International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(4): 1580-1585

Received: 20-05-2020
Accepted: 24-06-2020

S Murali
Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

D Jawahar
Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

T Chitdeshwari
Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Jagadesh
Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
S Murali
Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Synthesis of organic iron chelates and evaluation of their efficiency in improving the growth and yield of Blackgram

S Murali, D Jawahar, T Chitdeshwari and M Jagadesh

DOI: https://doi.org/10.22271/chemi.2020.v8.i4o.9838

Abstract
Supplying a sufficient amount of available iron (Fe) for plant growth in pot culture is a great challenge. The chelators commonly used to supply Fe in pot culture have several advantages and positively affect plant growth. In this research study we have synthesized certain Fe – amino acid and Fe – organic acid chelates, including Fe – glycinate, Fe – citrate and Fe – EDTA and evaluated their efficacy as a Fe source for blackgram grown in pot culture. FTIR analysis shows unbound glycine from chelated one. Free glycine exhibit a vibration peak at 2920 cm^{-1} that disappear upon chelate formation. The peak at 2920 cm^{-1} due to twisting vibrating of NH_2 groups. Disappearing of this peak indicates that new coordinate bond is formed through the terminal amine groups. The peak at 3153.04 cm^{-1} and 1327.75 cm^{-1} is due to weak stretching of symmetric and asymmetric amine vibration. As compared to the position of bands in the spectrum of iron glycinate confirms the chelation of said amino acid with Fe^{2+} ions. The pot experiment was conducted to study the effect of foliar and soil application of amino acid and organic acid chelated iron on growth of blackgram. The results showed that the foliar spraying of 1% ferrous glycinate resulted in maximum Fe, Zn content, leaf active iron, Catalase and Peroxidase activity. Higher activity of Catalase and Peroxidase confirmed the improvement in plant Fe status. Between the soil and foliar application foliar spraying is better in improving the plant micronutrient content which was evidenced from higher enzyme activities.

Keywords: Ferrous glycinate, leaf active iron, FTIR, catalase, peroxidase, micronutrients

Introduction
Iron is one of the most important micronutrient necessary to life and growth of plants because it helps in the formation chlorophyll (Curie *et al.*, 2008) [5] and enzymes (Rivero *et al.*, 2005) [14]. Iron is a constituent of many enzymes like, cytochrome oxidase, catalase, peroxidase, acotinase, and nitrogenase. The deficiency of iron causes chlorosis leaves and exhibit in the young leaves of plants. Susceptibility to iron deficiency is varied with varieties of same crops. Crops such as sorghum, field beans and soybeans can show severe iron chlorosis, where corn or alfalfa may appear normal. Some new varieties of soyabean can use iron efficiently than other, therefore selecting the appropriate crop and variety is important (Schulte and Kelling, 2004) [17]. Decomposition of organic matter releases some chelating agents like organic acids, amino acids, ligninsulfonates, ligninpolycarboxylates, sugar acids, derivatives, phenols, poly flavonoids, siderophores and phyto siderophores. Both classes of chelating/complexing agents increase micronutrient solubility (Sekhon, 2003) [18]. Chelating agents like citric acid and gluconic acid have the capacity to form water soluble chelates due to the presence of carboxylic and hydroxyl group combined with metal ions over coordinated covalent bonding. The hydroxyl groups are unattached complex with the metal ions under slightly acidic conditions (Clemens *et al.*, 1990) [4]. Calcium salts in soils have been found to increase the sorption of chelates (Wallace and Lunt, 1956) [21]. With increased concentrations of Ca-salts, there is a suppression of electrical double layer around the negatively charged surfaces, which permit anionic species to adsorb to sorption sites (Bolt and Warkentin, 1958). Carboxylate is one of the constituent in the root exudates which act as a chelating agent to alleviate nutrient deficiencies (Marschner and Römheld, 1994) [10].

Results from foliar applications may be only temporary and actually depress the plant's Fe stress mechanisms by preventing the increase in Fe-reducing capacity of the roots that would normally occur during Fe deficiency (Römheld and Marschner, 1986) [15].In solutions where Ca is the only competing cation, HEDTA was found to be a more efficient chelate than EDTA. Godsey *et al.* (2003) found that foliar-applied chelated Fe-HEDTA was not effective in increasing grain yield, indicating that another Fe source should be considered. However, seed-applied chelated Fe fertilizer (HEDTA) increased grain yield by approximately 55 percent for both tolerant and non-tolerant soybean varieties (Liesch *et al.*, 2011) [9].

Amino acids are chelated with micronutrients responsible extreme bioavailability, acceptability and protection. These amino acid chelated micronutrients are exactly used for foliar spraying and soil application. Schaffer *et al.* (2011) [16] examined that the natural amino acids are very small molecules they chelated with micronutrients therefore they 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 those take place in soil solution, in the presence of soil or on leaf surfaces. Sekhon (2003) [18] reported that the amino acid chelates are neutral charge and surface of the leaves negatively charged. They are neither attracted to nor repulsed from leaf surface and liberally permit through this barrier. So, amino acid chelate is taken into the cell very quickly and efficiently. Keeping this in view the present study was carried out to prepare amino acid Fe chelates and evaluated their efficacy on blackgram growth and yield.

Material and Methods

Synthesis of iron chelates

1,000 grams of water was boiled for 30 minutes to remove dissolved air and 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. Separately 150 grams of glycine was dissolved in 500 ml of deaerated water and the acid solution was added to the ferrous sulfate solution with stirring. The temperature of the mixture was maintained at 80° C and the mixture was filtered to remove any undissolved materials. The metal amino acid citrate was dried at 110°C and the dry material was ground to a fine powder (Hsu, 1996) [7].

Chelate analysis

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

Pot experiment

The pot experiment was conducted at Tamil Nadu Agricultural University, Coimbatore to find out the effect of amino acid and organic acid chelated iron on growth and productivity of blackgram. The seeds of blackgram were obtained from Department of Pulses, TNAU, Coimbatore and soils were collected from farmer's field of Thondamuthur, Coimbatore. Seeds were sown in the pots at three seeds $pots^{-1}$ with nine treatments involving T_1 - NPK control, T_2 – $FeSO_4$ 25 kg ha^{-1} as basal soil application, T_3 - Ferrous glycinate chelate @ 5 kg ha^{-1}, T_4 - Ferrous citrate chelate @ 5 kg ha^{-1}, T_5 - Fe – EDTA chelate @ 5 kg ha^{-1}, T_6 - 1% $FeSO_4$ as foliar spraying on 25 & 45 DAS, T_7 - 1% Ferrous glycinate as foliar spray on 25 & 45 DAS, T_8 - 1% Ferrous citrate as foliar spraying on 25 and 45 DAS, and T_9 – 1% Fe – EDTA as foliar spray on 25 and 45 DAS. Five randomly selected plants from net plot area were used to record the dry matter production at different growth stages. The sampled plants were partitioned into leaves, stem and pods and were dried at 65°C till they attained constant dry weight. Dry weight of stem, leaves, pods and total dry matter production per plant were recorded at all the growth crop stages and expressed as g per plant.

Elemental analysis

Plant samples were collected at three stages *viz*., vegetative, flowering and harvest stages, shade dried and then kept in a hot air oven at 65°C and ground in a Willey Mill. The processed plant samples were analyzed for total nutrient content of Fe, Cu, Mn and Zn by adopting standard procedure (Hessey, 1971) [6]. Nutrient uptake computed by multiplying the nutrient content of the plant with dry matter production and expressed on oven dry weight basis.

Active iron content

Two grams of chopped plant sample was weighed and transferred to 100ml glass bottles. Twenty ml of o-phenanthroline solution was added and the content of the bottles were stirred gently in order to embathe the plant sample with the extractant. The bottles were stoppered and allowed to stand for 16 hrs at room temperature. The contents were filtered through Whatman No. 1 filter paper. The active iron was estimated directly in the filtrate by measuring the transmittance at 510 nm in spectrophotometer (Katyal and Sharma, 1980) [8].

Estimation of catalase activity

Catalase activity was determined by following titration method using potassium permanganate (NC, 1963) and expressed as µg H_2O_2 g^{-1} min^{-1}. 250 mg of leaf sample was macerated with 10 ml of sodium phosphate buffer (0.2 M, pH 6.8) and the content was centrifuged at 10,000 rpm at 4°C for 20 minutes. The supernatant solution of 1 ml was taken in four beakers, 5 ml of 1.5 per cent sodium perborate, 1.5 ml of sodium phosphate buffer (0.2 M, pH 6.8) and 10 ml of sulphuric acid was added in a first four beakers at the time interval of 1, 2, 3, 4 minutes respectively. In 5th beaker, 10 ml of sulphuric acid was added first, then 5 ml of sodium perborate (1.5%), 1.5 ml of sodium phosphate buffer (0.2 M, pH 6.8) and 1 ml of enzyme extract was taken and kept as blank. The solution was titrated against with 0.05 per cent potassium permanganate and the development of pink colour, which persists for 30 seconds, is the end point.

Estimation of peroxidase activity

Peroxidase activity (change in absorbance value at 430 nm g^{-1} min^{-1}) was determined according to Perur (1962) [13] and Angelini *et al.* (1990) [1]. 100 mg of leaf sample was extracted using 0.1M phosphate buffer (pH 7.0) and a known volume of the extract was added to a cuvette containing 3 ml phosphate buffer and 3 ml pyrogallol and increase in absorbance at 430 nm was recorded. The change in absorbance in minutes was used to calculate the enzyme activity.

Statistical analysis

The data obtained from different experiments was analysed statistically to find out the effects of various treatments and

their interactions. Analysis of variance was calculated as suggested by Panse and Sukhatme (1985) [12]. Simple correlation and regression co-efficient were also worked out between certain inter-related parameters to observe their degree of dependence as suggested by Snedecor and Cochran (1967) [20].

Results

Characteristics of iron chelates

The iron amino acid and iron organic acid chelates were synthesized by reacting of $FeSO_4$ and glycine, citric acid and EDTA in 1:2 mole ratio and the reaction yield was more than 80%. The amino acid ligand generally act as a bidenate (N, O) chelates with respect to pH. The FTIR spectra of the complexes show an absorption pattern in 4000 – 400 cm^{-1} region, similar to amino acid ligands. FTIR analysis shows unbound glycine from chelated one. Free glycine exhibit a vibration peak at 2920 cm^{-1} that disappear upon chelate formation (Fig 1).

Dry matter weight

The root and shoot dry matter production of black gram was influenced by the foliar spraying of 1% ferrous glycinate on 25 and 45 DAS and registered the highest mean root DMP of 132, 145, and 157 kg ha^{-1} at vegetative, flowering and harvest stages respectively and highest mean shoot DMP (T_7) of 351, 385 and 415 kg ha^{-1} at vegetative, flowering and harvest stages respectively, which was on par with soil application of ferrous glycinate at 5kg ha^{-1} (T_3) (Table 1).

Leaf active iron content

The foliar spraying of 1% ferrous glycinate twice at 25 and 45 DAS at vegetative, flowering and harvest stages increase the leaf active iron content followed by soil application of ferrous glycinate at 5 kg ha^{-1} (T_3). This influence is due to the application of higher concentration of iron at different stages and thereby increased the absorption of iron in black gram.

Iron content

The gradual increase in iron content of above ground bio mass with advancement of growth has observed. The foliar spraying of 1% ferrous glycinate on 25 and 45 DAS at vegetative, flowering and harvest stages increase the shoot and root iron content (Fig 2). 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.

Zinc content

The different iron chelates brought out significant variations in the content of zinc. The highest mean shoot zinc content of 14.4, 17.3 and 16.1 mg kg^{-1} at vegetative, flowering and harvest stages respectively was recorded with 1% of ferrous glycinate as foliar spraying on 25 and 45 DAS (T_7). The highest mean root zinc content of 7.40, 10.5 and 9.70 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 (T_7) (Fig 3).

Enzyme activity in plants

Foliar spraying of 1% ferrous glycinate registered the highest mean catalase activity of 11.4 and 14.8 µg of H_2O_2 g^{-1} min^{-1} at vegetative and flowering stages (Table 2) and on par with soil application ferrous glycinate at 5 kg ha^{-1}. The enzyme peroxidase activity showed an upheaval trend for the application of iron chelates. Foliar spraying of 1% ferrous glycinate (T_7) registered the highest mean peroxidase activity of 6.90 and 7.40 Δ 430 nm min^{-1} g^{-1} at vegetative and flowering stages (Table 2).

Discussion

The peak at 2920 cm^{-1} due to twisting vibrating of NH_2 groups. Disappearing of this peak indicates that new coordinate bond is formed through the terminal amine groups. The peak at 3153.04 cm^{-1} and 1327.75 cm^{-1} is due to weak stretching of symmetric and asymmetric amine vibration. As compared to the position of bands in the spectrum of iron glycinate confirms the chelation of said amino acid with Fe^{2+} ions (Yin *et al.*, 2017). The iron amino acid and organic acid chelates were synthesized using Glycine, Citric acid, and EDTA. These are abundant in the plant rhizosphere and play significant role in plant and human nutrition of Fe. The stability of metal complexes of these amino acids is also high in water.

The root and shoot dry matter production of black gram was influenced by the foliar spraying of 1% ferrous glycinate on 25 and 45 DAS**.** 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 (Chavan and Banerjee, 1980) [3]. The foliar spraying of 1% ferrous glycinate twice at 25 and 45 DAS at vegetative, flowering and harvest stages increase the leaf active iron content. Similar results were reported by Shuedzhen (1991) [19] where the higher active iron content in the foliar application of 1% ferrous glycinate treatment could be due to the fact that plant absorbs and translocates iron efficiently in citrate form. 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 which is accordance with findings Yadav and Sehgal (2002) [22]. The supply of iron with higher concentration at critical stages resulting in increased absorption and translocation of zinc. In case of root the zinc content was found to decrease from flowering to harvesting stages, which can be attributed to dilution effect (Chavan and Banerjee, 1980) [3].

Higher activity in the presence of ferrous glycinate is due to the role of glycine expression of genes encoding the catalase activity. The activity of these enzyme increased with decreasing iron rates. Iron is a constituent of enzyme system and so it helps for carrying out different enzymatic reactions in plant like, cytochrome oxidase, catalase, peroxidase, acotinase, and nitrogenase. This has been proved by Zahra Karimi (2014) [23] who ascribed that comparison effect of nano-iron chelate and iron chelate on growth parameters and antioxidant enzymes activity of green gram.

Table 1: Effect of iron chelates on dry matter production at different stages of black gram (kg ha^{-1})

Treatments	Root							
	Vegetative	Flowering	Harvest	% increase over control	Vegetative	Flowering	Harvest	% increase over control
T_1 - NPK control	320	359	391	-	105	119	135	-
T_2 - $FeSO_4$ @ 25 kg ha^{-1} as basal soil application	325	365	397	1.59	115	125	145	7.24
T_3 - Ferrous glycinate chelate @ 5 kg ha^{-1}	346	376	409	5.70	126	138	155	16.7
T_4 - Ferrous citrate chelate @ 5 kg ha^{-1}	336	369	395	2.80	117	128	141	7.52
T_5 - Fe – EDTA chelate @ 5 kg ha^{-1}	339	365	401	3.27	119	127	139	7.24
T_6 - 1% $FeSO_4$ as foliar spraying on 25 & 45 DAS	329	362	403	2.24	110	119	146	4.46
T_7 - 1% Ferrous glycinate as foliar spraying on 25 & 45 DAS	351	385	415	7.57	132	145	157	20.9
T_8 - 1% Ferrous citrate as foliar spraying on 25 & 45 DAS	340	373	405	4.49	124	135	151	14.2
T_9 - 1% Fe – EDTA as foliar spraying on 25 & 45 DAS	338	368	400	3.36	121	133	149	12.3
S.Ed	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 enzymes activity at different stages of black gram (µg of H_2O_2/g/min)

Treatments	Catalase activity (µg of H_2O_2/g/min)		Peroxidase activity (Δ 430 nm $min^{-1}g^{-1}$)	
	Vegetative	Flowering	Vegetative	Flowering
T_1 - NPK control	3.83	6.80	5.44	5.92
T_2 –$FeSO_4$ 25 @ kg ha^{-1} as basal soil application	10.2	12.7	5.94	6.44
T_3 - Ferrous glycinate chelate @ 5 kg ha^{-1}	10.6	13.1	6.54	7.12
T_4 - Ferrous citrate chelate @ 5 kg ha^{-1}	5.10	7.65	6.10	6.68
T_5 - Fe – EDTA chelate @ 5 kg ha^{-1}	4.68	7.65	5.51	6.00
T_6 - 1% $FeSO_4$ as foliar spraying on 25 & 45 DAS	8.50	11.4	6.54	7.08
T_7 - 1% Ferrous glycinate as foliar spraying on 25 & 45 DAS	11.4	14.8	6.90	7.40
T_8 - 1% Ferrous citrate as foliar spraying on 25 & 45 DAS	5.53	7.65	6.74	7.16
T_9 – 1% Fe – EDTA as foliar spraying on 25 & 45 DAS	8.08	11.9	5.97	6.44
Mean	7.54	10.4	6.69	6.19
SEd	0.02	0.05	0.07	0.15
CD (P=0.05)	0.06	0.11	0.16	0.31

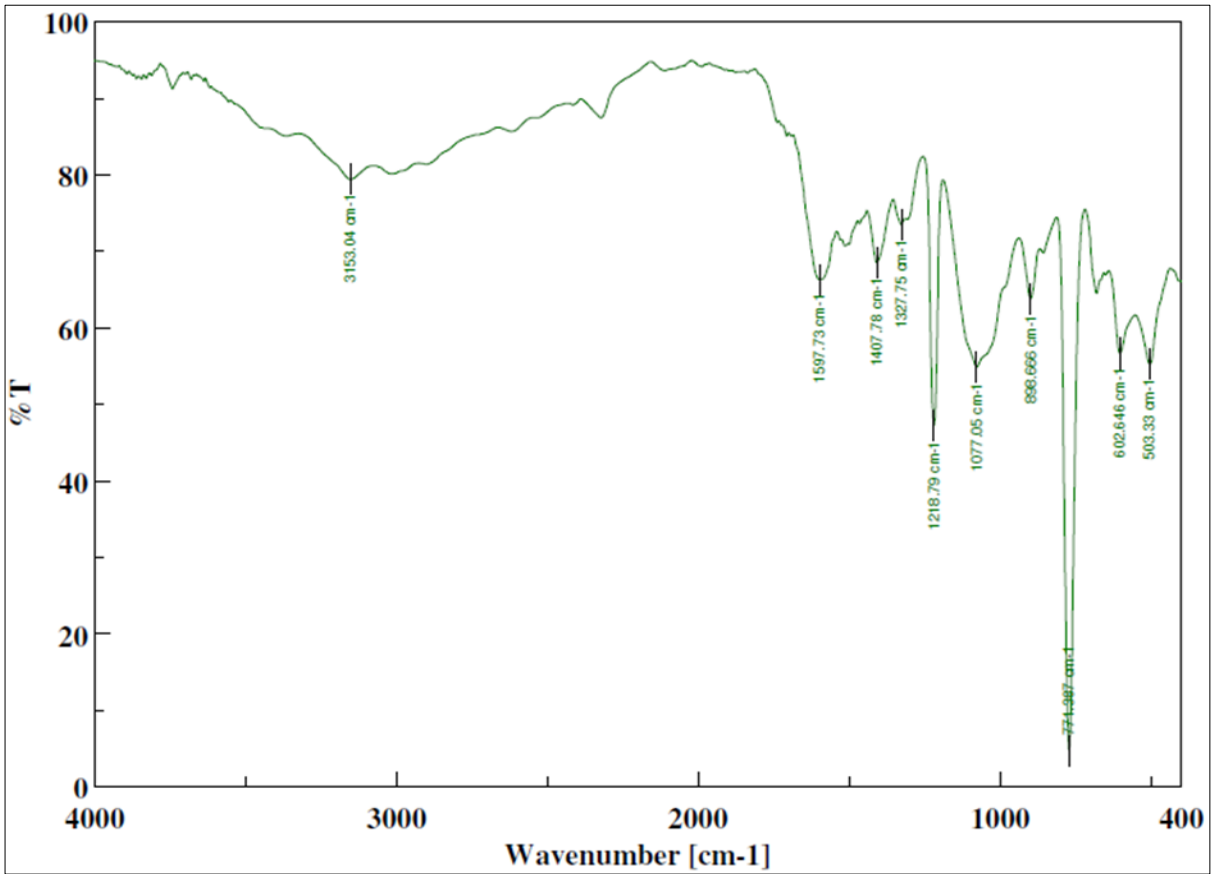

Fig 1: FTIR spectrum of Fe – Glycinate

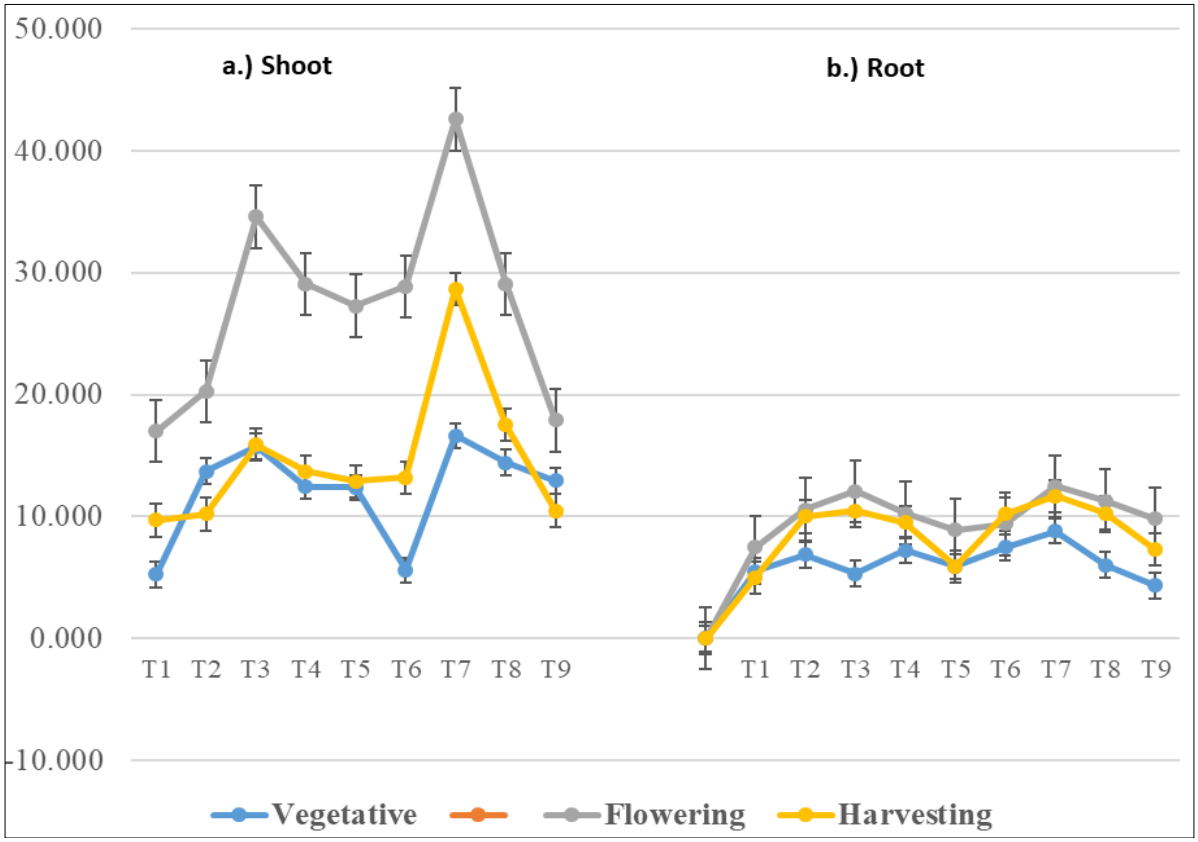

Fig 2: Effect of iron chelates on iron content at different stages of black gram (mg kg^{-1})

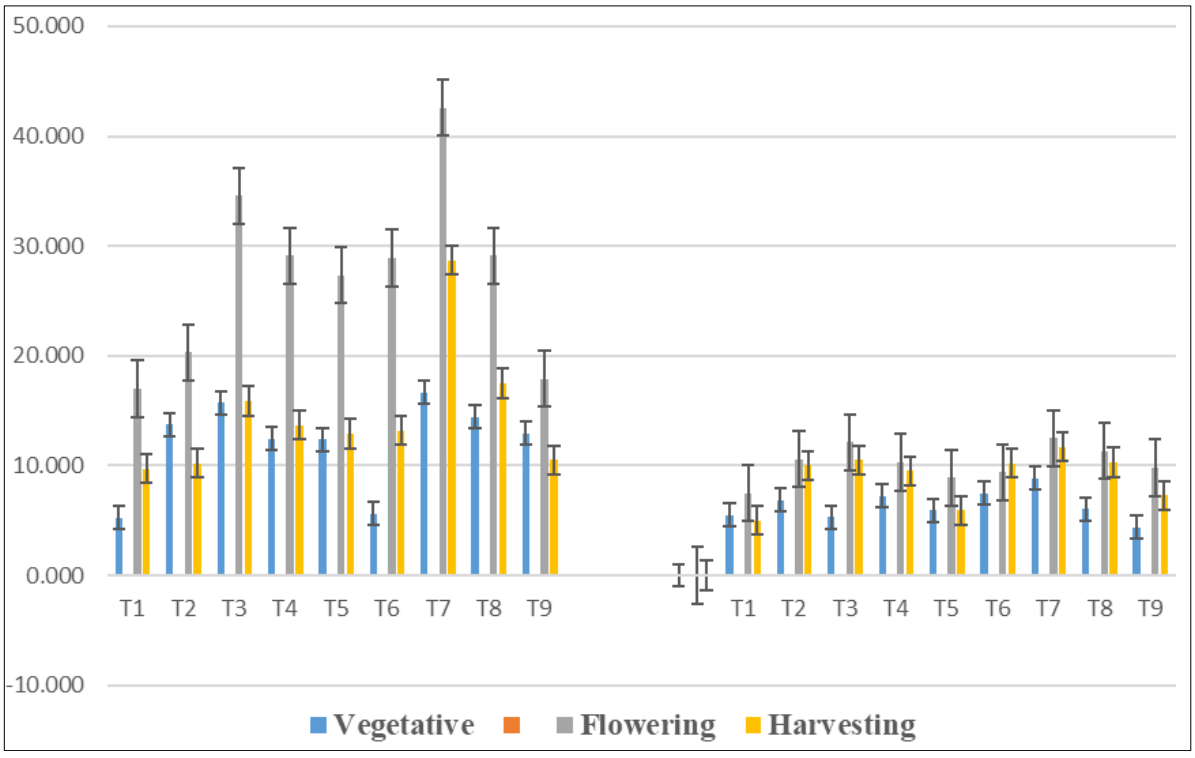

Fig 3: Effect of iron chelates on zinc content at different stages of black gram (mg kg^{-1})

Conclusion

Foliar application of 1% ferrous glycinate increased the dry matter production, leaf active iron, enzyme activity and micronutrient content of iron, zinc. The use of organic iron chelates for the correction of micronutrient deficiencies has increased in farms, as chelated micronutrients are soluble in solution and do not enter into adverse reaction in soil and easily absorbed and translocated by plants. Amino acid chelated micronutrient deliver selected nutrients with maximum bioavailability and safety to environment.

References

1. Angelini R, Manes F, Federico R. Spatial and functional correlation between diamine-oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chick-pea stems. Planta. 1990; 182(1):89-96.

2. Bolt G, Warkentin B. The negative adsorption of anions by clay suspensions. Kolloid-zeitschrift. 1958; 156(1):41-46.
3. Chavan A, Banerjee N. Iron-zinc interaction in a black loamy soil as studied on rice crop. Journal of the Indian Society of Soil Science. 1980; 28(2):203-205.
4. Clemens D, Whitehurst B, Whitehurst G. Chelates in agriculture. Fertilizer research. 1990; 25(2):127-131.
5. Curie C, Cassin G, Couch D, Divol F, Higuchi K, Le Jean M *et al*. Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters. Annals of botany. 2008; 103(1):1-11.
6. Hessey P. A text book of soil chemical analyses: London: Murray, 1971
7. Hsu HJ. Metal amino acid chelate: Google Patents, 1996.
8. Katyal J, Sharma B. A new technique of plant analysis to resolve iron chlorosis. Plant and Soil. 1980; 55(1):105-119.
9. Liesch A, Diaz D, Martin K, Olson B, Mengel D, Roozeboom K. Management strategies for increasing soybean yield on soils susceptible to iron deficiency. Agronomy Journal. 2011; 103(6):1870-1877.
10. Marschner H, Römheld V. Strategies of plants for acquisition of iron. Plant and Soil. 1994; 165(2):261-274.
11. NC G. Changes in activities of certain oxidizing enzymes during germination and seedling development of *Phaseolus mungo* and *Sorghum vulgare*. Indian journal of experimental biology. 1963; 1(2):98-102.
12. Panse V, Sukhatme P. Statical methods for Agricultural workers. ICAR. New Delhi, 1985.
13. Perur N. Measurement of peroxidase activity in plant leaf tissue. Current Science. 1962; 31(1):17-18.
14. Rivero R, Ruiz J, Romero L. Iron metabolism in tomato and watermelon plants: influence of grafting. Journal of plant nutrition. 2005; 27(12):2221-2234.
15. Römheld V, Marschner H. Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. Plant Physiology. 1986; 80(1):175-180.
16. Schaffer B, Crane JH, Li C, Li Y, Evans EA. Re-Greening of lychee (*Litchi chinensis* Sonn.) leaves with foliar applications of iron sulfate and weak acids. Journal of plant nutrition. 2011; 34(9):1341-1359.
17. Schulte E, Kelling A. Understanding plant nutrients: Soil and applied copper. *A2527*. University of Wisconsin-Extension, 2004.
18. Sekhon B. Chelates for micronutrient nutrition among crops. Resonance. 2003; 8(7):46-53.
19. Shuedzhen AK. Foliar application of trace elements to rice. Khimizatisya sel s Kogo Khozyzaistva. 1991; 3:46-50.
20. Snedecor GW, Cochran W. Statistical methods, Iowa State Univ., Ames, 1967, 593.
21. Wallace A, Lunt O. Reactions of Some Iron, Zinc, and Manganese Chelates in Various Soils 1. Soil Science Society of America Journal. 1956; 20(4):479-482.
22. Yadav S, Sehgal S. Effect of domestic processing and cooking methods on total, HCl extractable iron and in vitro availability of iron in spinach and amaranth leaves. Nutrition and health. 2002; 16(2):113-120.
23. Zahra Karimi LP, Hassan Feizi. Comparison Effect of Nano-Iron Chelate and Iron Chelate on Growth Parameters and Antioxidant Enzymes Activity of Mung Bean (*Vigna Radiata* L.). Adv. Environ. Biol. 2014; 8(13):916-930.