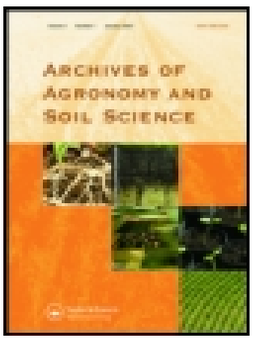

Archives of Agronomy and Soil Science

ISSN: 0365-0340 (Print) 1476-3567 (Online) Journal homepage: http://www.tandfonline.com/loi/gags20

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To cite this article: Haining Chen, Zhaoping Hu, Xinzhu Li, Fuqian Zhang, Jianqiu Chen & Min Zhang (2016): Iron fertilizers applied to calcareous soil on the growth of peanut in a pot experiment, Archives of Agronomy and Soil Science, DOI: 10.1080/03650340.2016.1173674

To link to this article: http://dx.doi.org/10.1080/03650340.2016.1173674

Accepted author version posted online: 04 Apr 2016.
Published online: 18 Apr 2016.

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Iron fertilizers applied to calcareous soil on the growth of peanut in a pot experiment

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ABSTRACT

A greenhouse pot experiment was conducted with peanuts (*Arachis hypogaea* L., Fabceae) to evaluate iron compound fertilizers for improving within-plant iron content and correcting chlorosis caused by iron deficiency. Peanuts were planted in containers with calcareous soil fertilized with three different granular iron nitrogen, phosphorus and potassium (NPK) fertilizers (ferrous sulphate ($FeSO_4$)–NPK, Fe–ethylendiamine di (*o*-hydroxyphenylacetic) (EDDHA)–NPK and Fe–citrate–NPK). Iron nutrition, plant biomass, seed yield and quality of peanuts were significantly affected by the application of Fe–citrate–NPK and Fe–EDDHA–NPK to the soil. Iron concentrations in tissues were significantly greater for plants grown with Fe–citrate–NPK and Fe–EDDHA–NPK. The active iron concentration in the youngest leaves of peanuts was linearly related to the leaf chlorophyll (via soil and plant analyzer development measurements) recorded 50 and 80 days after planting. However, no significant differences between Fe–citrate–NPK and Fe–EDDHA–NPK were observed. Despite the large amount of total iron bound and dry matter, $FeSO_4$–NPK was less effective than Fe–citrate–NPK and Fe–EDDHA–NPK to improve iron uptake. The results showed that application of Fe–citrate–NPK was as effective as application of Fe–EDDHA–NPK in remediating leaf iron chlorosis in peanut pot-grown in calcareous soil. The study suggested that Fe–citrate–NPK should be considered as a potential tool for correcting peanut iron deficiency in calcareous soil.

ARTICLE HISTORY
Received 11 August 2015
Accepted 30 March 2016

KEYWORDS
Iron chlorosis; Fe–citrate; Fe–EDDHA; ferrous sulphate ($FeSO_4$); active iron

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Introduction

Iron is an essential element for several plant metabolic functions (Taiz & Zeiger 1998). Iron deficiency chlorosis (IDC) is a common nutritional disordering affecting higher plant grown on calcareous soils (Sánchez-Rodríguez et al. 2014). In addition, low iron contents impair the nutritional value of plant products. Iron deficiency can lead not only to anemia but also to negative effects on work capacity and on motor and mental development of infants, children and adolescents (Erin et al. 2009).

High pH and bicarbonate concentrations are supposed to be mainly responsible for iron chlorosis in calcareous soils (Chakraborty et al. 2014). Surface coverage of lime affected or naturally found calcareous soils in the earth is estimated to be almost 30% (Chen & Shenker 2003). Often, in calcareous soils, the reduction of Fe^{3+} to Fe^{2+} by ferric chelate reductase in the leaves hampered by the high pH environment of the apoplast, although sufficient quantity of iron is translocated from

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the roots to the leaves (Mengel & Geurtzen 1988; Brüggemann et al. 1993; Mengel 1994; González-Vallejo et al. 2000). Iron chlorosis has been reported to result in decreased yield and poor quality of crops and fruits due to the decrease in leaf photosynthetic pigment concentrations, especially chlorophyll (Abadía & Abadía 1993).

Some efficient methods to correct iron chlorosis have been developed such as trunk injection, soil or foliar iron amendments including ferrous sulphate ($FeSO_4$), Fe–citrate and Fe(III) chelates. Some insoluble products, vivianite and siderite mixed with the soil evenly obtained better results to alleviate iron chlorosis (Rosado et al. 2002; Díaz et al. 2010; Sánchez-Alcalá et al. 2012a, 2012b; Cañasveras et al. 2014). Foliar application is beset with problems including soil salinization–alkalization, inefficient absorption, translocation and utilization resulting in a need for frequent spraying (Hamzé et al. 1985). However, the frequent applications required are very time-consuming and could not offer a good alternative for the full control of iron chlorosis (Álvarez-Fernández et al. 2004). Iron injection is also a useful alternative (Cañasveras et al. 2014), but pathogens and bacterial infections often occur simultaneously. Management may become more complicated when they coexist. Moreover, trunk injection is expensive and mainly used in garden trees. Currently, soil iron fertilizers applications are widely used, and have been regarded as an important remediation technique influencing plant iron uptake. $FeSO_4$ applied to the soil has been the major therapy against iron chlorosis and is still widely used by farmers especially in the developing countries due to its low costs. However, soil-applied $FeSO_4$ is of little or no agronomic value in calcareous soils where the Fe^{2+} is converted into non plant-available forms (hydroxide) rapidly (Abadía et al. 2011). Nowadays, iron chelates are the most efficient way to cure iron deficiency (Álvarez-Fernández et al. 2004). However, they are very expensive and could be easily washed from the soil. In addition, iron chelates can trigger nutritional disorders because they can chelate Mn and reduce plant uptake of this element (Cañasveras et al. 2014). Estimates made in Southern Europe indicate that iron chelates represent up to 60% of the total fertilizer costs and often amount to more than 250 Euros per ha per year (Álvarez-Fernández et al. 2004). In addition, some synthetic Fe(III) chelates, such as Fe–EDDHA, Fe–ethylendiamine di (*o*-hydroxy-*p*-methylphenylacetic) (EDDHMA), Fe– ethylendiamine di (2-hydroxy-5-sulfophenylacetic) (EDDHSA) and Fe–ethylendiamine di (5-carboxy-2-hydroxyphenylacetic) (EDDCHA) contain large amounts of potentially polluting compounds (Cremonini et al. 2001; Álvarez-Fernández et al. 2002). Obviously, there is a need for a cheaper and useful alternative to iron chelates for preventing or alleviating iron deficiency in calcareous soils.

Peanut is one of the major oilseed crops contributing to China's edible oil. IDC usually limits the yield and quality of peanut especially in calcareous soils of North China, which accounts for one-third of the total oilseed production in China (Zuo et al. 2007). Traditionally, all the nitrogen, phosphorus and potassium (NPK) fertilizer is broadcasted in the field and then cultivated before sowing in order to save labors. There are no other nutrients input at the whole growth stage of peanut. Production of NPK fertilizer frequently involves the addition of small quantities of trace elements is time and labor forces consuming for farmers when they apply. China is one of the world's largest consumers and producers of NPK compound fertilizers. Most researches focused on the single application of iron fertilizer in agricultural production. Little research has been performed on the combination of iron products and industrial production. Sustainable agriculture production on calcareous soils requires persistent and cost-effective strategies to overcome IDC.

The purpose of this study was to test the effects of three iron NPK fertilizers so as to determine some justified iron fertilizer and provide a practical iron source for industrial production. In this paper, we compared the effectiveness of Fe–EDDHA–NPK, Fe–citrate–NPK and $FeSO_4$–NPK, at the same rate of iron, to assess the capability of these iron NPK fertilizers for correcting iron chlorosis of peanut grown in calcareous soil under pot conditions, and to evaluate iron nutritional indexes, seed yield and quality.

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Materials and methods

Soil

Soil sample was collected from important peanut growing area of Dezhou city of Shandong province, China. The soil developed from alluvial deposits of the Yellow River terraces is typical for Dezhou City. The soil sample was taken to a depth of 20 cm from the soil surface. The sample was air-dried and ground to pass through a 3-mm sieve for a pot experiment. A sub-sample was ground to pass through a 2-mm sieve for laboratory analysis. The soil is classified as Car-Och-Aquic Primosols (Gong et al. 1999). The soil had the following characteristics: pH 8.27, organic matter 8.70 g kg^{-1}, free CO_3^{2-} 6.33%, total CO_3^{2-} 33.7%, NH_4^+–N 2.81 mg kg^{-1}, NO_3–N 2.41 mg kg^{-1}, available P 8.74 mg kg^{-1}, available K 119.98 mg kg^{-1}, DTPA–Fe 3.81 mg kg^{-1}.

Peanut

The variety of peanut in this experiment is 'Luhua 12', which is an iron-sensitive species provided by Shandong Peanut Research Center, China.

Preparation of Fe–NPK

In producing granular compound fertilizer (N:P_2O_5:K_2O:Fe = 20:5:10:2) containing iron, NH_4NO_3, potassium phosphate, potassium sulphate were used as the nitrogen, phosphorus source and potassium source, respectively. $FeSO_4 \cdot 7H_2O$ (20.14% Fe), Fe–EDDHA (6% Fe) and Fe–citrate (16.5% Fe) were used as the iron sources. There was used a pan granulation method comprising mixing the raw materials, additives and adhesives for the fertilizer, adding a suitable amount of water to the resulting mixture, granulating the mixture by rolling it in a rotary granulator and drying the resulting granules.

Plant culture in soil

The pot experiment was conducted in a greenhouse with average temperature of 25/18°C (day/night), 15/9 h light/dark regime, and the relative humidity at 70–75%. Peanut seeds susceptible to iron chlorosis were provided by Shandong Peanut Research Institute. The seeds of uniform size were surface-sterilized with 5% (v/v) H_2O_2 for 30 min, then washed three to four times with deionized water and then germinated in disinfected coarse quartz sand (2 mm diameter) at 25°C in the dark. Treatments included: (1) Control (no Fe added); (2) $FeSO_4$ compound fertilizer (FeSO4–NPK); (3) Fe–EDDHA compound fertilizer (Fe–EDDHA–NPK); (4) Fe–citrate compound fertilizer (Fe–citrate–NPK). In order to maintain the normal growth of peanut, iron was added at the rate of 90 mg kg^{-1} soil as $FeSO_4$–NPK, Fe–EDDHA–NPK, Fe–citrate–NPK. N, P_2O_5, K_2O were applied to each pot at total rate of 100, 100 and 200 mg kg^{-1} soil in the form of NH_4NO_3, KH_2PO_4 and K_2SO_4 according to above soil analysis data, respectively. All fertilizers were mixed with the soil before planting. Potted soil was moistened to field capacity and equilibrated for 1 day before sowing. Eight uniform pre-germinated seeds were sown in plastic pots with 13 kg soil per pot. The pots were arranged in a complete randomized design with three replicates for each treatment. Ten days after planting, seedlings were thinned to four plants per pot. The water content was periodically maintained with deionized water to the approximate field capacity to avoid plant wilting. The watering weights were adjusted periodically to compensate for plant growth.

Soil solution sampling and chemical analysis

Soil solution was collected directly from soil using rhizon soil moisture samplers. One soil moisture sampler was inserted to soil per pot prior to planting, and the stoppers were sealed with protective

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caps. Vacuum is kept by keeping plunger in place with wooden spacer. Solution was sampled at maturity stage. The pH values were measured directly after collection. Available iron concentrations were measured by atomic absorption spectrometry (AAS) (AA370MC, Shanghai Precision and Scientific Instrument Co., Ltd., China).

Determination of soil properties

Soil pH was measured in a 1:2 soil–water (w/v) suspension. Organic matter was determined by dichromate oxidation (Walkley & Black 1934). Free $CaCO_3$ was estimated by the method outlined by Puri (1950). DTPA-extractable Fe was determined by AAS. Total N content of soil was determined by the Kjeldahl method (Bremner & Breitenbeck 1983), and available P content by 0.5 M $NaHCO_3$ (pH 8.5) extraction (Olsen & Sommers 1982), exchangeable K by 1 M NH_4OAc, pH 7.0 extraction (Knudsen et al. 1982). Available P was analyzed colorimetrically, and exchangeable K was analyzed directly by flame photometry.

Chlorophyll measurement

At 20, 35, 50, 65, 80, 100 days after planting, leaf chlorophyll concentrations were monitored with a soil and plant analyzer development (SPAD) chlorophyll meter (Minolta 502, Osaka, Japan) on young, healthy, and fully expanded leaves.

Plant analyses

At 50 and 80 days after planting, after SPAD measurements, leaves were collected, carefully washed in 0.1 M HCl and rinsed in deionized water. A fresh leaf sub-sample was taken for measurement of HCl-extractable iron (so-called 'active' iron) according to the procedure of Takkar and Kaur (1984). The remainder of the samples was mill-ground and after a dry digestion in a muffle furnace at 500°C for about 5 h, the ashes were digested using 1 M HCl, according to method of Lopez-Moreno et al. (2010). Total iron was determined by AAS following standard methods (Belkhodja et al. 1998). The plants were harvested after 120 days. The shoots were harvested by cutting at 1 cm above the soil surface. Plants were separated into leaves, stems and roots. The soil in the sampling pot was used for soil analysis. Plant tissues were dried in an oven maintained at 105°C for 15 min, and then dried at 70°C for 72 h, and dry matter weights were determined for all tissues. The concentrations of total iron in leaves, stems and roots were also determined by AAS. Yield components such as plant height, branch length, number of pegs per plant were determined in each pot before harvest. Seed yield was determined after harvesting. Peanut kernel protein was fractionated according to the continuous extracting method (He 1985). Fat was accomplished using the method of He (1985). Fatty acids were determined using a gas chromatograph (GC2010, Shimadzu Co., Ltd., Japan) (Zhou et al. 2007). Soluble sugar content was determined by enthrone colorimetric method (Spiro 1966).

Date analysis

Data were analyzed using the SAS 8.1 software package (SAS Institute, Cary, NC), expressed as means of three replicates with standard deviation, and the means were subject to another test by using the least significant difference (LSD) method at 5% probability level.

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Results

Leaf chlorophyll concentrations and symptoms of iron deficiency

SPAD reading in the youngest leaves fluctuated with time (Figure 1). The influence of the Fe–EDDHA–NPK on the degree of chlorosis was illustrated best when chlorosis in control is most severe. Fe–EDDHA–NPK increased the SPAD reading with average value of 31.8. The Fe–citrate–NPK-treated plants also had a healthy appearance with the average value of 30.5. Application of $FeSO_4$–NPK only caused SPAD reading increase up to 28.3. The SPAD reading of leaf was significantly increased due to Fe–EDDHA–NPK application over control. The plants treated with Fe–citrate–NPK had the same enhancing effect on leaves SPAD reading and significantly different to control and $FeSO_4$–NPK-treated leaves. No significant difference was observed between Fe–EDDHA–NPK and Fe–citrate–NPK at these growth stages, and none of them showed clear chlorosis symptoms. Application of $FeSO_4$–NPK caused a slight SPAD reading increase compared to control. In this concern, $FeSO_4$–NPK treatment at two sampling date (50 and 80 days) induced the higher significant improving effect in SPAD reading. Treatments with iron were much more effective in severely iron-deficient leaves than in control leaves in this experiment.

Concentration of available iron in the soil solution and iron uptake in young leaves

The uptake of iron by peanut plants, as influenced by source of iron, was reported in Table 1. Data showed that in Fe–EDDHA–NPK and Fe–citrate–NPK treatments, levels of active iron were enhanced significantly compared to control and $FeSO_4$–NPK treatment. Application of Fe–EDDHA–NPK recorded significantly higher active iron concentration than control and other soil applications. Compared with control, $FeSO_4$–NPK-treated plants still depressed the active iron concentration. Total iron concentrations in the youngest leaves increased along with iron supply, and specifically, Fe–EDDHA–NPK and Fe–citrate–NPK treatments significantly increased total iron concentration. Plants treated with Fe–EDDHA–NPK had the largest amount of this element from the second sampling time. AAS data indicated that active iron in the youngest leaves was affected by the iron compounds speciation. Total iron uptake in Fe–EDDHA–NPK and Fe–citrate–NPK treatments, however, did not show many differences. But, all iron applications were superior to control. The enhancements in total iron levels in Fe–EDDHA–NPK and Fe–citrate–NPK treatments were approximately 29.0 and 27.6% of the control, respectively.

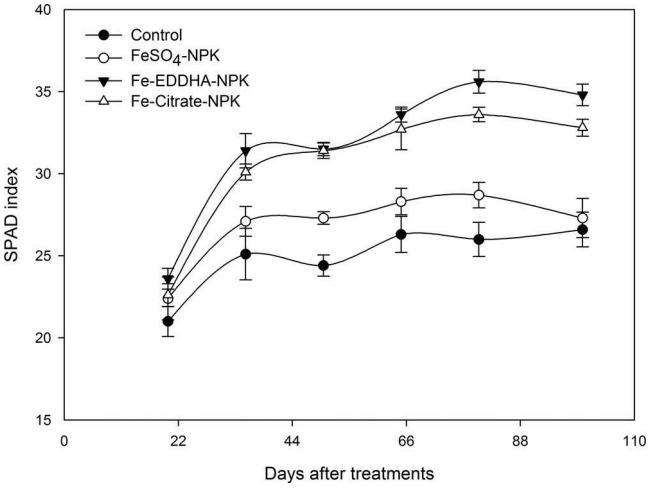

Figure 1. Times course of the changes in SPAD reading of the youngest leaves of peanuts treated with $FeSO_4$–NPK, Fe–EDDHA and Fe–citrate during the experimental periods. Error bars indicate standard deviations. Error bars represent standard deviations ($P < 0.05$).

Table 1. Effect of iron treatments on active iron, total iron in the youngest leaves of peanut and available iron concentration in soil solution at two sampling days.

	50 Days			80 Days		
Treatments	Active iron (mg kg^{-1} FW)	Total iron (mg kg^{-1} DW)	Available iron (mg kg^{-1})	Active iron (mg kg^{-1} FW)	Total iron (mg kg^{-1}DW)	Available iron (mg kg^{-1})
Control	30.679 ± 0.338[b]	136.413 ± 2.669[d]	6.647 ± 0.898[d]	29.160 ± 1.191[c]	103.123 ± 2.769[c]	6.267 ± 1.012[b]
$FeSO_4$–NPK	34.080 ± 1.526[b]	146.967 ± 4.506[c]	9.425 ± 0.327[c]	32.154 ± 1.113[c]	172.267 ± 5.477[b]	8.645 ± 0.379[b]
Fe–EDDHA–NPK	43.010 ± 1.190[a]	190.500 ± 1.686[a]	17.087 ± 0.665[a]	50.669 ± 1.035[a]	220.500 ± 1.686[a]	15.726 ± 0.797[a]
Fe–citrate–NPK	42.990 ± 1.095[a]	180.433 ± 4.562[b]	14.593 ± 0.386[b]	45.893 ± 1.407[b]	208.933 ± 10.133[a]	13.457 ± 0.560[a]

Means within columns followed by different letters were significantly different at $P < 0.05$ in the least significant difference (LSD) test.

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The total iron concentrations in soil solution were determined to show the availability of iron in soil (Table 1). Iron concentrations in soil solution were generally higher for Fe–EDDHA–NPK and Fe–citrate–NPK treatments than $FeSO_4$–NPK treatment and control, and iron concentrations at the second sampling time (about 80 days after seedling) were lower than the first sampling time.

Correlation between active iron concentration and SPAD reading in the young leaves of peanuts

Correlation relationships between active iron concentration and SPAD reading in the youngest leaves of peanut at two sampling dates (50 and 80 days) are given in Figure 2. Particularly notable was that active iron induced from iron applications were positively correlated with chlorophyll meter readings after 50 and 80 days.

Iron distribution in peanut plants

Considering roots, stems, and leaves of peanuts, mean iron concentrations increased significantly in soils treated with iron fertilizers compared with the control (Table 2). Total iron in old leaves was significantly higher than that in young leaves. No significant differences were found for roots,

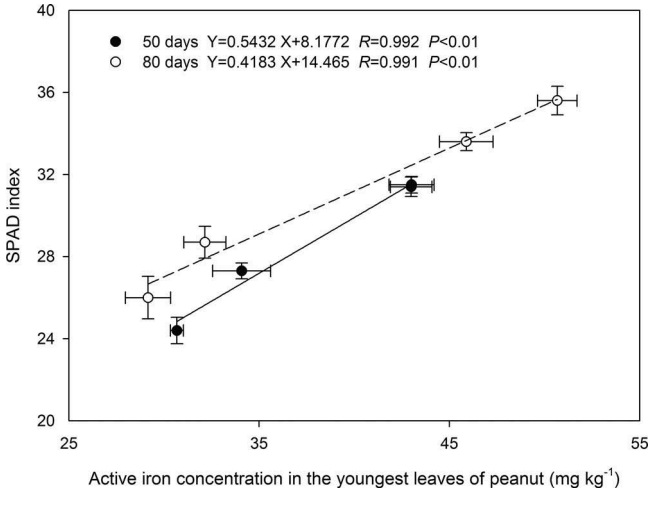

Figure 2. Relationship between active iron concentration and SPAD reading in the youngest leaves of peanut plants grown on calcareous soil after 50 and 80 days. Error bars indicate standard deviations ($P < 0.05$).

Table 2. Effect of iron treatments on total iron of leaf, stem, root and kernel of peanut grown in calcareous soil.

Treatments	Young leaves	Old leaves	Stem	Root	Kernel
			(mg plant^{-1} DW)		
Control	0.107 ± 0.009^{d}	1.027 ± 0.046^{b}	1.060b ± 0.095^{b}	1.140 ± 0.072^{b}	0.137 ± 0.008^{c}
$FeSO_4$–NPK	0.170 ± 0.016^{c}	1.193 ± 0.011^{b}	1.304 ± 0.088^{b}	1.740 ± 0.201^{a}	0.173 ± 0.014^{c}
Fe–EDDHA–NPK	0.234 ± 0.016^{b}	2.157 ± 0.110^{a}	2.175 ± 0.101^{a}	1.976 ± 0.172^{a}	0.392 ± 0.013^{a}
Fe–citrate–NPK	0.292 ± 0.011^{a}	1.879 ± 0.098^{a}	2.059 ± 0.130^{a}	1.789 ± 0.236^{a}	0.321 ± 0.012^{b}

Means within columns followed by different letters were significantly different at $P < 0.05$ in the LSD test.

stems, and leaves between Fe–EDDHA–NPK and Fe–citrate–NPK. The total iron of stems and leaves was also higher in control conditions than in $FeSO_4$–NPK. However, $FeSO_4$–NPK application did not show any significant effect on iron of leaves, stems and kernel compared to the control ones.

Yield components and seed yield

Applications of Fe–EDDHA–NPK and Fe–citrate–NPK significantly increased plant height, pegs, and dry matter compared to control, whereas $FeSO_4$–NPK led to no significant effects on these traits (Table 3). Without iron application, the control treatment showed a significantly smaller dry matter accumulation. Fe–citrate–NPK supply gave the highest rate of increase for plant height (14.22%), branch length (14.30%) and dry matter (41.41%) over control. The Fe–EDDHA–NPK treatment significantly increased the numbers of pegs with a 60% increase over the control plants. Overall, application of these iron fertilizers to containerized peanuts in calcareous soil significantly increased dry matter yields.

Significant differences between $FeSO_4$–NPK and Fe–citrate–NPK treatments were observed for yield components whereas no significant effects were found for any traits in control and $FeSO_4$–NPK. Similar results were found for $FeSO_4$–NPK and Fe–EDDHA–NPK treatments.

Only Fe–EDDHA–NPK and Fe–citrate–NPK significantly increased the seed yields as well (Table 3). Of these, Fe–EDDHA–NPK was numerically highest with 54.40% more than the control, which was numerically lowest. However, the $FeSO_4$–NPK application showed only marginal yield improvements with no significant difference compared with the control, which indicates low plant availability. These results suggest that Fe–citrate–NPK and Fe–EDDHA–NPK could be recommended for cultivation of peanuts in iron-deficient soils.

Kernel quality

Protein, fat, oleic acid, linoleic acid, palmitic acid and soluble sugar were measured to estimate the kernel quality of peanuts. Iron fertilization generally improved kernel quality comparing with the control treatment (Table 4). Kernel protein and fat were significantly affected by Fe–EDDHA–NPK and Fe–citrate–NPK fertilization. Oleic acid followed this trend. Seed protein content from the Fe–citrate–NPK treatments as about 1.5 times higher than the control, while the $FeSO_4$–NPK treatment yielded a 1.2-fold increase. Numerically, the highest protein content was found in the Fe–citrate–NPK, whereas fat and oleic acid levels were numerically highest for Fe–EDDHA–NPK, and were each 1.1 times higher than the control. There were no significant differences among treatments in the

Table 3. Effect of iron treatments on plant height, branch length, pegs and dry matter (DM) weight of peanut grown in calcareous soil.

Treatments	Plant height (cm)	Branch length (cm)	Pegs (plant^{-1})	DM weight (g plant^{-1})	Seed yield (g plant^{-1})
Control	22.500 ± 0.721^{b}	24.367 ± 0.376^{bc}	9.867 ± 0.145^{b}	17.223 ± 0.629^{c}	8.290 ± 0.589^{b}
$FeSO_4$–NPK	23.500 ± 0.500^{b}	23.767 ± 0.926^{c}	9.033 ± 0.176^{b}	19.203 ± 0.572^{b}	8.657 ± 0.532^{b}
Fe–EDDHA–NPK	25.633 ± 0.521^{a}	26.667 ± 0.491^{ab}	14.433 ± 0.353^{a}	23.330 ± 0.359^{a}	12.797 ± 0.327^{a}
Fe–citrate–NPK	25.700 ± 0.569^{a}	27.167 ± 0.521^{a}	13.833 ± 0.233^{a}	24.347 ± 0.418^{a}	11.707 ± 0.512^{a}

Means within columns followed by different letters were significantly different at $P < 0.05$ in the LSD test.

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Table 4. Effect of iron treatments on kernel quality of peanut grown in calcareous soil.

Treatments	Protein	Fat	Oleic acid	Linoleic acid	Palmitic acid	Sugar	O/L
Control	19.423 ± 1.296[b]	44.890 ± 1.061[b]	41.323 ± 0.812[b]	33.130 ± 2.009[a]	11.287 ± 0.640[a]	7.347 ± 0.215[a]	1.257 ± 0.052[b]
$FeSO_4$–NPK	22.647 ± 2.350[ab]	44.710 ± 2.558[b]	43.283 ± 1.546[b]	34.183 ± 1.017[a]	12.527 ± 0.485[a]	6.900 ± 0.666[a]	1.267 ± 0.050[b]
Fe–EDDHA–NPK	27.313 ± 1.122[a]	50.930 ± 1.501[a]	49.547 ± 1.620[a]	35.587 ± 1.798[a]	11.580 ± 0.797[a]	7.993 ± 0.194[a]	1.397 ± 0.047[a]
Fe–citrate–NPK	28.240 ± 1.446[a]	50.103 ± 1.703[a]	48.857 ± 1.653[a]	34.607 ± 2.547[a]	12.640 ± 0.714[a]	8.207 ± 0.238[a]	1.420 ± 0.062[a]

Means within columns followed by different letters were significantly different at $P < 0.05$ in the LSD test.

concentrations of linoleic acid, palmitic acid and soluble sugar. The numerically highest ratio of oleic acid and linoleic acid (O/L) was obtained from fertilization by Fe–citrate–NPK, but this did not differ significantly from the Fe–EDDHA–NPK treatment.

Discussion

The soil used in this study was high in pH and $CaCO_3$, but low in organic matter, hence, it showed typical calcareous soil characteristics. Biometric parameters are widely used to determine plant responses in greenhouse experiments with containerized plants. In this study, biometric data helped to distinguish iron nutritional statuses between control and iron-treated plants. Here, plant height, branch length, number of pegs, and dry matter weight of peanuts were well correlated with the degree of chlorosis. This suggested the parameters can help in determining plant iron nutritional status when only iron limited the growth of plants. Fe–citrate–NPK was as effective at re-greening iron-chlorotic plants growing in calcareous soil as the more well-known Fe–EDDHA. However, plants treated with $FeSO_4$ differed from the untreated control only in dry matter weight.

Leaf chlorophyll density measured by the intensity of green color using a SPAD meter appeared to work better for determining iron nutritional status. Chlorophyll density usually reflects iron nutritional status in plants partly because iron is essential for the synthesis of chlorophyll. Higher chlorophyll density (indicated by SPAD measurements) suggested increased iron uptake in peanuts (Figure 1). This was similarly observed by Sánchez-Rodríguez et al. (2014), who noted that if iron was not supplied in sufficient quantity, the synthesis of chlorophyll precursors was reduced, hence leading to lower chlorophyll contents. In this study, iron fertilizers were generally effective in increasing the chlorophyll density (SPAD reading) of iron-deficient peanut leaves. Active iron was reflected on increasing the chlorophyll content to the level of healthy plants. Thus, application of active iron helped the plants overcome the adverse effects of $CaCO_3$. Hamzé and Nimah (1982) using citrus roots stock as well as Olsen and Brown (1981) reported that total iron or Fe^{3+} was a poor indicator of iron metabolism in plants, and stressed the significance of active iron or Fe^{2+} in chlorotic leaves. Hamzé et al. (1985) reported that the active iron correlated better than Fe^{3+} with the degree of chlorotic in plants. Significant correlations were found between active iron concentration and SPAD reading in the young leaves of peanuts, which agrees with the results of Zuo et al. (2007) and Sánchez-Rodríguez et al. (2014). However, this positive effect contrasts with the well-known 'Fe chlorosis paradox' (Römheld 2000), in which the leaf chlorophyll index was not correlated with leaf iron concentration. Application of Fe–EDDHA–NPK and Fe–citrate–NPK resulted in higher active iron concentrations in leaves. Several factors may influence how effective and useful fertilization with Fe–citrate–NPK can be. First, acidic solutions may prove more effective when significant amounts of pre-existing iron pool occur, such as in leaves of peanut plants showing the 'chlorosis padadox'. Furthermore, the acidic solutions may improve the solubility and chemical stability of applied iron, as well as the cell iron uptake through the Fe(III) chelate reductase FCR enzyme. Hence, the Fe–citrate–NPK treatment also may be very important because applied acidic solutions may decrease the apoplast pH, and thus potentially improve iron utilization. Chatterjee et al. (2015) reported that more iron released from the calcareous soils with the increase of citrate concentration.

Treatment with $FeSO_4$–NPK was less effective than the other two products. $FeSO_4$–NPK did not improve levels of leaf available iron possibly because of large quantities of $CaCO_3$ in the soil. When iron became limiting, the chlorophyll synthesis slowed down and the chlorophyll gated diluted due to continuous leaf expansion. The concentration of iron in the soil solution increased as iron applied. The application of Fe–EDDHA–NPK and Fe–citrate–NPK to calcareous soil appeared to have a positive effect on improving concentrations of iron present in the active form as Fe^{2+} in peanut leaves. On the earliest sample date, differences among iron fertilizers were not significant probably because of insufficient time for the plants to absorb enough fertilizer to show effects from the treatments.

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Iron ratios presented the advantages of using an internal reference for the iron content in the peanut plant. Presumably, because of greater solubility of soil iron, plants provide with Fe–EDDHA–NPK and Fe–citrate–NPK had higher active iron concentrations than plants from the $FeSO_4$–NPK treatment. Our results showed that plant roots had the ability to accumulate large quantities of iron and are in agreement with results found by Yamauchi & Peng (1995) and Chatterjee et al. (2006). The stem showed lowest iron accumulation, which might indicate that most of the iron was translocated to leaves. Despite having low effective iron concentrations, concentrations for leaves in the $FeSO_4$–NPK treatment were apparently higher than for the untreated control. Olsen and Brown (1981) reported that iron concentration in the plant tissue is not a reliable prediction of iron nutrition because yellow leaves frequently contain as much or even higher Fe^{3+} than green leaves. In addition, increased Fe^{3+} may result from the inhibition of photo reduction of Fe^{3+} in chlorotic leaf tissue. Hamzé and Nimah (1982) noted that these discrepancies have led to an emphasis on the physiological role of iron rather instead of its total concentration. In total, analyzing leaf material for levels of iron may not provide a proper diagnosis of the deficiency. For example, iron deficiency symptoms have resulted from inactivation of iron, which led to greater iron concentrations in chlorotic than in green leaves (Katyal & Sharma 1980). Therefore, estimation of HCl-extractable iron contents from fresh leaf material has been suggested as a more effective measure of the iron status in peanuts.

Iron deficiency is generally believed to decrease yields of peanuts, though few references have been provided in recent reviews on iron deficiency in peanuts. In this study, seed yield increased with the application of iron fertilizers, which is consistent with this general belief. In soybeans, photosynthesis was improved and assimilates transportation and seed yield increased with the application of nano-iron (Mohammadi 2015). We found that on calcareous soil, iron utilization by peanuts improved with increasing iron supply. By applying $FeSO_4$–NPK, however, only a moderate increase in seed yield was observed. In addition to decreasing iron uptake by the plants, bicarbonates also impaired root nodule formation and nitrogen fixation by *Bradyrhizobium* strains in peanuts (Tang et al. 1991).

Decreasing peanut kernel quality because of iron deficiency can result from many factors. These include changing concentrations of chemical compounds in the kernel including proteins, fats, oleic and linoleic acids, which in turn may affect organoleptic characteristics.

Peanuts are believed to consist mainly of proteins and oils and are a source of very high energy (5.64 cal g^{-1}). Among treatments in this study, peanut seeds treated with Fe–EDDHA–NPK yielded the highest levels of fat, oleic acid and O/L with results from the Fe–citrate–NPK treatment slightly lower. High ratios of oleic to linoleic acids have resulted in greatly enhanced peanut shelf life and decreased spoilage. High oleic to linoleic acid ratios may also confer a major health advantage to consumers and can greatly enhance the marketability of peanuts.

Further evidence supporting effects of iron deficiency on peanut plants is found in results from fertilization trials with other crops. Iron fertilization has been found to increase yields in many other field crops and vegetables, such as dry beans (Zaiter et al. 1992), groundnut (Papastylianou 1993), lentils (Erskine et al. 1993), ginger (Wilson & Ovid 1993), tomatoes (Chatterjee et al. 2006), soybeans (Schenkeveld et al. 2008), and rice (He et al. 2013). Hence, it would be expected to also increase in peanut yields. Our study found that applying Fe–EDDHA–NPK and Fe–citrate–NPK at the same concentrations of active ingredients resulted in increased growth parameters, yield components, and kernel quality compared to $FeSO_4$–NPK and the untreated control. Application of Fe–citrate–NPK was as effective as applying Fe–EDDHA–NPK in reducing leaf chlorosis in highly calcareous soil, and was also more favorable to plant growth.

Thus, a commercial product containing Fe–citrate was more effective correcting iron chlorosis than one with Fe–EDDHA. Our study indicated that leaf active iron concentrations, plant growth, and seed quality can be increased with Fe–EDDHA–NPK or with Fe–citrate–NPK fertilization in calcareous soil. However, because of high cost, potential for environment damage, and that its effects are not better than those of Fe–citrate–NPK, using Fe–EDDHA–NPK may not be justified and hence does not represent a sustainable way to prevent or cure iron chlorosis.

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Conclusion

Fe–citrate–NPK was as effective at re-greening peanut plants with iron chlorosis as the better-known Fe–EDDHA. However, applying $FeSO_4$–NPK resulted in lower growth parameters, probably because the Fe^{2+} converted to forms that were not available to plants. Thus, using Fe–citrate in iron treatments to peanut plants does provide any advantage over the less costly and more environmentally friendly iron salts. Future research should develop and test controlled release iron fertilizers to minimize nutrient losses and leaching.

Disclosure statement

No potential conflict of interest was reported by the authors.

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