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Synthesis and Characterization of Iron Chelates Using Organic and Amino acids as a Chelating Agents and Evaluation of Their Efficiency in Improving the Growth, Yield and Quality of Blackgram

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ABSTRACT

Deficiency of iron in plants is the most serious problem in recent agricultural practices due to the introduction of high yielding varieties, loss through leaching and reduced farm yard manure application. Chelating agents are widely used to increase the solubility of micronutrients, for stable and sustainable crop production. A chelate refers to a ring system that results when a metal ion combines with two or more electron donor groups of a single molecule. The lab 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. A pot experiment was conducted to study the effect of foliar and soil application of amino acids and organic acids chelated micronutrients on growth yield and quality of blackgram. The plants were sprayed with single dose of organically chelated iron (1%) along with common ferrous sulphate on 25 and 45 DAS and untreated control. The results showed that foliar application of 1% ferrous glycinate chelate at resulted in maximum plant height, SPAD value, number of pods plant^{-1}, number of seeds pod^{-1}, pod length and 100 grain weight, Starch content and Protein content of blackgram in calcareous black soil.

KEYWORDS

Iron deficiency, Ferrous glycinate, Blackgram, Starch and Protein content

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INTRODUCTION

Deficiency of iron in plants is the most serious problem in recent agricultural practices due to the introduction of high yielding varieties, loss through leaching and reduced farm yard manure application. A chelate refers to a ring system that results when a metal ion combines with two or more electron donor groups of a single molecule. The compound with these characters is very much useful in agriculture. By addition of some micro-nutrients fertilizers, there is a chance of getting conversion of form that is unable to utilize by the plants. This is said to be plant unavailable form. But by application of micronutrient fertilizers in chelated forms can be the solution for available form in plants (Nurchi *et al*, 2020).

Chelation is a term that explains an encapsulation process. For example, a mineral like calcium, reacts with another material to form a guarding shell around the particular mineral or metal. The word chelate derives from the Greek word “chel”, meaning a crab’s claw and refers to the pincer-like manner in which the mineral is bound. Some chelating chemicals are shaped like a letter ‘C’ and surround the mineral with just one molecule. This type of binding is called a “complex”. When two molecules of the same material surround a mineral, it is known as a chelate. The thing is to be noted here is, some minerals like boron and molybdenum are not favorable for forming complex because the nature of having one chemical bond. So, we can conclude that above mentioned minerals cannot be chelated but still they are supplied in market as chelating minerals (Clemens *et al*, 1990).

“A chelate refers to a ring system that results when a metal ion combines with two or more electron donor groups of a single molecule. Actually, unidentate water molecules, which are coordinated with a metal ion, are replaced by the most stable bi, trio poly dentate groups of the chelating agent. This results in the ring formation. Metals bound in chelate rings have essentially lost their cationic characteristics. In some chemical reaction precipitation of these chelates are rare. The compound with these characters is very much useful in agriculture. By addition of some micro-nutrients fertilizers, there is a chance of getting conversion of form that is unable to utilize by the plants. This is said to be plant unavailable form. But by application of micro-nutrient fertilizers in chelated forms can be the solution for above mentioned form” (Sekhon, 2003).

Plant available form of micronutrient can be estimated by determining the stability of chelate bond with the specific metal. An effective chelate relationship is one in which the rate of substitution of the chelated micronutrients for cations already in the soil is low, thus maintaining the applied

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micronutrient in the chelated form for length of time sufficient to be absorbed by the plant roots (Sekhon, 2003). Micronutrients are required in lower concentration, for crop plants, nevertheless are vital to growth and productivity of many crops. Among the micronutrients, iron plays an important role by involving electron transport, redox reactions and functions as cofactors (Benepal, 1967) . A relatively large amount of iron is accumulated in plant tissue and its essential role in metabolic reactions make it as the most important nutrient among transition metals. The chloroplast contains relatively larger amount of iron compared to other cell organ (Terry and Abadía, 1986)and (Morrissey and Guerinot, 2009). It is essential for chlorophyll synthesis (Duy *et al*, 2007). Iron deficiency in plants occurs mostly in calcareous and alkaline soils (Zhang *et al*, 1995) and Mengel (2001). Deficiency of iron in plants is the most serious problem in recent agricultural practices due to the introduction of high yielding varieties, loss through leaching and reduced farm yard manure application (Bose *et al*, 2006) . A chelate describes a kind of organic chemical complex in which the metal part of the molecule is held so tightly that it cannot be 'stolen' by contact with other substances, or could convert it to an insoluble form. Chelating agents are organic molecules that can trap or encapsulate certain metal ions like Ca, Mg, Fe, Co, Cu, Zn and Mn and then release these metal ions slowly so that they become available to plants (Sekhon, 2003) .

MATERIALS AND METHODS

Lab Experiment

The lab 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 500ml 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 500m of deaerated water and the acid solution was added to the ferrous sulfate solution with stirring (Figure 1). 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, 1996)

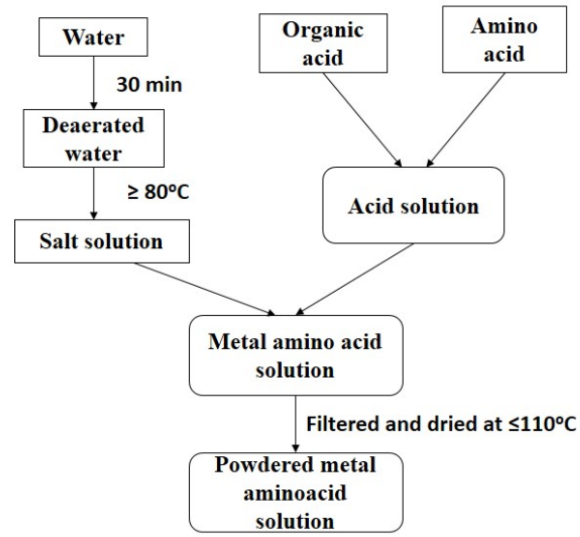

Fig. 1: Flow chart for synthesis of iron chelates

Characterization of iron chelates

The synthesized iron chelates were characterized for the following parameters

- Physical properties: colour and solubility
- Physico - chemical properties: pH and EC
- Chemical properties: Fe content, and amino acid content

Colour

The colour was determined by visual observations.

Solubility

One gram of the chelate was dissolved in 50 ml of distilled water. If not completely soluble, the insoluble fraction was separated and dried, to determine the solubility in percentage.

pH

Chelate was mixed with distilled water in the proportion of 1:5 and the pH was analyzed using digital pH meter.

Electrical conductivity

Chelate was mixed with distilled water in the proportion of 1:5. Electrical conductivity was determined using digital auto ranging conductivity bridge (Equiptronics- EQ-664A).

Iron content

Dissolved the chelate in water to prepare approximately 1000 ppm solution. The solution was deaerated with required of dilute H_2SO_4 prepare sample solution. The sample was feed to AAS to get the absorbance valves, which were converted to concentration in ppm.

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, Tamil Nadu Agricultural University, 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. The growth characters like plant height, root length and SPAD value (chlorophyll content) number of pods $plant^{-1}$, number of seeds pod^{-1},100 grain weight, starch content and protein content of blackgram in calcareous black soil.

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 Panse and Sukhatme (1985). Simple correlation and regression co-efficient were also worked out between certain inter-related parameters to observe their degree of dependence as suggested by Gw and Cochran (1967) .

RESULTS AND DISCUSSION

Characterization of iron chelates

The lab experiment was conducted for synthesizing twenty iron chelates for different characters. Those iron chelates are ferrous aspartic acid, ferrous asparagine, ferrous alanine, ferrous arginine, ferrous citrate, ferrous cysteine, ferrous cystine, ferrous DTPA, ferrous EDTA, ferrous glycinate, ferrous histidine, ferrous leucine, ferrous lysine, ferrous malate, ferrous malonic acid, ferrous oxalic acid, ferrous proline, ferrous succinate and ferrous valine. In that different iron chelate shown different characteristics. The characteristics of the prepared chelates are tabulated (Table 1). The chelates are almost completely soluble except Fe oxalate and Fe succinate. Fe glycine, Fe proline, hygroscopicity was observed and the others were non hygroscopic. The colour varies from white to black including yellow, pink and among chelates.

The pH of the chelates range from 3.51 to 9.01 and electrical conductivity varies from 0.39 to 2.02 dSm^{-1} in 1:10 aqueous solution. The iron content was as low as 8.6 per cent in Fe leucine and Fe histidine and highest value of 18.2 per cent in Fe succinate. The cost of making the chelate per 100g ranges from Rs. 25 to Rs. 1100 in the lab scale.

Plant height

The data on plant height at different growth stages increase with the advancement of crop growth period and furnished in Table 2 . The highest plant height at all the growth stages was registered with treatment T_7 (1% ferrous glycinate as foliar spraying on 25 & 45) and it was on par with (T_3) soil application of ferrous glycinate @ 5 kg ha^{-1} and (T_4) soil application ferrous citrate chelate @ 5 kg ha^{-1}. The lowest plant height was noticed in absolute control (T_1) and the mean values varied from 13.1 cm at vegetative, 25.7 cm at flowering and 28.3 cm at harvest stages respectively. The application of ferrous glycinate has enhanced the plant height significantly which might be due to the production phenolic acid, enhanced nutrient concentration, and pod vitamin C. The data shows that iron is a critical nutrient in phenolic biosynthesis, ascorbic acid and protein synthesis. This results can also be recognized due to the enhancement of other nutrient status owing to ferrous glycinate application (Marschner *et al*, 2011).

Root length

The data on root length of the crop at different growth stages increased with the advancement of growth stages of black gram and furnished in Table 2 . The highest root length at all the growth stages was registered with treatment T_3 (soil application of ferrous glycinate @ 5 kg ha^{-1}) and it was on par with T_5 (soil application of Fe – EDTA @ 5 kg ha^{-1}) and T_7 (1% ferrous glycinate as foliar spraying on 25 and 45). The lowest root length was observed in absolute control (T_1) and mean valves varied from 11.9 cm at vegetative, 14.9 cm at flowering and 15.5 cm at harvest stages respectively.

Application of amino acid chelates significantly increase the iron, copper, manganese, calcium and potassium nutrient concentration and plant growth compared to absolute control. Also, ferrous glycinate chelates improved the shoot nutrient concentration and enhanced the growth of blackgram plants when compared with control (Garcia *et al*, 2011).

SPAD values

The chlorophyll content of black gram was significantly influenced by the application of iron chelates. The chlorophyll content increased from vegetative to flowering stages and thereafter decreased drastically at harvest stages. Higher chlorophyll content in the leaves indicated the iron sufficiency due to the application of chelates to black gram crop. The highest mean chlorophyll content of 52.5, 55.2 and 53.7 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) (Table 2). Iron plays an important role in the synthesis of chloroplastic mRNA and rRNA, which regulate chlorophyll synthesis. The continuous supply of iron in soluble chelated form increased chlorophyll content in these treatments because of its involvement in the pathway of chlorophyll synthesis as discussed in earlier report by Beale (1999).

Number of pods per plant

Application of iron chelates influenced the number of pods per plant and it ranged from 16.0 to 26.0 (Table 3). Among the treatments, the highest number of pods $plant^{-1}$ was recorded in treatment receiving foliar spraying of 1% ferrous glycinate on 25 and 45 DAS (T_7) of 26.0 followed by T_3 (soil application ferrous glycinate @ 5kg ha^{-1} 23.0), while control recorded the least number of pods $plant^{-1}$ (16.0).

The results showed that amino acids remains a key role in several plant metabolic responses viz., synthesis of peptides, proteins, enzymes, nitrogen transformation and assimilation and several secondary important metabolics (Causin, 1996).

Table 1: Properties of synthesized iron chelates

Chelate Name	Solubility in water (%)	Hygroscopy	Colour	pH (1:5 ratio)	EC (dSm^{-1}) (1:5 ratio)	Fe – content (%)	Cost (100g)
Fe- Glycine	100	Hygroscopic	Brown	5.35	1.47	15.2	95
Fe- Citrate	100	Non hygroscopic	White	3.65	1.32	14.4	25
Fe- EDTA	100	Non hygroscopic	Yellow	4.27	1.05	8.53	81
Fe- DTPA	100	Non hygroscopic	White	3.51	2.02	13.3	210
Fe- Malate	100	Non hygroscopic	White	3.57	0.56	14.6	120
Fe- Oxalate	80	Non hygroscopic	Yellow	3.59	1.10	6.67	255
Fe- Succinate	80	Non hygroscopic	Pink	4.01	1.13	18.2	155
Fe- Cysteine	100	Non hygroscopic	White	5.35	0.60	12.5	290
Fe- Arginine	100	Non hygroscopic	Black	9.01	0.75	13.5	300
Fe- Alanine	100	Non hygroscopic	Black	4.11	0.39	10.1	1100
Fe- Aspartic acid	100	Non hygroscopic	Yellow	3.85	0.75	11.6	700
Fe- Proline	100	Hygroscopic	Yellow	5.64	1.21	14.2	315
Fe- Lysine	100	Non hygroscopic	Black	3.55	1.23	9.6	450
Fe- Valine	100	Non hygroscopic	Brown	4.01	0.68	8.4	1125
Fe- Malonoic acid	100	Non hygroscopic	White	3.58	0.98	12.2	325
Fe- Leucine	100	Non hygroscopic	White	3.55	0.67	8.6	870
Fe- Histidine	100	Non hygroscopic	Yellow	6.78	0.54	8.6	885
Fe- Humic acid	100	Non hygroscopic	Black	5.86	1.01	12.4	155
Fe- Aspragine	100	Hygroscopic	Yellow	5.95	0.84	10.5	170
Fe- Cystine	100	Non hygroscopic	White	5.29	0.58	11.6	150

Number of seeds per pod

The results envisaging that the number of seeds per plant varied from 4.40 to 6.40 (Table 3) and the highest number of seeds pod^{-1} was registered in the treatment receiving foliar spraying of 1% ferrous glycinate on 25 and 45 DAS (T_7 of 6.40) followed by T_3 (soil application ferrous glycinate @ 5kg ha^{-1} 6.10) in black calcareous soil, while control recorded the lesser number of seeds pod^{-1} (4.40). This was in agreement with the findings of Marschner *et al* (2011); Causin (1996) and Näsholm *et al* (2009).

Pod length

Application of iron chelates influenced the pod length significantly and it ranged from 3.90 to 6.02 in black calcareous soil (Table 2). Among the treatments, the highest pod length was recorded in the treatment receiving foliar spraying of 1% ferrous glycinate on 25 and 45 DAS (T_7). Besides, iron application increases the photosynthetic activity, root length, and number branches is all growing environment and greater partitioning of metabolites and adequate translocations of nutrients to developing structure leads to increased number of pods per plant in blackgram (Marschner *et al*, 2011).

Test weight

A significant influence was confabulated on the 100 seed weight by applying iron chelates and it varied from 4.90 to 6.30 g (Table 2). The highest hundred grain weight of 6.30 g was recorded with foliar spraying of 1% ferrous glycinate on 25 and 45 DAS (T_7). This was augmented that foliar spraying of ferrous glycinate increases the yield by increasing iron from source (assimilate) to sink (seed) which would have increased the 100 seed weight (Garcia *et al*, 2011).

Starch content

Foliar spraying of 1% ferrous glycinate at 25 and 45 DAS (T_7) significantly influenced the starch content of blackgram. The highest starch content (45.3%) were recorded in (T_7) foliar spraying of 1% ferrous glycinate (Table 3). Application of iron chelates increase in the carbohydrate content in blackgram which responsible amylase and amylopectin acivity (Ziaeian and M, 2001).

Table 2: Effect of iron chelates on growth and yield attributes of blackgram

Treatments	Plant height (cm)			Root length (cm)			SPAD value			Pod length (cm)	Test weight (g)
	Vegetative	Flowering	Harvest	Vegetative	Flowering	Harvest	Vegetative	Flowering	Harvest		
T_1	13.1	25.7	39.5	11.9	14.9	15.5	39.5	43.4	41.6	5.90	4.90
T_2	14.7	27.8	43.8	11.2	13.7	14.3	43.8	46.9	44.7	4.90	4.80
T_3	17.9	33.4	47.9	15.3	19.3	19.9	47.9	51.2	49.5	5.50	5.70
T_4	17.3	32.7	45.3	10.9	13.2	14.1	45.3	49.1	47.3	4.80	5.50
T_5	15.0	30.2	42.1	14.8	18.8	19.4	42.1	45.5	43.9	5.20	5.60
T_6	15.8	28.0	41.5	12.3	13.6	14.1	41.5	44.2	42.8	5.00	5.10
T_7	19.4	36.5	52.5	14.0	16.3	17.1	52.5	55.2	53.7	6.02	6.30
T_8	17.2	31.7	45.8	11.8	13.3	13.9	45.8	48.3	46.5	5.40	5.80
T_9	16.3	26.4	42.5	10.9	12.8	13.5	42.5	45.7	42.1	5.30	5.70
Mean	16.3	30.3	44.5	12.6	15.1	15.7	44.5	47.7	45.8	5.11	5.49
SEd	0.84	2.05	0.93	0.24	0.98	0.98	0.93	0.88	1.06	0.07	0.12
CD (P=0.05)	1.77	4.30	1.97	0.51	2.06	2.07	1.97	1.85	2.22	0.16	0.25

T_1 – NPK control; T_2 –$FeSO_4$ 25 kg ha^{-1} as basal soil application; T_3 - Ferrous glycinate chelate @ 5kg ha^{-1}; T_4 - Ferrous citrate chelate @ 5kg ha^{-1}; T_5 - Fe – EDTA chelate @ 5kg 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 & 45 DAS; T_9 – 1% Fe – EDTA as foliar spray on 25 and 45 DAS

Table 3: Effect of iron chelates on yield attributes and quality parameter of blackgram

Treatments	No. of pods per plant	No. of seeds per pod	Protein (%)	Starch (%)
T_1	16.0	4.40	16.2	26.4
T_2	19.0	5.20	20.2	34.6
T_3	23.0	6.10	23.9	44.5
T_4	20.0	5.50	21.2	37.7
T_5	21.0	5.40	22.5	36.5
T_6	18.0	5.10	23.6	34.8
T_7	26.0	6.40	24.8	45.3
T_8	22.0	5.60	21.6	40.5
T_9	21.0	5.70	22.3	35.2
Mean	20.7	5.49	21.8	37.2
SEd	0.54	0.14	0.38	0.85
CD (P=0.05)	1.15	0.29	0.80	1.78

T_1 – NPK control; T_2 –$FeSO_4$ 25 kg ha^{-1} as basal soil application; T_3 - Ferrous glycinate chelate @ 5kg ha^{-1}; T_4 - Ferrous citrate chelate @ 5kg ha^{-1}; T_5 - Fe – EDTA chelate @ 5kg 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 & 45 DAS; T_9 – 1% Fe – EDTA as foliar spray on 25 and 45 DAS

Protein content

Foliar spraying of 1% ferrous glycinate (T_7) at 25 and 45 DAS and soil application of ferrous glycinate at 5 kg ha^{-1} enhanced the protein content of blackgram. The highest protein content (24.8%) was recorded in (T_7) foliar spraying of 1% ferrous glycinate (Table 3). This might be due to the role of iron on the enzymatic activity in plants which could bring about significant changes in the protein content in blackgram grain (Zhang *et al*, 1995).

CONCLUSION

Results obtained from the present study showed that foliar spraying of 1% ferrous glycinate was effective in increasing the growth, yield and quality of blackgram in black calcareous soil. Between the soil and foliar application foliar spraying is better in improving the plant micronutrient content which was evidenced from higher enzyme activities. The ferrous glycinate application increased the efficiency in utilization of solar energy through increases in chlorophyll content resulting in higher photosynthetic activity and increased dry matter production, as expressed by higher yields and better yield attributes.

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