



## Research Article

## Synthesis of iron chelates for remediation of iron deficiency in an alkaline and calcareous soil

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**Abstract**

The present study was aimed to investigate the using iron chelates *viz.*, ferrous glycinate and ferrous citrate for the remediation of iron deficiency in alkaline and calcareous soil. The lab experiment was carried out to study the synthesis of Fe chelates by using organic and amino acid based chelating agents. The Fe chelates were synthesized based on 2:1 molar ratio of chelating agents and metal ions. The synthesized iron chelate was characterized by using Fourier transform infrared spectrophotometer (FT-IR). Finally, the synthesized amino acid and organic acid chelated iron were used to remediate the calcareous soil with black gram as a test crop. Iron content in black gram (above ground mass) tended to fluctuate at different growth stages. The highest shoot iron content of 325, 351 and 347 mg kg<sup>-1</sup> at vegetative, flowering and harvest stages were recorded with 1% ferrous glycinate as foliar spraying on 25 and 45 Day after sowing (DAS). The root iron content was also higher in 1% ferrous glycinate as foliar spraying on 25 and 45 DAS. The current investigation affirmed that the utilizing different chelating agents like the ferrous glycinate were powerful than ferrous sulfate, which may build the iron substance and iron take-up of blackgram in various development stages.

**Keywords:** Black gram, Calcareous soil, Ferrous glycinate, Iron uptake

**INTRODUCTION**

Iron deficiency is a typical issue for some plants developed in alkaline and calcareous soils, causing the indication known as iron chlorosis. Fe deficiency (FD) often occurs in alkaline soils. Fe becomes insoluble and immobile, leading to lesser root uptake from soil due to high soil pH; an excessive amount of calcium carbonate, nitrate, and heavy metals; poor aeration; unbalanced cation ratios; and temperature changes (Kobayashi *et al.*, 2014). The use of iron chelates solves the problem of iron deficiency in alkaline and calcareous soils. Iron is a catalyst in the production of

chlorophyll and is involved in several plant enzyme systems. The uptake form of iron is Fe<sup>++</sup> cation. Iron deficiency symptoms show interveinal chlorosis, i.e. yellow to white leaf color in which veins remain green. The appearance of deficiency symptom on younger leaves develops, which remain nearer to the plant top (Schulte and Kelling, 2004).

Natural amino acids are very small molecules that chelate with micronutrients are quickly absorbed, translocated and metabolized by plants. The merits of amino acid chelates are that the amino acid ligand frame and defend the micronutrients from adverse relations usually take place in soil solution, in the presence of soil or

on leaf surfaces (Schaffer *et al.*, 2011). Various examinations have demonstrated that the likely limit of iron compounds to address iron lacks in plants developed in alkaline and calcareous soils relies upon two fundamental features (Chaney and Bell, 1987) viz. (i) the limit of iron compounds to keep up solvent iron in soil arrangement and (ii) the limit of plant roots to absorb the iron from the iron compounds present in soil solution.

Definitely the two components are validly identified with the solvency and steadiness of the iron compounds in soil solution. When the plant ability to acclimatize iron from the iron compound has been shown under hydroponic conditions, a potential method to assess the possible viability of the iron compound under soil conditions is study the variety in grouping of the total iron and the iron compound in soil solution after some time (Josemaría *et al.*, 2003). In spite of the fact that foliar application is by all accounts compelling in taking care of issues of micronutrients, notwithstanding, leaf fertilizer with an inorganic mineral structure scarcely diffuses from leaf surface into the plant due to high weight molecular structure (El-Seginy *et al.*, 2003).

Amino acids are tolerably hard chelating agents. When they enter inside the plant, the mineral is discharged and the plant utilizes leftover amino acids that shaped the defensive shell as a wellspring of water-solvent nitrogen. Amino acids are building blocks in cell apparatus. Everything is utilized and nothing is lost. Then again, EDTA is a synthetic particle, and plants do not normally utilize EDTA. Amino acid chelates are commonly fundamental in the plant, meaning they move and travel to where they are required. They can do this since the plant perceives amino acids as building squares and are utilized in almost every tissue in the plant. Amino acid chelates are accessible as fluids or powders and, by and large accessible for use in natural food creation. Glycine chelates (otherwise called glycinate) are a subset of amino acid chelates. It is the minutest amino acid and it is frequently utilized as a chelating agent. Since glycine is little, it makes a little last item that goes through leaf stomata more effectively than other bigger molecules, in this way upgrading plant take-up. At the point when glycine is isolated from the mineral in the plant, the plant utilizes glycine. As amino acid chelates effectively enter the plant, they are amazingly valuable for rectifying supplement inadequacies rapidly. The amino acid chelates do not cause a burning impact in plants; then again, EDTA - metal chelates are phytotoxic or show consumption of plant tissues when appropriate consideration is not taken (Datir *et al.*, 2010).

Foliar application is credited with the advantage of quick and efficient utilization of nutrients, elimination of losses through leaching and fixation, besides helping in regulating the uptake of nutrient by plants (Manonmani

and Srimathi, 2009). Foliar application of nutrients using water-soluble fertilizer is one of the possible ways to enhance the productivity of pulses like green gram and black gram. Hence the present study was proposed to develop iron chelates to increase the iron use efficiency and to evaluate its effect on crop yield in calcareous soil.

## MATERIALS AND METHODS

### Laboratory experiment

The laboratory experiment was carried out to study the synthesis of Fe chelates by using organic and amino acid based chelating agents. The synthesized iron chelate was characterized.

### Synthesis of iron chelates

1,000 grams of water was boiled for 30 minutes to remove dissolved air. 170 grams of ferrous sulfate monohydrate was dissolved in 500 ml of the deaerated water and the solution was maintained at 80° C and 30 grams of citric acid was mixed to it (Fig. 1). Separately 150 grams of glycine was dissolved in 500ml of deaerated water and the acid solution was added to the ferrous sulfate solution with stirring. The temperature of the mixture was maintained at about 80° C. The mixture was filtered to remove any undissolved materials. The metal amino acid citrate was dried at about less than about 110°C and the dry material was ground to a fine powder. (Hsu, 1995)

### Chelate analysis

The analysis was carried out in Fourier transform infrared spectrophotometer (FT-IR) 6800 (Jasco, Japan) equipped with ATR PRO ONE accessory and TGS detector. Registration was carried out in the region 400 – 4000  $\text{cm}^{-1}$  (resolution 4  $\text{cm}^{-1}$  with a number of scans 40). The report was then processed using origin © 8.0 software and interpreted.

### Schrodinger maestro suite

The software used for the research work is Schrodinger maestro suite v. 2015-16. It is a collection of tools and interfaces that are designed to assist calculations which are significant to biological molecules. The maestro interface organizes access to the interactive tools for use in biological projects. Maestro helps in the basic structure manipulation, demonstration and organization characteristics of the interface and is an integrated interface for all the Schrodinger software.

### Characterization of structure

The molecular models of ferrous glycinate and ferrous citrate were generated initially using ACD chemsketch. The 3D optimization was performed and the 3D atomic coordinates were collected. The modules were further

visualized and analysed using Schrodinger's maestro interface.

### Pot culture experiment

The pot culture experiment was conducted on black gram (*Vigna mungo* L) at Tamil Nadu Agricultural University, Coimbatore to find out the effect of amino acid and organic acid chelated iron on growth and productivity of black gram in iron-deficient calcareous soil with nine treatments involving T<sub>1</sub> - NPK control, T<sub>2</sub> - FeSO<sub>4</sub> 25 kg ha<sup>-1</sup> as a basal soil application, T<sub>3</sub> - Ferrous glycinate chelate @ 5 kg ha<sup>-1</sup>, T<sub>4</sub> - Ferrous citrate chelate @ 5 kg ha<sup>-1</sup>, T<sub>5</sub> - Fe - EDTA chelate @ 5 kg ha<sup>-1</sup>, T<sub>6</sub> - 1% FeSO<sub>4</sub> as foliar spraying on 25 & 45 DAS, T<sub>7</sub> - 1% Ferrous glycinate as foliar spray on 25 & 45 DAS, T<sub>8</sub> - 1% Ferrous citrate as foliar spraying on 25 and 45 DAS, and T<sub>9</sub> - 1% Fe - EDTA as foliar spray on 25 and 45 DAS was planned in potted plants with three replicates. The plant analysis of iron content was carried out with the help of atomic absorption spectrophotometer.

### Statistical analysis

The data obtained from the experiments was analysed statistically to find out the effects of various treatments and their interactions. Data recorded from three replications were subjected to single way analysis of variance (ANOVA), and critical differences were calculated at p= 0.05 level.

## RESULTS AND DISCUSSION

### FTIR spectrum characteristics of ferrous glycinate

FTIR analysis shows unbound glycine from chelated one (Fig. 2). Free glycine exhibited a vibration peak at 2920 cm<sup>-1</sup> that disappeared upon chelate formation. The peak at 2920 cm<sup>-1</sup> was due to the twisting and vibration of NH<sub>2</sub> groups. The disappearance of this peak indicates that a new coordinate bond was formed through the terminal amine groups. The peak at 3153.04 cm<sup>-1</sup> and 1327.75 cm<sup>-1</sup> was due to the weak stretching of symmetric and asymmetric amine vibration. As compared to the position of bands in the spectrum of iron glycinate, it confirms the chelation of amino acid with Fe<sup>2+</sup> ions. Similar results were reported by Ahamed *et al.* (2019) in infrared spectroscopy of pure glycine revealed several peaks which ranged from 2500 cm<sup>-1</sup> to 3200 cm<sup>-1</sup> where 2520.51 cm<sup>-1</sup>, 2603.43 cm<sup>-1</sup>, 2703.71 cm<sup>-1</sup>, 3001.66 cm<sup>-1</sup> and 3149.17 cm<sup>-1</sup> (OH groups) observed sharp peaks. A peak at 1056.8 cm<sup>-1</sup> characteristics of sulphate was observed in zinc sulphate heptahydrate. The FT-IR spectral investigations of synthesized chelate indicated peaks at 1065.48 cm<sup>-1</sup> (SO<sub>4</sub><sup>2-</sup>), 1393.33 cm<sup>-1</sup> (COO<sup>-</sup>) and 3169.44 cm<sup>-1</sup> (OH). Broadband from 2700 cm<sup>-1</sup> to 3300 cm<sup>-1</sup> with a

centroid at 3169.44 cm<sup>-1</sup> (NH<sub>2</sub> broad peak) was also observed.

### FTIR Spectrum characteristics of ferrous citrate

FTIR spectrum of citric acid at wavelength 3317.93 cm<sup>-1</sup>, 3219.88 cm<sup>-1</sup> and 3014.19 cm<sup>-1</sup> was due to broad stretching of carboxyl groups (COO<sup>-</sup>). The peaks at 2668.03 cm<sup>-1</sup>, 2551.36 cm<sup>-1</sup> and 1950.64 cm<sup>-1</sup> was assigned to weak stretching of OH groups and aromatic over ton vibrations. The peak in FTIR at 1718.26 cm<sup>-1</sup> and 1205.20 cm<sup>-1</sup> was assigned to the very sharp stretching of C=O group and C-O group (Fig. 3). Spectrum of Fe<sup>2+</sup> - citrate was compared with citric acid, shown that the spectrum of Fe<sup>2+</sup> citrate contains only one weak broadband of high intensity in the range from 3000 cm<sup>-1</sup> to 3500 cm<sup>-1</sup> with centroid at 3281.29 cm<sup>-1</sup>, which could have been attributed to valance vibrations of OH stretching. The wavelength of 1742.37 cm<sup>-1</sup> and 1695.12 cm<sup>-1</sup> was assigned to sharp and strong C=O bending vibrations. As compared to positions of these bonds in the spectrum of citric acid, it confirms the presence of C=O groups in the structure of Fe<sup>2+</sup> - citrate. Two bonds originate from valance vibrations of carboxyl anion (COO<sup>-</sup>), which interacts with iron ion. Compared to positions in the spectrum of Fe<sup>2+</sup> - citrate, these bonds are exhibited in the citric acid spectra, indicating coordination of carboxyl group and Fe<sup>2+</sup> ion. Similar results were reported by Ahamed *et al.* (2019), zinc sulphate heptahydrate, the peak corresponding to the sulphate bond at 1056.8 cm<sup>-1</sup> was obtained. However, in the new chelate *viz.*, of zinc citrate sulphate, FT-IR analysis pointed out sharp peaks at 1641.13 cm<sup>-1</sup>, 3742.19 cm<sup>-1</sup>, 3828.97 cm<sup>-1</sup> and 3844.4 cm<sup>-1</sup>. A weak broadband with a width of 2700 cm<sup>-1</sup> to 3400 cm<sup>-1</sup> with a centroid at 3281.29 cm<sup>-1</sup> (OH group) was noticed.

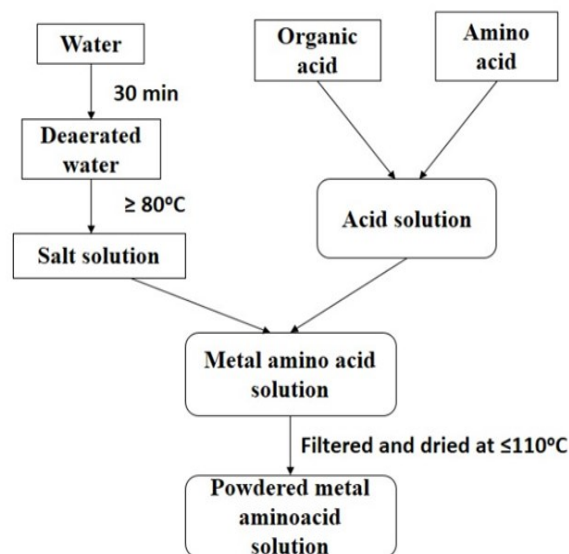


Fig. 1. Synthesis of iron chelate.

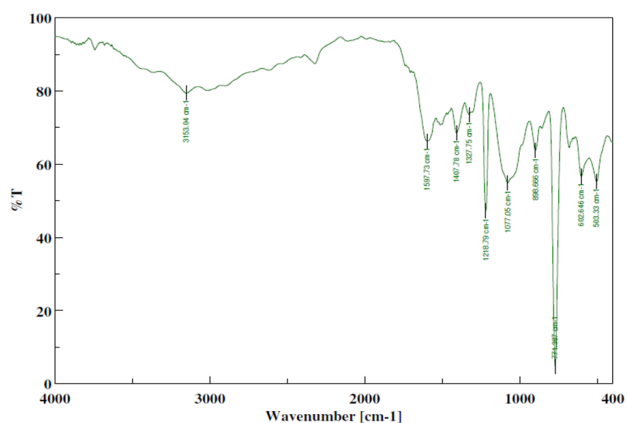


Fig. 2. FTIR Spectrum of ferrous glycinate.

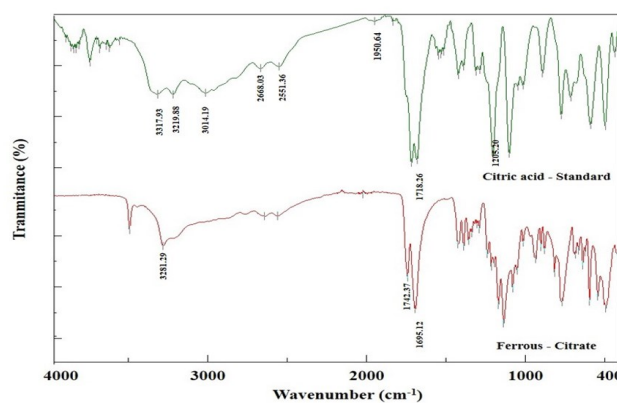


Fig. 3. FTIR spectrum of Fe - Citrate.

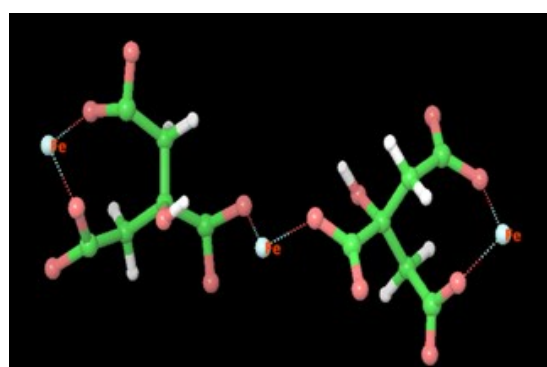
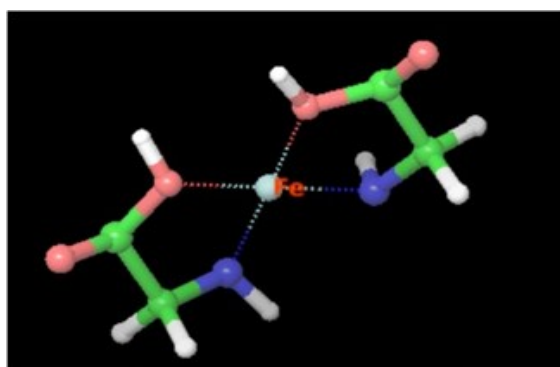


Fig. 4 &amp; 5. Structure of ferrous glycinate and ferrous citrate.

### Characterization of iron chelates

The coordination geometry of chelated ferrous glycinate and ferrous citrate have been presented in Fig. 4 and Fig. 5. The predicted energy value of the chelated molecule in  $38.47 \text{ k. cal mol}^{-1}$  indicated that the molecule was relatively stable. The ferrous glycinate structure, the carboxyl group and  $\alpha$ - amino group of glycine both donate electron pairs into the ferrous iron cation forming a coordinate covalent bond.

Dry matter production of root and shoot

An increase in DMP with the advancement of crop growth and the increase between flowering and maturity were also marked. The increase in DMP at flowering might be mainly related to the increase in branches, number and size of leaves and higher photosynthesis due to an increase in leaf area. The highest DMP at vegetative, flowering and harvest stages recovered by treatment  $T_7$ , namely foliar spraying of 1% ferrous glycinate at 25 and 45 DAS (Table 1) which is on par with soil application ferrous glycinate ( $T_3$ ) at  $5 \text{ kg ha}^{-1}$ . Kumar *et al.* (2015), also observed that sources and mode of iron application also had a significant effect on dry matter production. The lowest dry matter accumulation was recorded with the control plot, which was significantly lower than 2 and 3 foliar sprays of 2.0% iron sulphate and 0.5% iron chelate at all the stages. The better performance of foliar spraying of 1% ferrous glycinate ( $T_7$ ) at 25 and 45 DAS treatment on DMP in the present

study could be due to the fact that the plants absorb and transport iron efficiently in its glycinate form, i.e., glycine helps maintain the iron in its soluble form within the plants. In the present study, the treatment  $T_7$  viz., foliar spraying of Fe chelate at 25 and 45 DAS (15.2% present in iron) which might be attributed to the 15.2% of iron supply by the ferrous glycinate. Further, the iron availability and translocation to crop in iron-deficient soil under the treatment  $T_7$  could be the reason for enhanced DMP observed in the present study.

Soil application of  $\text{FeSO}_4$  at  $25 \text{ kg ha}^{-1}$  as inorganic salt did not perform well in increasing the DMP in comparison with  $T_7$ . According to Pal *et al.* (2008), the effectiveness of Fe supplements through inorganic source may be attributed to its quick conversion from  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  under field condition, which is a highly stable and insoluble form. These findings corroborate our results since experimental soils were calcareous in nature.

### Iron content (Shoot and root)

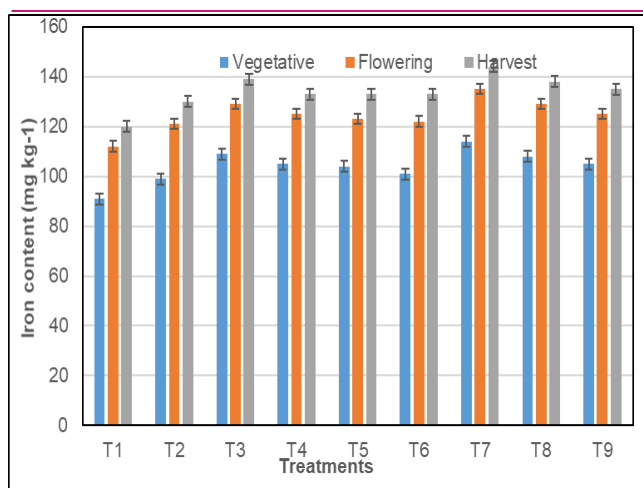
The increase in plant growth was mainly due to a gradual increase in iron content in the shoot. (Table 2). Iron content in black gram (above ground mass) tended to fluctuate at different growth stages. The highest shoot iron content of 325, 351 and  $347 \text{ mg kg}^{-1}$  at vegetative, flowering and harvest stages respectively were recorded with 1% ferrous glycinate as foliar spraying on 25 and 45 DAS, which were on par with foliar spraying of

**Table 1.** Effect of iron chelates on dry matter production at different stages of black gram ( $\text{g ha}^{-1}$ ).

Treatments	Stages									
	Shoot (Above ground mass)					Root				
	Vegetative	Flowering	Harvest	Mean	% increase over control	Vegetative	Flowering	Harvest	Mean	% increase over control
T <sub>1</sub> - NPK control	320	359	391	357	-	105	119	135	120	-
T <sub>2</sub> - FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup> as basal soil application	325	365	397	362	1.59	115	125	145	128	7.24
T <sub>3</sub> - Ferrous glycinate chelate @ 5 kg ha <sup>-1</sup>	346	376	409	377	5.70	126	138	155	140	16.7
T <sub>4</sub> - Ferrous citrate chelate @ 5 kg ha <sup>-1</sup>	336	369	395	367	2.80	117	128	141	129	7.52
T <sub>5</sub> - Fe - EDTA chelate @ 5 kg ha <sup>-1</sup>	339	365	401	368	3.27	119	127	139	128	7.24
T <sub>6</sub> - 1% FeSO <sub>4</sub> as foliar spraying on 25 & 45 DAS	329	362	403	365	2.24	110	119	146	125	4.46
T <sub>7</sub> - 1% Ferrous glycinate as foliar spraying on 25 & 45 DAS	351	385	415	384	7.57	132	145	157	145	20.9
T <sub>8</sub> - 1% Ferrous citrate as foliar spraying on 25 & 45 DAS	340	373	405	373	4.49	124	135	151	137	14.2
T <sub>9</sub> - 1% Fe - EDTA as foliar spraying on 25 & 45 DAS	338	368	400	369	3.36	121	133	149	134	12.3
Sed	8.42	8.06	9.13			2.65	3.75	2.35		
CD (P=0.05)	17.6	16.9	19.1			5.57	7.88	4.94		

**Table 2.** Effect of iron chelates on iron content at different stages of black gram ( $\text{mg kg}^{-1}$ ).

Treatments	Stages									
	Shoot (Above ground mass)					Root				
	Vegetative	Flowering	Harvest	Mean	% increase over control	Vegetative	Flowering	Harvest	Mean	% increase over control
T <sub>1</sub> - NPK control	283	311	308	301	-	128	141	136	135	-
T <sub>2</sub> - FeSO <sub>4</sub> @ 25 kg ha <sup>-1</sup> as basal soil application	305	332	327	321	6.87	131	146	141	139	3.21
T <sub>3</sub> - Ferrous glycinate chelate @ 5 kg ha <sup>-1</sup>	315	343	339	332	10.5	138	151	146	145	7.16
T <sub>4</sub> - Ferrous citrate chelate @ 5 kg ha <sup>-1</sup>	311	340	336	329	9.42	134	148	142	141	4.69
T <sub>5</sub> - Fe - EDTA chelate @ 5 kg ha <sup>-1</sup>	307	338	331	325	8.20	135	147	144	142	5.19
T <sub>6</sub> - 1% FeSO <sub>4</sub> as foliar spraying on 25 & 45 DAS	306	336	329	324	7.65	137	149	145	144	6.67
T <sub>7</sub> - 1% Ferrous glycinate as foliar spraying on 25 & 45 DAS	325	351	347	341	13.4	145	159	153	152	12.8
T <sub>8</sub> - 1% Ferrous citrate as foliar spraying on 25 & 45 DAS	318	346	341	335	11.4	136	145	139	140	3.70
T <sub>9</sub> - 1% Fe - EDTA as foliar spraying on 25 & 45 DAS	312	341	338	330	9.87	130	143	138	137	1.48
Sed	6.28	6.33	7.81			2.46	3.30	3.36		
CD (P=0.05)	13.2	13.3	16.4			5.17	6.94	7.06		



**Fig. 6.** Effect of iron chelates on iron content at different stages of black gram ( $\text{mg kg}^{-1}$ ).

1% ferrous citrate ( $T_8$ ) of 318, 346 and 341  $\text{mg kg}^{-1}$  at vegetative, flowering and harvest stages respectively (Fig. 6). Foliar application of ferrous glycinate in black gram expressed a higher amount of Fe in their shoots than other treatments. Our study indicated that using ferrous glycinate chelate in the foliar application could supply sufficient Fe for plant uptake and improve the shoot and root growth of black gram. Mohammadipour *et al.* (2013) also observed that highest of plant Fe value (454.5 ppm) was obtained from the  $\text{FeSO}_4$  and Fe-EDTA with 91.17 ppm caused to the lowest Fe value. Fe value in  $\text{FeSO}_4$ , EDDHA, iron nano fertilizer treatments was more than the optimum range (50-300 ppm) for *Spathiphyllum* plant.

Amino acids induce biosynthesis of chlorophyll and thereby improve the photosynthesis rate (Amin *et al.*, 2011; Zeid 2009). The highest root Fe content of 145, 159 and 153  $\text{mg kg}^{-1}$  at vegetative, flowering and harvest stages respectively was recorded in the treatments that received 1% ferrous glycinate as foliar spraying on 25 and 45 DAS which followed by soil ap-

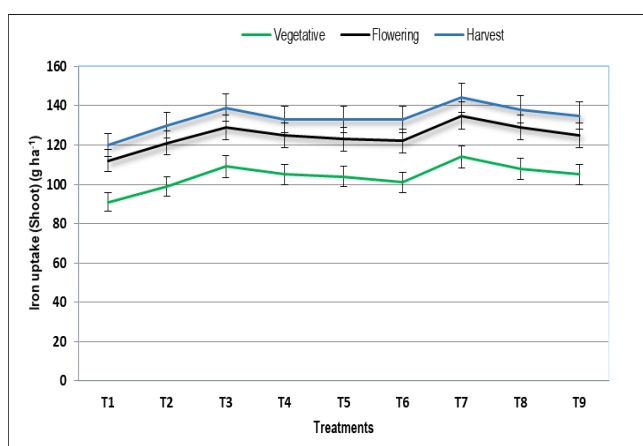
plication of ferrous glycinate at  $5\text{kg ha}^{-1}$  ( $T_3$ ) of 138, 151 and 146  $\text{mg ha}^{-1}$ . Although amino acids used in the present study stimulated plant growth, it is not easy to dissect whether the effect is due to better Fe uptake, more nitrogen supplied in the form of amino acids, or the hormonal effect of amino acids (Ghasemi *et al.*, 2012).

### Iron uptake (Shoot and root)

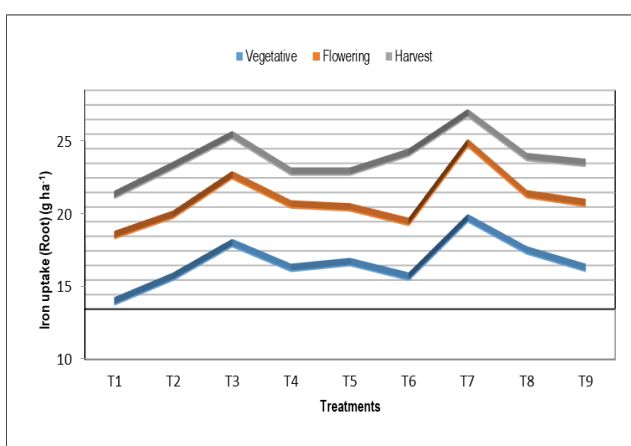
The results showed an increase in the iron uptake by shoot and root at different stages and maximum Fe uptake was recorded at harvesting stages (Fig.7). The different treatments significantly influenced the Fe uptake at different stages and a similar trend was noticed in the case of DMP. Increased DMP coupled with higher iron concentration resulted in higher uptake of Fe in the present investigation. Foliar spraying of 1% ferrous glycinate @ 25 & 45 DAS recorded the maximum iron uptake by both shoot and root at all the growth stages. Zimbovskaya *et al.* (2020) also reported that 70–75% higher iron content in washed and dried wheat shoots compared to a coordination complex of ferric ions and ethylene diamine tetraacetic acid (Fe-EDTA). The higher uptake of iron from the ferrihydrite (FeH) stabilized with HS was related to the enhanced wettability of the wheat leaves.

### Conclusion

Among the organic and amino acids, iron chelates tested in the present investigation, the foliar spraying of iron chelates was superior in increasing the iron content and iron take-up of black gram under pot situations because of its mainly higher iron uptake. Amino acids prompt the biosynthesis of chlorophyll and in this manner, improve the photosynthesis rate. Although amino acids used in the present study stimulated plant growth, it was not easy to dissect whether the effect was due to better Fe uptake, more nitrogen supplied in the form of



a.) Shoot



b.) Root

**Fig. 7.** Effect of iron chelates on iron uptake at different stages of blackgram ( $\text{g ha}^{-1}$ ).

amino acids or the hormonal effect of amino acids. So, amino acid chelated iron was more effectively explaining the iron inadequacy in calcareous and alkaline soil.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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