harvest values may reflect differences in nutrient cycling, root activity and nutrient transformations associated with improved crop growth. The increases in DTPA-extractable Mn, Zn and Cu similarly indicate that Fe biofortification was associated with changes in the available micronutrient pool.

The results are broadly consistent with previous reports on Fe application in spinach and other crops. Patel *et al.* (2021) [3] reported positive effects of Fe sources and application methods on spinach under calcareous soil. Priyadarshini *et al.* (2019) [4] reported improved Fe availability from Fe-EDTA in calcareous soils, while Rengel *et al.* (1999) [2] highlighted agronomic approaches for improving micronutrient density in crops.

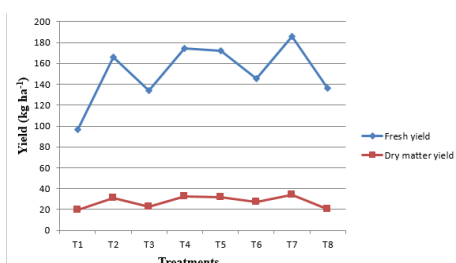


Figure 1. Effect of iron biofortification on yield of spinach.

#### 5. Conclusion

The field experiment demonstrated that iron biofortification improved the growth and yield performance of spinach grown under calcareous soil conditions. Among the treatments, combined soil and foliar application of Fe-EDTA along with RDF (T<sub>7</sub>) recorded higher plant height, number of leaves, leaf area, fresh yield and dry matter yield.

The application of iron did not significantly alter soil pH, EC, organic carbon and calcium carbonate content during the experimental period. However, iron biofortification resulted in higher post-harvest availability of N, P and K and increased DTPA-extractable Fe, Mn, Zn and Cu compared with the control.

The findings indicate that combined soil and foliar application of Fe-EDTA can improve spinach productivity and maintain a higher available nutrient pool in calcareous soil. The response of soil micronutrients, particularly Fe, was more pronounced than the changes observed in basic soil physico-chemical properties.

Payal Chaudhari

Department of soil Science

Vasanthrao Naik Marathwada

Agricultural University, Parbhani

Maharashtra, India

S. L. Waikar

Department of soil Science

Vasanthrao Naik Marathwada

Agricultural University, Parbhani

Maharashtra, India

H. K. Kausadikar

Department of soil Science

Vasanthrao Naik Marathwada

Agricultural University, Parbhani

Maharashtra, India

S.P. Zade

Department of soil Science

Vasanthrao Naik Marathwada

Agricultural University, Parbhani

Maharashtra, India

G.s. Pawar

Department of Plant Physiology

Vasanthrao Naik Marathwada

Agricultural University, Parbhani

Maharashtra, India

P. H. Vaidya

Department of soil Science

Vasanthrao Naik Marathwada

Agricultural University, Parbhani

Maharashtra, India

A.L. Dhamak

Department of soil Science

Vasanthrao Naik Marathwada

Agricultural University, Parbhani

Maharashtra, India

Corresponding Author

Chaudhari Payal

Department of soil Science

Vasanthrao Naik Marathwada

Agricultural University, Parbhani

Maharashtra, India

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## Authors

### Chaudhari Payal

Department of Soil Science, Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani (Maharashtra), India

### SL Waikar

Department of Soil Science, Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani (Maharashtra), India

### HK Kausadikar

Department of Soil Science, Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani (Maharashtra), India

### SP Zade

Department of Soil Science, Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani (Maharashtra), India

### GS Pawar

Department of Soil Science, Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani (Maharashtra), India

### PH vaidya

Department of Soil Science, Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani (Maharashtra), India

### AL Dhamak

Department of Soil Science, Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani (Maharashtra), India