

# Effect of Different Doses of Iron Chelate on Plant Growth and in Preventing Iron Deficiency Chlorosis in Soybean

## Abstract

The objective of this research work is to solve the problem due to iron deficiency in soybean. Iron deficiency chlorosis is a widespread agricultural problem in soybean crops, especially in alkaline and calcareous soils resulting in complete crop failure. To eradicate this problem, the methodology adopted in this work was conducted with a factorial experimental design of different doses (0, 2.5, 5 and 7.5 mgkg<sup>-1</sup>) of FeDTPA in 15 different types of soil categorized into low (L1-L5), medium (M1-M5) and high (H1-H5) iron content during Kharif season 2020 at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The experiment ended after 60 days of seed sowing. Plant height and total chlorophyll content were determined at 30 and 60 days after sowing. The highest plant height and total chlorophyll content were observed in 7.5 mgkg<sup>-1</sup> dose of Fe in low iron content soil (L1-L5) is 102.36cm and 45.63 SPAD value respectively. Increasing the application of iron doses from 0 to 7.5 mgkg<sup>-1</sup> increases the plant height and total chlorophyll content in low iron content soil while in high iron content soil (H1-H5) it decreases the plant height and total chlorophyll content. In conclusion, the use of FeDTPA in iron-deficient soils of the Indo-Gangetic plain will be beneficial for the growth of soybean crops as well as for the eradication of iron deficiency chlorosis.

Keywords: Soybean, FeDTPA, SPAD

## Introduction

Soybean, from the Fabaceae family, is a significant agronomic crop with uses in both human and animal nutrition (Farhadi, Souri, and Alirezalu 2016). In comparison to alfalfa, soybeans contain a significant amount of protein (Souri, Naiji, and Aslani 2018). It has a prominent place as an important seed legume, with a 25% contribution to the production of vegetable oil globally along with two-thirds of its protein concentrate used for livestock feeding (Agarwal et al., 2013). India is the 4th-largest producer of soybeans in the world. This crop has huge potential for elevating farmers' economic status in many different regions of the country. The diverse uses of soybean include its intake as dal and soya milk, and also its role as an ingredient for bakery products (Tripathi and Mishra 2005).

Iron (Fe) is one of the most essential nutrients for all organisms (Zuo and Zhang, 2011). Fe is a crucial micronutrient for plants and is involved in a variety of physiological activities, such as RNA synthesis, chlorophyll biosynthesis, the activation of numerous enzymes, respiration, and redox reactions. (Ye et al.,2015; Zargar et al.,2015). Many crops, especially those grown in semi-arid regions and calcareous soils, suffer from iron deficiency, which is a widespread issue in agriculture.

Chlorosis in young leaves of soybean is frequently used to diagnose deficiencies, which are typically present in sensitive crops grown in calcareous soils, which make up over 30% of the world's surface soil. Deficiencies of N, P, Fe, B and S may cause soybean yield losses up to 10 %, 29-45 %, 22-90 %, 100 % and 16-30 %, respectively, depending on soil fertility, climate and plant factors (Hellal et al., 2013). Crop failure may result from severe iron deficiency (Lindsay and Schwab, 1982; Chen and Barak, 1982). In the Indo-Gangetic Plain of India, most of the soils are alkaline and low in available Fe content (Kreye et al., 2009). The excess rainfall during Kharif season also causes a lack of oxygen around the roots, which inhibits the plant's capability to take up bioavailable Fe from the soil. Because plants usually uptake  $Fe^{+2}$  from the soil. When the soil-plant-animal-human food chain is considered, Fe deficiency does not only affect plant growth and development but also leads to anemia in animals and humans (Li et al.,2014). Fe is a component of the nitrogenase enzyme, which is used in symbiotic nitrogen fixation. As a result, the availability of this nutrient in the soil has a direct impact on the symbiotic nitrogen fixation in legumes. Immobilization of Fe in the apoplast of the mesophyll cells, partly through high apoplastic pH (Ortiz et al 2007). Soil  $HCO_3^{-1}$  levels have been found to play a role in this increase in apoplastic pH, and subsequent immobilization of Fe (Zhang et al 2014). Additionally,

plants' biological nitrogen binding and bacterial activity are significantly impacted by the iron shortage (Souri and Hatamian 2019; Souri, Naiji, and Aslani 2018).

Soil application of synthetic iron chelates is effective in counteracting iron deficiency chlorosis of plants grown on calcareous soils and is the most commonly applied technique in agriculture (Lucena et al 2006). In contrast, it was significantly correlated with the clay content and with the amounts of Fe extracted with oxalate, citrate/ascorbate, and diethylenediaminepentaacetic acid (FeDTPA) (Benitez et al., 2002). The objective of this study was to investigate the effects of soil iron chelate fertilizer on the eradication of chlorosis in soybean seedlings.

## Material and methods

To test the efficacy of chelate in preventing Fe deficiency in soybean the experiment was conducted according to a factorial experimental design with three replications under controlled conditions in the glass house during Kharif 2020 at the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

To conduct the pot experiment, bulk surface soil samples (0-15 cm) were collected from the Narainpur block of Mirzapur District of Uttar Pradesh. Soils are tested for DTPA extractable iron by Lindsay and Norvell (1978) and grouped in low (L1 to L5), medium (M1 to M5), and high (H1 to H5) iron contents. 5 soils from each category are taken and on a dry weight basis, five kilograms of soil were placed in polythene lined pots and recommended dose of NPK @ 20kg N: 80 kg P<sub>2</sub>O<sub>5</sub>: 50 kg K<sub>2</sub>O (kg ha<sup>-1</sup>). Iron @ 0, 2.5, 5 and 7.5 mg kg<sup>-1</sup> was given in solution form through Fe-DTPA as per the plan of the experiment. Eight soybean seeds were sown in the rainy season in each pot finally three seedlings were kept for further growth and yield. SPAD measurements from the leaves and plant height were taken on 30 DAS and 60 DAS to compare leaf chlorophyll content and height between treatments. The relation between SPAD-index and chlorophyll content is widely accepted and has been proven by Schenkeveld et al. (2008). After 60 days DAS, the crop was harvested for dry matter yield and chemical analysis. Treatment details are given in table 1. Chlorophyll content and plant height was tested by adopting the procedure for Factorial Completely Randomized Design (FCRD) for pot experiment as

recommended by Federer (1967). Least significance difference (LSD) at a 0.05 level of probability was used to test the significance of differences among treatment means.

## Result and Discussions

A perusal of the data from table 2 shows the effect of different doses of Fe DTPA on the eradication of chlorosis and increase in total chlorophyll content in leaves. A significant variation in leaf chlorophyll was found in 30 DAS (fig.1) in each treatment having 15 types of soil divided into 3 categories low (L1 to L5), medium (M1 to M5) and high (H1 to H5). The leaf chlorophyll content ranged from 21.98 to 29.65 SPAD in T<sub>1</sub> (Fe @ 0 mgkg<sup>-1</sup>), 24.64 to 32.02 SPAD in T<sub>2</sub> (Fe @ 2.5 mgkg<sup>-1</sup>), 31.6 to 35.11 SPAD in T<sub>3</sub> (Fe @ 5mgkg<sup>-1</sup>) and 32.21 to 38.67 in T<sub>4</sub> (Fe @ 7.5 mgkg<sup>-1</sup>) which are statistically significant at 5% level (p<0.05). The maximum increase of chlorophyll content found in T<sub>4</sub> with low iron content soil (L1 to L5) is 38.67 which is 43.16 % higher than the control (T<sub>1</sub>). This result is consistent with the study of Rodríguez et al (2010) that Fe chelates increase the SPAD values after 21 DAS of soybean. Bin et al (2016) also concluded that Fe chelates significantly increased the SPAD-index in the first 4 weeks of the pot experiment compared to the control treatment.

At 60 DAS the leaf chlorophyll content (fig.2) ranged from 24.61 to 34.71 SPAD in T<sub>1</sub> (Fe @ 0 mgkg<sup>-1</sup>), 28.34 to 36.56 SPAD in T<sub>2</sub> (Fe @ 2.5 mgkg<sup>-1</sup>), 35.21 to 42.41 SPAD in T<sub>3</sub> (Fe @ 5mgkg<sup>-1</sup>) and 36.84 to 45.63 in T<sub>4</sub> (Fe @ 7.5 mgkg<sup>-1</sup>). The maximum increase of chlorophyll content found in T<sub>4</sub> with low iron content soil (L1 to L5) is 45.63. The response of crops towards iron uptake is more in low iron content soil. Supplementation of plants with 1.8 mM Fe intensified the decrease in the concentrations of chlorophyll *b* and carotenoids and also resulted in a decrease in chlorophyll *a* (Dellias et al.,2022).

The data pertaining to the effect of soil application of Fe on plant height has been presented in table 3. A significant variation in plant height was found in 30 DAS (fig.3) in each treatment having 15 types of soil divided into 3 categories low (L1 to L5), medium (M1 to M5) and high (H1 to H5). The plant height at 30 DAS ranged from 33.22 to 40.91 cm in in T<sub>1</sub> (control Fe @ 0 mg kg<sup>-1</sup>), 35.77 to 45.43 cm in T<sub>2</sub> (Fe @ 2.5 mg kg<sup>-1</sup>), 38.96 to 49.97 cm in T<sub>3</sub> (Fe @ 5mgkg<sup>-1</sup>) and 47.31 to 52.20 in T<sub>4</sub> (Fe @ 7.5 mg kg<sup>-1</sup>) which are statistically significant at 5% level (p<0.05). The maximum increase in plant height found in T<sub>4</sub> with low iron content soil (L1 to L5) is 52.20 which is 36.60% higher than the control (T<sub>1</sub>). It was reported by Gulser et al (2019)

that the highest mean values of plant growth criteria were generally found in 15 mg kg<sup>-1</sup> iron doses of Fe-EDDHA and nano-Fe.

The plant height at 60 DAS (fig.4) ranged from 70.31 to 81.32 cm in T<sub>1</sub> (control Fe @ 0 mg kg<sup>-1</sup>), 75.12cm to 85.46 in T<sub>2</sub> (Fe @ 2.5 mg kg<sup>-1</sup>), 84.35 to 91.46cm in T<sub>3</sub> (Fe @ 5mgkg<sup>-1</sup>) and 84.21 to 102.36 in T<sub>4</sub> (Fe @ 7.5 mg kg<sup>-1</sup>). The maximum increase in plant height found in T<sub>4</sub> with low iron content soil (L1 to L5) is 52.20 which is 36.60% higher than the control (T<sub>1</sub>). It is because a particular nutrient-deficient soil gives a better response when that nutrient is supplied in adequate amounts. The decrease in plant height of the high iron content soil in T<sub>4</sub> was observed due to the toxic effect of iron. Gulser et al (2019) reported that increasing iron doses generally caused decreases in the mean values of plant growth criteria, except for root length and compound of leaf number.

## **Conclusions**

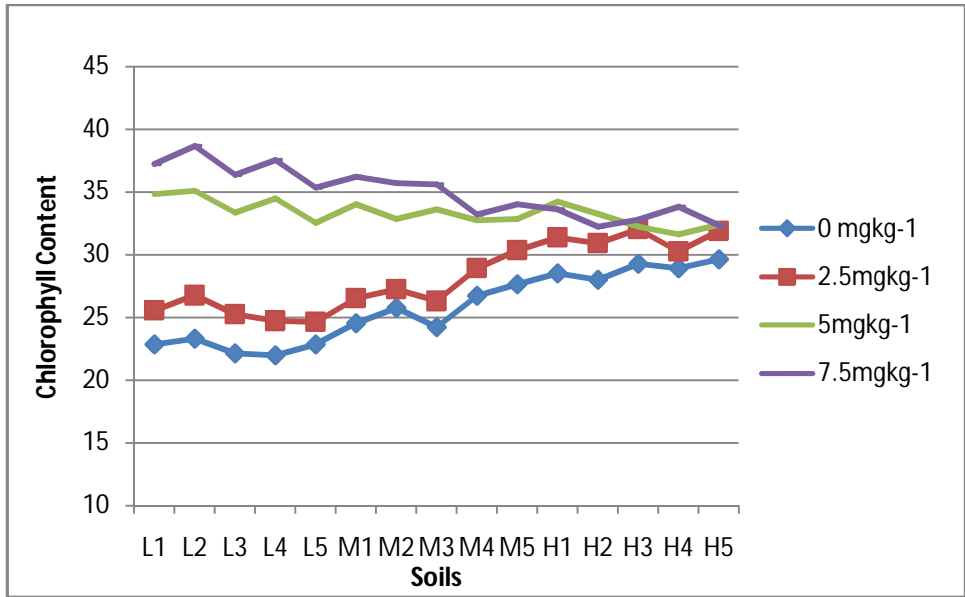
The soil application of different doses of FeDTPA soil application significantly influenced plant height and total chlorophyll content in soybean seedlings in 15 different types of soils categorized into 3 types low, medium and high. The highest plant growth height and total chlorophyll content were determined in 7.5 mg kg<sup>-1</sup> FeDTPA application in low iron content soils (L1 to L5) followed by Fe @ 5mgkg<sup>-1</sup> and then Fe @ 2.5 mg kg<sup>-1</sup> as compared to high iron content soil ( H1 to H5). A decrease in plant height and total chlorophyll content was found in high iron content soils with 7.5 mg kg<sup>-1</sup>. This may be due to less uptake and the toxic effect of high iron. As a result, it can be suggested that using FeDTPA in iron deficient soils of Indo Gangetic plain will be beneficial for soybean crop growth as well as in eradication of iron deficiency chlorosis.

## **Acknowledgments**

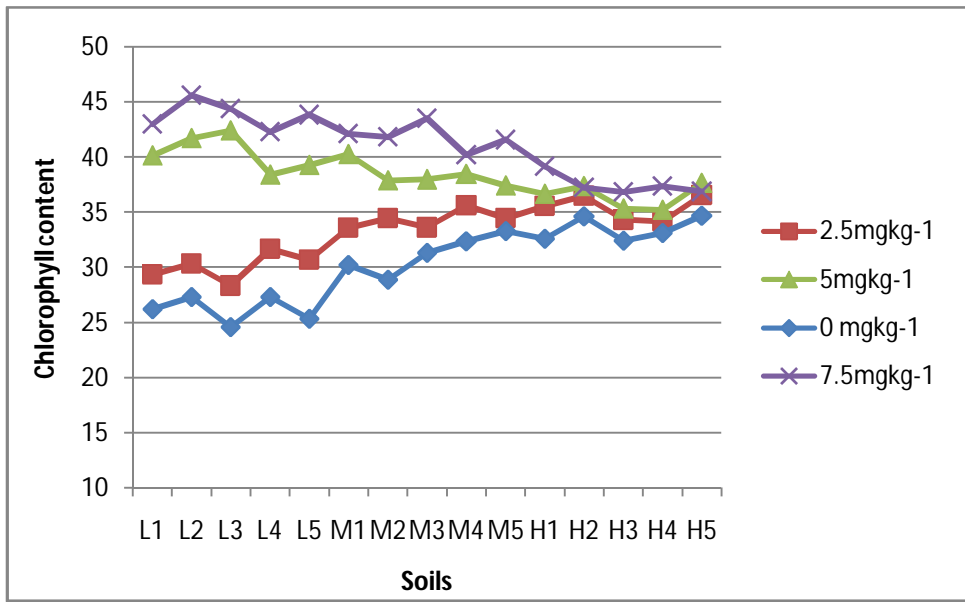
The authors would like to acknowledge the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University for providing infrastructure and research for this research study.

## **Competing Interests**

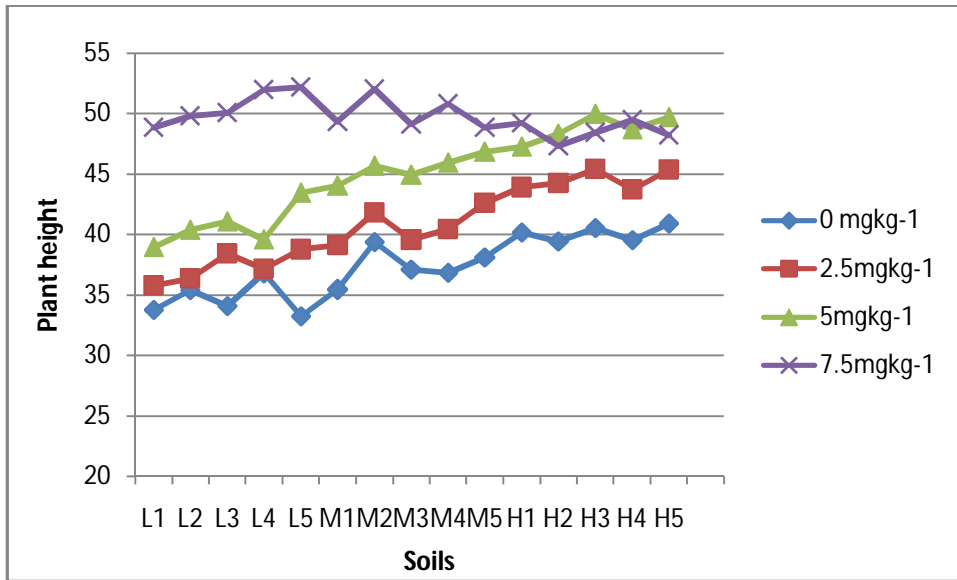
Author has declared that no competing interests exist.



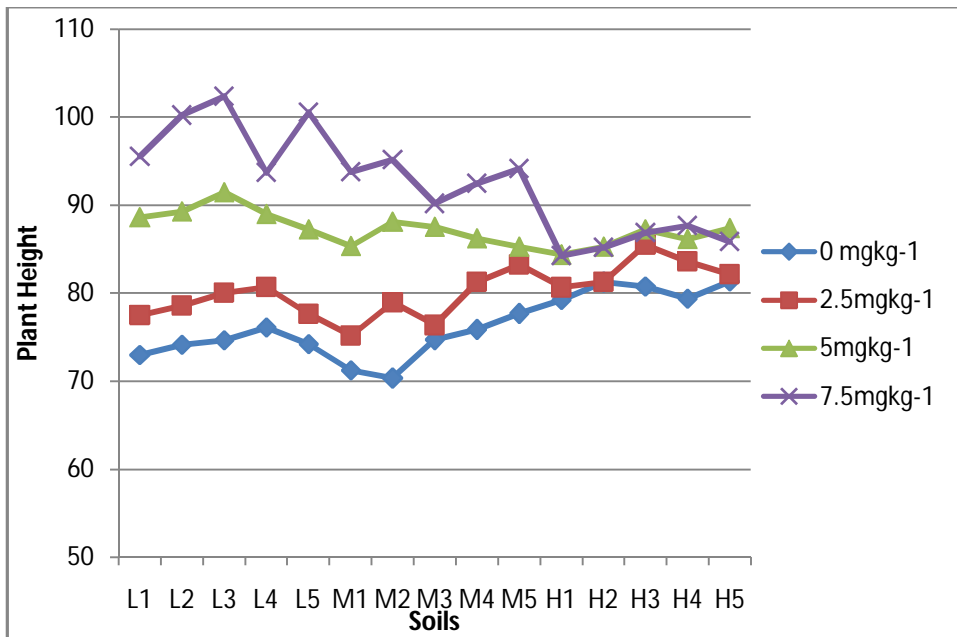
**Figure 1. Chlorophyll content SPAD 30 DAS**



**Figure 2. Chlorophyll content SPAD 60 DAS**



**Figure 3. Plant height (cm) 30 DAS**



**Figure 4. Plant height (cm) 60 DAS**



**Table 1. Treatment details**

<b>Treatments</b>	<b>Treatment details</b>
T <sub>1</sub>	Recommended dose of NPK fertilizers (RDF)
T <sub>2</sub>	RDF + 2.5 Fe mgkg <sup>-1</sup>
T <sub>3</sub>	RDF + 5 Fe mgkg <sup>-1</sup>
T <sub>4</sub>	RDF + 7.5 Fe mgkg <sup>-1</sup>

**Table 2. Effect of soil application of iron on chlorophyll content at 30 and 60 days after sowing (DAS) of soybean**

Soil No.	Chlorophyll content SPAD 30 DAS				Chlorophyll content SPAD 60 DAS				
		Iron applied mg kg <sup>-1</sup>				Iron applied mg kg <sup>-1</sup>			
		0	2.5	5	7.5	0	2.5	5	7.5
<b>1</b>	L1	22.85	25.54	34.84	37.21	26.21	29.37	40.14	43.02
<b>2</b>	L2	23.31	26.75	35.11	38.67	27.32	30.35	41.75	45.63
<b>3</b>	L3	22.14	25.24	33.33	36.35	24.61	28.34	42.41	44.38
<b>4</b>	L4	21.98	24.71	34.47	37.56	27.35	31.68	38.42	42.30
<b>5</b>	L5	22.84	24.64	32.52	35.35	25.36	30.68	39.31	43.85
<b>6</b>	M1	24.54	26.51	34.02	36.21	30.25	33.6	40.28	42.14
<b>7</b>	M2	25.75	27.25	32.84	35.68	28.87	34.49	37.88	41.82
<b>8</b>	M3	24.24	26.29	33.61	35.61	31.32	33.63	37.98	43.51
<b>9</b>	M4	26.71	28.94	32.76	33.21	32.38	35.64	38.48	40.22
<b>10</b>	M5	27.64	30.34	32.84	34.04	33.32	34.47	37.44	41.58
<b>11</b>	H1	28.51	31.38	34.21	33.62	32.63	35.59	36.69	39.18
<b>12</b>	H2	27.99	30.9	33.26	32.21	34.62	36.52	37.35	37.25
<b>13</b>	H3	29.29	32.02	32.21	32.81	32.43	34.32	35.33	36.84
<b>14</b>	H4	28.94	30.24	31.6	33.82	33.13	34.16	35.21	37.38
<b>15</b>	H5	29.65	31.89	32.39	32.31	34.71	36.56	37.65	36.88
SEm±		0.06	0.01	0.27	0.03	0.08	0.006	0.008	0.01
<b>LSD</b> (P=0.05)		0.18	0.05	0.79	0.10	0.24	0.01	0.02	0.05

**Table 3. Effect of soil application of iron on plant height (cm) at 30 and 60 days after sowing (DAS) of soybean**

Soil No.		Plant height (cm) 30 DAS				Plant height (cm) 60 DAS			
		Iron applied mg kg <sup>-1</sup>				Iron applied mg kg <sup>-1</sup>			
		0	2.5	5	7.5	0	2.5	5	7.5
<b>1</b>	L1	33.75	35.77	38.96	48.87	72.98	77.45	88.61	95.54
<b>2</b>	L2	35.42	36.38	40.40	49.82	74.12	78.56	89.26	100.21
<b>3</b>	L3	34.08	38.43	41.06	50.06	74.65	80.01	91.46	102.36
<b>4</b>	L4	36.81	37.15	39.58	51.98	76.12	80.66	88.95	93.71
<b>5</b>	L5	33.22	38.77	43.45	52.20	74.21	77.62	87.24	100.56
<b>6</b>	M1	35.44	39.10	44.02	49.33	71.23	75.12	85.31	93.74
<b>7</b>	M2	39.40	41.80	45.69	52.01	70.31	78.91	88.12	95.14
<b>8</b>	M3	37.11	39.57	44.95	49.12	74.67	76.35	87.54	90.16
<b>9</b>	M4	36.82	40.43	45.94	50.79	75.84	81.23	86.21	92.48
<b>10</b>	M5	38.09	42.59	46.84	48.85	77.65	83.21	85.26	94.13
<b>11</b>	H1	40.17	43.91	47.25	49.24	79.21	80.64	84.35	84.21
<b>12</b>	H2	39.42	44.27	48.33	47.31	81.22	81.23	85.26	85.20
<b>13</b>	H3	40.56	45.43	49.97	48.44	80.74	85.46	87.23	86.84
<b>14</b>	H4	39.52	43.74	48.71	49.51	79.36	83.61	86.15	87.67
<b>15</b>	H5	40.91	45.36	49.71	48.23	81.32	82.12	87.35	85.86
SEm±		0.01	0.03	0.09	0.02	0.18	0.11	0.18	0.33
<b>LSD</b> (P=0.05)		0.05	0.09	0.27	0.06	0.52	0.34	0.34	0.95

## References

Agarwal, D.K.; Billore, S.D.; Sharma, A.N.; Dupare, B.U.; Srivastava, S.K. Soybean: Introduction, improvement, and utilization in India—Problems and prospects. *Agric. Res.* 2013, 2, 293–300.

Benítez ML, Pedrajas VM, Del Campillo MC, Torrent J. Iron chlorosis in olive in relation to soil properties. *Nutrient Cycling in Agroecosystems.* 2002 Jan;62(1):47-52.

Bin LM, Weng L, Bugter MH. Effectiveness of FeEDDHA, FeEDDHMA, and FeHBED in preventing iron-deficiency chlorosis in soybean. *Journal of agricultural and food chemistry.* 2016 Nov 9;64(44):8273-81.

Chen Y, Barak P. Iron nutrition of plants in calcareous soils. *Advances in agronomy.* 1982 Jan 1;35:217-40.

Delias DS, Da-Silva CJ, Martins AC, de Oliveira DS, do Amarante L. Iron toxicity increases oxidative stress and impairs mineral accumulation and leaf gas exchange in soybean plants during hypoxia. *Environmental Science and Pollution Research.* 2022 Mar;29(15):22427-38.

Farhadi, N., M. K. Souri, and A. Alirezalu. 2016. Effect of sowing dates on quantity and quality of castor bean (*Ricinus communis* L.) under semi-arid condition in Iran. *Zeitschrift für Arznei- und Gewürzpflanzen* 18 (2):72–77.

Farhadi N, Souri MK, Alirezalu A, Moghaddam M. Effect of sowing dates on quantity and quality of castor bean (*Ricinus communis* L.) under semi-arid condition in Iran. *Zeitschrift für Arznei- & Gewürzpflanzen.* 2013;18(2):72-7.

Federer, W.T. *Experimental Design.* Oxford & IHB Publication Co., New Delhi, 1967.

Gülser F, Yavuz Hİ, Gökkaya TH, Sedef M. Effects of iron sources and doses on plant growth criteria in soybean seedlings. *Eurasian Journal of Soil Science.* 2019;8(4):298-303.

Hellal FA, Abdelhamid MT. Nutrient management practices for enhancing soybean (*Glycine max* L.) production. *Acta Biológica Colombiana.* 2013 Aug;18(2):239-50.

Kreye C, Bouman BA, Castaneda AR, Lampayan RM, Faronilo JE, Lactaoen AT, Fernandez L. Possible causes of yield failure in tropical aerobic rice. *Field Crops Research.* 2009 Apr 3;111(3):197-206.

Li X, Gui X, Rui Y, Ji W, Yu Z, Peng S. Bt-transgenic cotton is more sensitive to CeO<sub>2</sub> nanoparticles than its parental non-transgenic cotton. *Journal of hazardous materials.* 2014 Jun 15;274:173-80.

Lindsay WL, Schwab AP. The chemistry of iron in soils and its availability to plants. *Journal of Plant Nutrition*. 1982 Jan 1;5(4-7):821-40.

Lucena JJ. Synthetic iron chelates to correct iron deficiency in plants. *Iron nutrition in plants and rhizospheric microorganisms*. 2006:103-28..

Ortiz PR, Meza BI, Francisco R, Flores GM, Barra JD. Evaluation of different iron compounds in chlorotic Italian lemon trees (*Citrus lemon*). *Plant Physiology and Biochemistry*. 2007 May 1;45(5):330-4.

Rodríguez Lucena P, Hernández Apaolaza L, Lucena JJ. Comparison of iron chelates and complexes supplied as foliar sprays and in nutrient solution to correct iron chlorosis of soybean. *Journal of Plant Nutrition and Soil Science*. 2010 Feb;173(1):120-6.

Schenkeveld WD, Dijcker R, Reichwein AM, Temminghoff EJ, Van Riemsdijk WH. The effectiveness of soil-applied FeEDDHA treatments in preventing iron chlorosis in soybean as a function of the o, o-FeEDDHA content. *Plant and Soil*. 2008 Feb;303(1):161-76..

Souri MK, Hatamian M. Aminocheletes in plant nutrition: a review. *Journal of Plant Nutrition*. 2019 Jan 2;42(1):67-78.

Souri MK, Naiji M, Aslani M. Effect of Fe-glycine aminochelete on pod quality and iron concentrations of bean (*Phaseolus vulgaris* L.) under lime soil conditions. *Communications in Soil Science and Plant Analysis*. 2018 Jan 19;49(2):215-24.

Tripathi, A.K.; Mishra, A.K. Soybean—A consummate functional food: A review. *J. Food Sci. Technol.-Mysore* 2005, 42, 111–119.

Ye L, Li L, Wang L, Wang S, Li S, Du J, Zhang S, Shou H. MPK3/MPK6 are involved in iron deficiency-induced ethylene production in *Arabidopsis*. *Frontiers in Plant Science*. 2015 Nov 3;6:953.

Zargar SM, Agrawal GK, Rakwal R, Fukao Y. Quantitative proteomics reveals role of sugar in decreasing photosynthetic activity due to Fe deficiency. *Frontiers in Plant Science*. 2015 Aug 3;6:592.

Zhang Y, Hu CX, Tan QL, Zheng CS, Gui HP, Zeng WN, Sun XC, Zhao XH. Plant nutrition status, yield and quality of satsuma mandarin (*Citrus unshiu* Marc.) under soil application of Fe-EDDHA and combination with zinc and manganese in calcareous soil. *Scientia Horticulturae*. 2014 Jul 22;174:46-53

Zuo Y, Zhang F. Soil and crop management strategies to prevent iron deficiency in crops. *Plant and Soil*. 2011 Feb;339(1):83-95.