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# Synthesis and crystal structure of tris (1, 10 phenanthroline)-iron (II) tetra (4-Nintro-phenyl-urea) bis chloride, [Fe(phen)$_3$][Cl$_2$·L$_4$] chelates and evaluation of their efficacy as iron source for tomato in nutrient solution culture

Sara Rafiei · Hossein Chiniforoshan ·
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**Abstract** Supplying a sufficient amount of available Fe for plant growth in hydroponic nutrient solutions is a great challenge. The chelators commonly used to supply Fe in nutrient solutions have several disadvantages and may negatively affect plant growth. In this research study we have synthesized certain Iron by 1,10-phenanthroline [(Fe(phen)$_3$)(Cl$_2$·L$_4$)] (L = 4-Nintro-phenyl-urea), and evaluated their efficacy as Fe source for a tomato cultivar grown in nutrient solution. Application of Fe-chelates significantly increased root and shoot dry matter yield tomato cultivar compared with Fe-EDTA and FeCl$_3$. Tomato plants supplied with [Fe(phen)$_3$][Cl$_2$·L$_4$] chelates also accumulated significantly higher levels of Fe and N in their roots and shoots compared with those supplied with Fe-EDTA and FeCl$_3$. The results obtained indicated that using Fe-phen chelates in the nutrient solution could supply a sufficient amount of Fe for plant uptake and also improve root and shoot growth of tomato plants. According to the results, Fe-phen chelates can be used as an alternative for Fe-EDTA to supply Fe in nutrient solutions.

## Introduction

Metal chelators are widely used in algal and plant growth solutions to maintain Fe solubility in hydroponic solutions, demonstrate limitation effects or examine metal transport mechanisms (Parker and Norvell 1999).

Metal chelate compounds are common components of fertilizers to provide micronutrients. These micronutrients (manganese, iron, zinc, copper) are required for the overall health of the plants. Most fertilizers contain phosphate salts that, in the absence of chelating agents, typically convert these metal ions into insoluble solids that are of no nutritional value to the plants. EDTA is the typical chelating agent for this purpose (Vadas et al. 2007).

Most studies involving chelators in hydroponic nutrient solutions have focused on the mineral nutrition of the plant. Typically, the elemental composition of both solution and plant are analyzed to determine the influence of the chelator on metal uptake or availability.

1,10-Phenanthroline (phen) complexes have been studied extensively. Phen is a well-known N-heterocyclic chelating agent with a rigid planar structure (Goel and Singh 2013). The metal chelating properties of 1,10-phenanthroline have been widely utilized in all aspects of

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**Fig. 1** Chemical structure of [Fe(phen)₃][Cl₂·L₄]
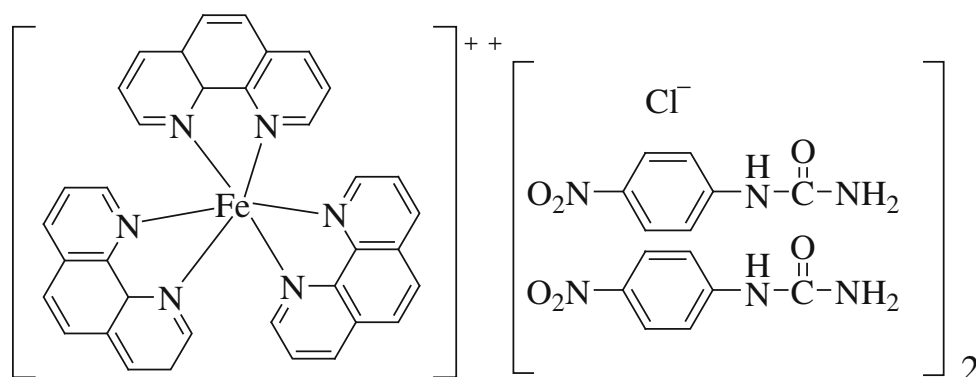


coordination chemistry such as ligand substitution, redox reactions, photochemical reactions and photophysical properties, as well as in its recent applications to develop biomimetic models of metalloenzymes and to prepare supramolecules, self-assembling systems, or metal complexes with interesting anticancer properties (MacDonall et al. 1999).

Fe is an important micronutrient that plays a role in several crop physiological processes such as photosynthesis, respiration, and synthesis of heme proteins, DNA, RNA, and hormones (Curie et al. 2009; Rivero et al. 2003). In nutrient solution cultures, synthetic Fe chelates are widely used to maintain a desirable concentration of this element for the plant (Parker and Norvell 1999; Vadas et al. 2007).

The most common Fe sources used in nutrient solutions are Fe-EDTA and Fe-DTPA (Vadas et al. 2007). Although these chelates maintain Fe solubility in hydroponic solutions, after Fe uptake by the plant, the concentration of free ligands is increased in the nutrient solution and, as a result, the possibility of complex formation between free ligands and other micronutrients (that is, Zn, Cu, and Mn) in the solution increases.

Fe deficiency chlorosis is a nutritional disorder characterized by a significant decrease of chlorophyll in the leaves, often observed in plants grown on alkaline and calcareous soils. It decreases crop yield both quantitatively and qualitatively, resulting in economic losses (Chaney 1984; Mortvedt 1991).

The application of synthetic Fe chelates is the most common practice to mend or to prevent Fe chlorosis. These chelates increase the solubility of Fe and function as a transporter through solution to the plant (Schenkeveld et al. 2010).

Supplying a sufficient amount of available Fe for plant growth in hydroponic nutrient solutions used in laboratory studies and commercial facilities is a great challenge.

## Materials and methods

### Synthesis of [Fe(phen)₃][Cl₂·L₄]

To a solution of $FeCl_2 \cdot 4H_2O$ (39 mg, 0.2 mmol) in 10 ml DMF (dimethylformamide) was placed in a two-neck round-bottomed flask. Then, the ligand 4-nitro phenylcyanamide, (4-NO₂·pcyd) (0.4 mmol) was added to the reaction flask while stirring. The reaction mixture was stirred at reflux temperature (95 °C) for 2 h under vacuum; the flask was placed under $N_2$ atmosphere. Then 1, 10-phenanthroline, (phen) (118 mg, 0.6 mmol) dissolved in 50 ml dimethylformamide was added dropwise to the reaction flask while stirring. During which time the solution turned a deep red. Then the mixture was stirred at room temperature in two hours. The precipitate was removed by filtration and dried while standing in air (Fig. 1).

### Analyses

All chemicals were purchased from Merck and Aldrich. Phenylcyanamide ligands were synthesized by the literature method (Crutchley 2001; Letcher et al. 1993). Elemental analyses were performed by Heraeus CHN-O-Rapid lemental analyzer. IR spectra were recorded as KBr pellets on a FT-IR JASCO 460 spectrophotometer and electronic spectra on a JASCO 7850 pectrophotome-ter. Atomic absorption measurements of Fe were recorded with atomic absorption spectrometry (PerkinEl-mer 3030; PerkinElmer, Wellesley, MA, USA).

### Plant

Seeds of tomato were thoroughly rinsed with distilled water and germinated on moist filter paper in an incubator at 28 °C. Tomato seedlings were transferred to 2-L pots with continuously aerated nutrient solution. The pots were wrapped with black polyethylene to prevent light from reaching the roots and solution. The nutrient solution had

the following composition: 1 mM $MgSO_4$, 1.75 mM $Ca(NO_3)_2$, 4 mM $KNO_3$, 2 mM $KH_2PO_4$, 2.5 mM $NH_4NO_3$, 1.25 mM $CaCl_2$, 2 μM $MnSO_4$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.25 μM $H_3BO_3$, 0.5 μM $H_2MoO_4$ and 50 μM KCl adjusted to pH 6. No Fe was added to the nutrient solution. Iron was supplied from three different sources (Fe-EDTA, $FeCl_3$ and $[Fe(phen)_3][Cl_2{\cdot}L_4]$) The Fe concentration in all treatments was 50 μM.

## Elemental analyses

Plants were harvested approximately 30 days after seedling and separated into roots and shoots. Plant materials were dried for 48 h at 70 °C, weighed, and digested by microwave (USEPA1995). About 0.100 g of plant shoot samples were digested in APCU-40 75 ml TFM Teflon vessel of microwave (Milestone Srl, START D, Sorisole, Italy) using 5 ml of $HNO_3$ and 3 ml of $H_2O_2$, then were filtered through Whatman no. 42 filters, transferred to 50-ml volumetric flasks, and diluted with deionized water. Analyses of Fe were carried out with an atomic absorption spectrophotometer (WFX 200, RAY LEIGH). Shoot and root N concentration was measured using Autotech (Model 300) according to Kjeldahl method (Bremmer and Mulvancey 1982).

N and Fe contents were calculated by multiplying N and Fe concentrations by dry weights.

## Catalase (CAT) assay

Catalase (EC 1.11.1.6) activity of the leaves was determined according to Cakmak and Marschner (1992). The reaction mixture contained 25 mM sodium phosphate buffer (pH 7.0) plus 10 mM $H_2O_2$ and 0.1 mL leaf extract in a total volume of 3 ml. The reaction was initiated by the addition of leaf extract, and the enzyme activity was determined by measuring the initial rate of disappearance of $H_2O_2$ at 240 nm (E = 39.4 mM$^{-1}$ cm$^{-1}$) for 70 s.

## Statistical analysis

The experiment was set up in a completely randomized factorial design; each treatment contained three replicates. All statistical analyses were conducted using the SAS (SAS Institute, 2000). Means were compared using least significant differences (LSD) at $P < 0.05$.

## Crystal structure determination and refinement

Crystals of $[Fe(phen)_3][Cl_2{.}L_4].2CH_3CN$ were grown by ether diffusion into an acetonitrile solution of the complex. The obtained crystal was characterized by single-crystal

**Table 1** Crystallographic and structure refinement data for $[Fe(phen)_3][Cl_2.L_4].2CH_3CN$

| Empirical formula | $C_{68}H_{58}Cl_2FeN_{20}O_{12}$ |
|---|---|
| Formula weight | 1,474.09 |
| Temperature (K) | 120(2) |
| Wavelength (Å) | 0.71073 |
| Crystal system | Monoclinic |
| Space group | $P2/n$ |
| Crystal size (mm$^3$) | $0.30 \times 0.20 \times 0.10$ |
| $a$ (Å) | 17.166(3) |
| $b$ (Å) | 11.900(2) |
| $c$ (Å) | 17.471(4) |
| $\beta$ (°) | 106.21(3) |
| Volume (Å$^3$) | 3,427.0(13) |
| Z | 2 |
| Density$_{calc}$ (g cm$^{-1}$) | 1.429 |
| $\theta$ Ranges for data collection (°) | 2.43–29.16 |
| F(000) | 1,524 |
| Absorption coefficient (mm$^{-1}$) | 0.376 |
| Index ranges | $-18 \leq h \leq 23$ |
| | $-14 \leq k \leq 16$ |
| | $-23 \leq l \leq 23$ |
| Data collected | 24,125 |
| Unique data | 9,230 |
| Parameters/restrains | 490/0 |
| Final $R_1/wR_2$[a] (I > 2σ(I)) | 0.1056/0.1340 |
| Final $R_1/wR_2$[a] (all data) | 0.2010/0.1583 |
| Goodness-of-fit on $F^2$ (S) | 1.089 |
| Largest diff. peak and hole (e Å$^{-3}$) | 0.427, −0.439 |

[a] $R_1 = \Sigma||F_o|-|F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma(w(F_o^2-F_c^2)^2)/\Sigma w(F_o^2)^2]^{1/2}$

X-ray analysis. The X-ray diffraction measurements were made on a STOE IPDS-2T diffractometer with graphite monochromated Mo-Kα radiation. For $[Fe(phen)_3][Cl_2{\cdot}L_4].2CH_3CN$, red plate shape crystal was chosen using a polarizing microscope and was mounted on a glass fiber which was used for data collection. Cell constants and orientation matrices for data collection were obtained by least-squares refinement of diffraction data from 9,230 unique reflections. Data were collected to a maximum 2θ value of 58.32° in a series of ω scans in 1° oscillations and integrated using the Stoe X-AREA (Stoe and Cie 2005a) software package. A numerical absorption correction was applied using X-RED (Stoe and Cie 2005b) and X-SHAPE (Stoe and Cie 2005c) software. The data were corrected for Lorentz and Polarizing effects. The structures were solved by direct methods (Sheldrick 1997a) and subsequent difference Fourier maps and then refined on $F^2$ by a full-matrix least-squares procedure using anisotropic displacement parameters (Sheldrick 1997b). The atomic factors were taken from the International Tables for X-ray

**Fig. 2** IR spectra of [Fe(phen)₃][Cl₂·L₄]
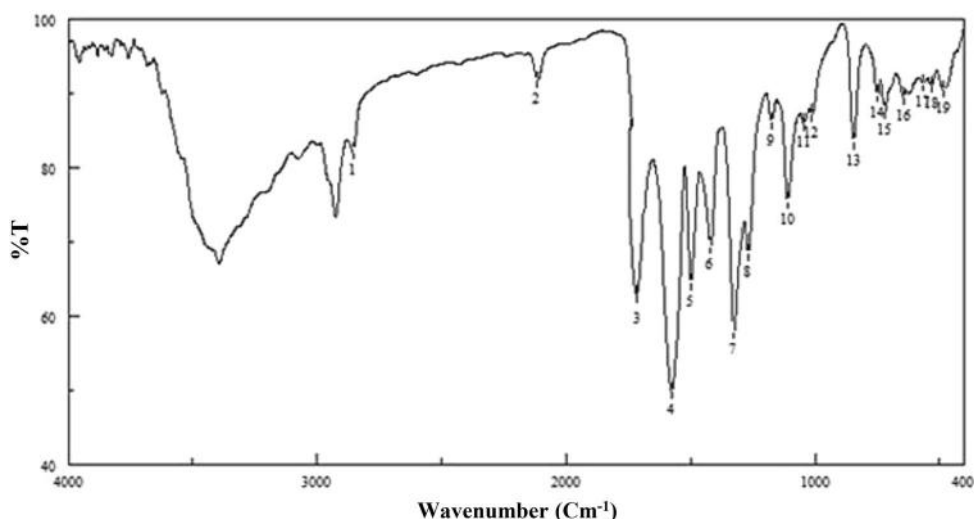
Crystallography (Wilson 1992). All refinements were performed using the X-STEP32 crystallographic software package (Stoe and Cie 2000). A summary of crystal data, experimental details, and refinement results is given in Table 1.

## Results

### Characteristics of Fe-phen chelates

The IR spectrum of 1, 10-Phenenthroline (phen) clearly exhibits a band at 1,586 cm$^{-1}$ that is ascribable to a stretching frequency of the C=N bands on the ligand (Amani et al. 2007). In these complexes, the ν(C=N) was shifted about 10 cm$^{-1}$ to the lower wave number. The IR spectrum of the [Fe(phen)₃][Cl₂·L₄] shows a strong band at 1,697 cm$^{-1}$ that is assigned to ν(C=O) of the (4-Nintrophenyl) urea.(Fig. 2).

The resulting red precipitate was filtered and recrystallized from water to yield the title complex as red crystals. *Anal*. Calc. for $C_{68}H_{58}N_{19}FeO_{12}Cl_2$ (1,474.09): C, 54.65; H, 4.29. N, 18.92 Found: C, 55.35; H, 4.13 N, 18.04 %. Fe, 3.78 %. Cl, 4.80 %.

### *Description of the molecular structures of [Fe(phen)₃][Cl₂·L₄].2CH₃CN*

Crystallographic data reveals that [Fe(phen)₃][Cl₂·L₄].2-CH₃CN complex crystallize in the monoclinic (space group, *P2/n*) crystal system. The asymmetric unit of the [Fe(phen)₃][Cl₂·L₄].2CH₃CN complex consists of half of the [Fe(phen)₃] species, one chloride ion, two 4-Nintrophenyl-urea and one CH₃CN molecule. An ORTEP

drawing of the structure is presented in Fig. 3. The coordination geometry around iron ion can be described as distorted octahedral geometry. Selected bond lengths and angles with their standard deviations for complex [Fe(phen)₃][Cl₂·L₄]·2CH₃CN is given in Table 2.

### Visual deficiency symptoms

At the second week of growth, leaves of plants grown in solutions with Fe-EDTA and FeCl₃ developed light green leaf color and chlorosis while plants grown in solution with [Fe(phen)₃][Cl₂·L₄] shown no Fe deficiency symptoms (Fig. 4). These symptoms were more sever in plants grown at FeCl₃ treatment.

### Shoot and root dry matter production

Results shown that [Fe(phen)₃][Cl₂·L₄] chelates significantly enhanced shoot and root dry matter yield compared to Fe-EDTA and FeCl₃ treatments (Fig. 5, 6).

Based on the obtained results, [Fe(phen)₃][Cl₂·L₄] increased the dry weight of tomato shoots 104 percent greater than Fe-EDTA therefore, the effect of combination [Fe(phen)₃][Cl₂·L₄] on the dry weight function in comparison with FeCl₃ and Fe-EDTA is outstanding.

### Shoot and root Fe concentration

The effect of Fe treatment on shoot Fe concentration was significant ($P < 0.05$) (Fig. 7). [Fe(phen)₃][Cl₂·L₄] treatment significantly increased shoot Fe concentration while there was no significant difference between shoot Fe content in Fe-EDTA and FeCl₃ treatments.

**Fig. 3** ORTEP diagram of [Fe(phen)$_3$][Cl$_2$·L$_4$] 2CH$_3$CN. Hydrogen atoms and solvent molecules have been omitted for clarity
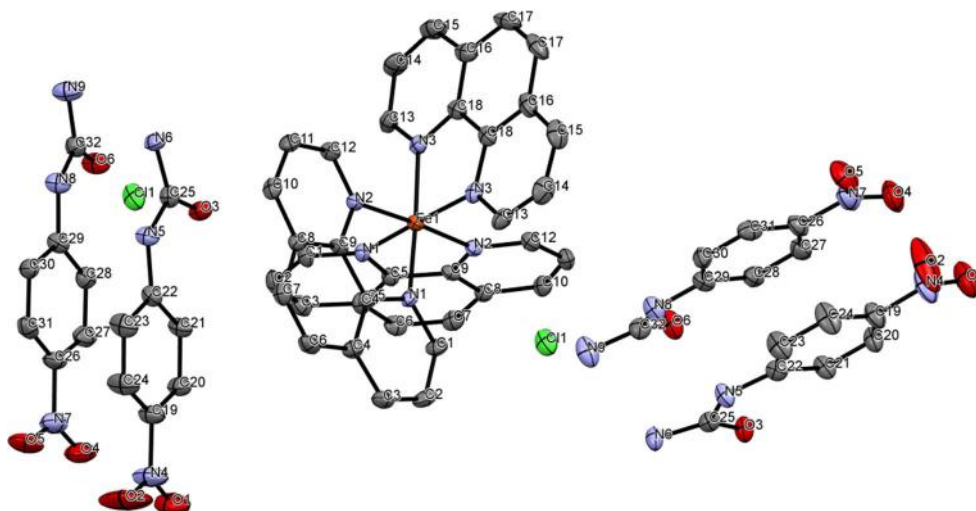


**Table 2** Selected bond distances (Å) and bond angles (deg) for [Fe(phen)$_3$][Cl$_2$·L$_4$]·2CH$_3$CN

| | | | |
|---|---|---|---|
| Fe(1)–N(1) | 1.976(3) | O(3)–C(25) | 1.235(5) |
| Fe(1)–N(2) | 1.983(3) | O(6)–C(32) | 1.230(5) |
| Fe(1)–N(3) | 1.967(4) | N(8)–C(32) | 1.376(5) |
| C(25)–N(6) | 1.344(6) | N(5)–C(25) | 1.383(5) |
| N(3)–Fe(1)–N(1) | 174.40(15) | N(3)–Fe(1)–N(2) | 92.48(15) |
| N(1)–Fe(1)–N(2) | 82.29(15) | N(10)–C(33)–C(34) | 178.8(6) |
| N(9)–C(32)–N(8) | 112.4(4) | N(6)–C(25)–N(5) | 113.9(4) |

The effect of Fe treatment on root Fe concentration was significant ($P < 0.01$) (Fig. 8). [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment remarkably increased root Fe concentration. [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment increased Fe concentration in shoots 169 and 304 % versus Fe-EDTA and FeCl$_3$, respectively.

Shoot and root N concentration

The effect of Fe treatment on shoot N concentration was significant ($P < 0.05$). [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment significantly increased shoot N concentration in comparison with FeCl$_3$ while there was no significant difference between shoot N concentration in Fe-EDTA and [Fe(phen)$_3$][Cl$_2$·L$_4$] treatments. [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment remarkably increased root Fe concentration. [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment increased N concentration in shoots 76/98 and 121 % versus Fe-EDTA and FeCl$_3$, respectively (Fig. 9).

The effect of Fe treatment on root N concentration was significant ($P < 0.05$) (Fig. 10). [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment significantly increased root N concentration while there was no significant difference between root N concentration in Fe-EDTA and FeCl$_3$ treatments (Fig. 10).

Shoot and root CAT activity

The effect of Fe treatment on shoot CAT activity was significant (Fig. 11). [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment significantly increased shoot CAT activity while there was no significant difference between shoot CAT activity in Fe-EDTA and FeCl$_3$ treatments (Fig. 11).

The effect of Fe treatment on root CAT activity was significant (Fig. 12). [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment significantly increased root CAT activity while there was no significant difference between root CAT activity in Fe-EDTA and FeCl$_3$ treatments (Fig. 12).

## Discussion

The [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment were synthesized using proper amounts of 4-nitro phenylcyanamide and phenanthroline. Interesting phenomenon in this synthesized is Hydrolysis of Phenylcyanamide to phenyl urea derivatives (Kukushkin and Pombeiro 2005) As it was mentioned in experimental section [Fe(phen)$_3$][Cl$_2$·L$_4$] compound obtains through 0.2 mmol FeCl$_2$·4H$_2$O reaction to 0.4 mmol ligand 4-nitrophenil cyanamide and adding 0.6 mmol white ligand 1, 10-phenanetrolin solution to demethyl formamide. Resulted product is in the form of red crystals and it can be solved in water. The notable consideration in this synthesis is that by adding ligand 4-nitrophenil cyanamide to salt solution of Fe(II) and regarding to the existence of a few water in reaction environment, salt of iron(II) can catalyze hydrolysis reaction of nitrile group of ligand 4-nitrophenil cyanamide and transformation of this group to amid group. Following this reaction ligand 4-nitrophenil cyanamide (Conn 1988) will change and it no longer can connect to metal. Transformation of nitrile

Fig. 4 The effect of [Fe(phen)$_3$][Cl$_2$·L$_4$], Fe-EDTA FeCl$_3$, on the shoot growth of tomato
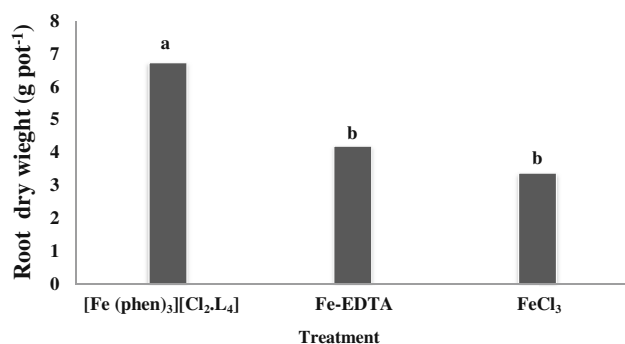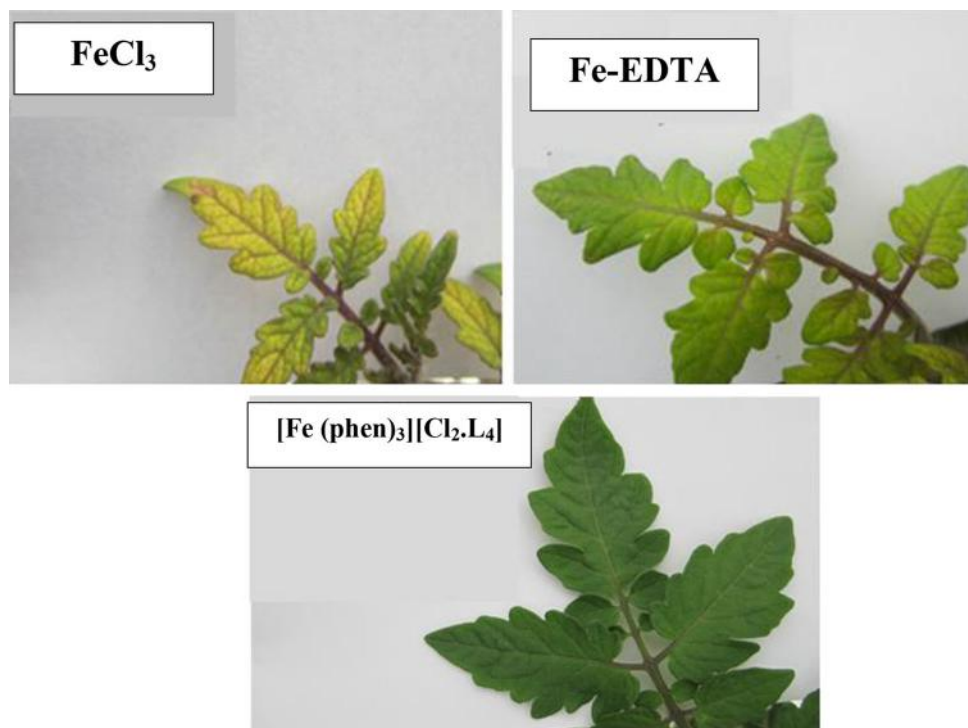




Fig. 5 Root dry of tomato plant grown in nutrient solution containing [Fe(phen)$_3$][Cl$_2$·L$_4$], Fe-EDTA, FeCl$_3$. *Bars* having different letters are significantly different at the 5 % level by LSD
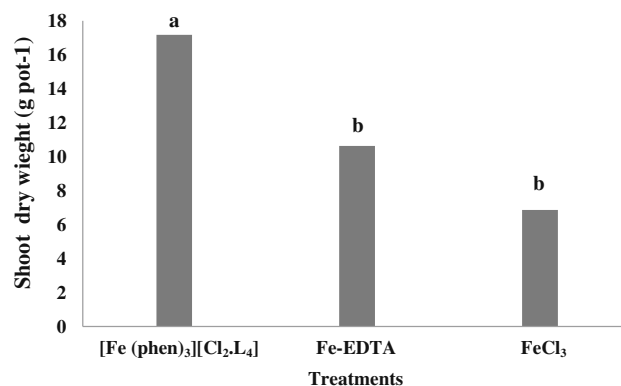
Fig. 6 Shoot dry of tomato plant grown in nutrient solution containing [Fe(phen)$_3$][Cl$_2$·L$_4$], Fe-EDTA, FeCl$_3$. *Bars* having different letters are significantly different at the 5 % level by LSD

ligand 4-nitrophenil cyanamide group to amid group caused that this ligand transforms to an effective treatment for plant growth in the agriculture (Noveron et al. 2001). The results obtained from our study indicated that using Fe-phen chelates in the nutrient solution could supply sufficient amounts of Fe for plant uptake (Figs. 7, 8) and also improve root and shoot growth of tomato plants (Figs. 4). Considering the positive and significant correlation between shoot dry matter yield and Fe and N uptake in tomato plant, it seems that both enhanced Fe and N uptakes play roles in improvement of tomato growth. Phenanthroline is a heterocyclic organic compound. As a bidentate

ligand in coordination chemistry, it forms strong complexes with most metal ions. EDTA is a hexadentate ("six-toothed") ligand.

$$Fe^{+2} + EDTA \rightarrow [Fe(EDTA)]^{3+} \qquad (2)$$

$$Fe^{+2} + phen \rightarrow [Fe(phen)_3]^{2+} \qquad (1)$$
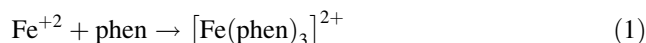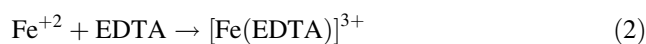
In reaction (1) the bidentate ligand phen forms a chelate complex with the iron ion. Chelation results in the formation of a two–membered ring. In reaction (2) the bidentate ligand EDTA forms a chelate complex with the iron ion. The chelate effect increases with the number of chelate rings so the
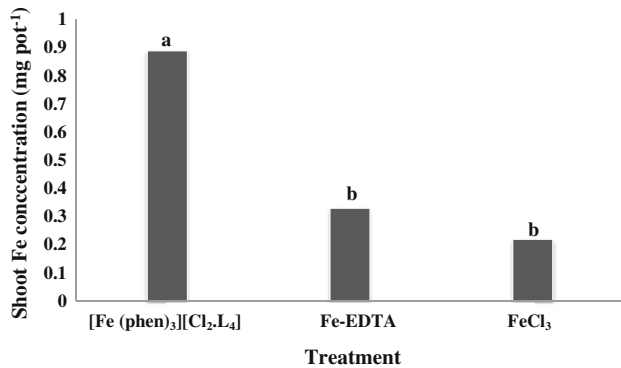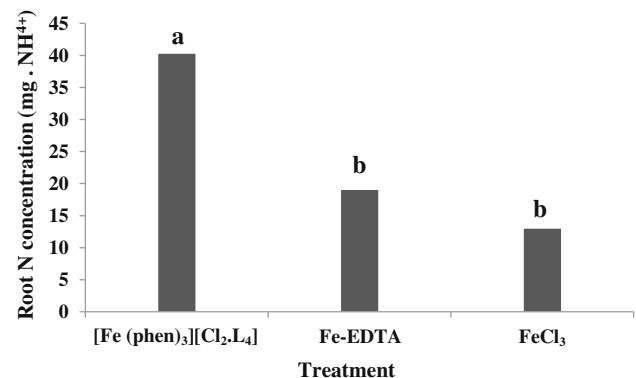
**Fig. 7** Shoot Fe concentration of tomato plant grown in nutrient solution containing [Fe(phen)$_3$][Cl$_2$·L$_4$], Fe-EDTA, FeCl$_3$. *Bars* having different letters are significantly different at the 5 % level by LSD
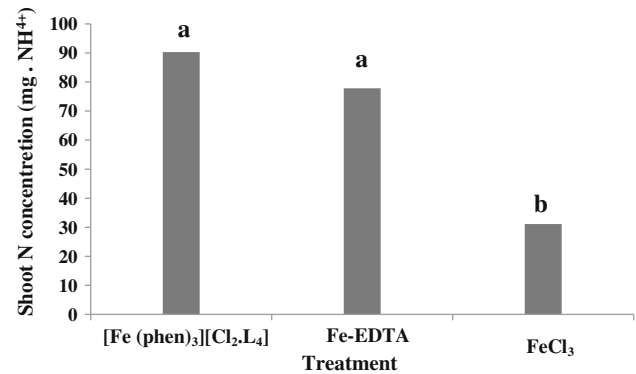


**Fig. 9** Shoot N concentration of tomato plant grown in nutrient solution containing [Fe(phen)$_3$][Cl$_2$·L$_4$], Fe-EDTA, FeCl$_3$. *Bars* having different letters are significantly different at the 5 % level by LSD
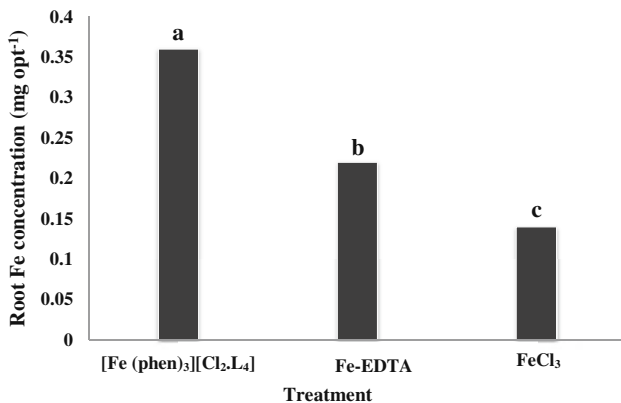


**Fig. 8** Root Fe concentration of tomato plant grown in nutrient solution containing [Fe(phen)$_3$][Cl$_2$·L$_4$], Fe-EDTA, FeCl$_3$. *Bars* having different letters are significantly different at the 5 % level by LSD
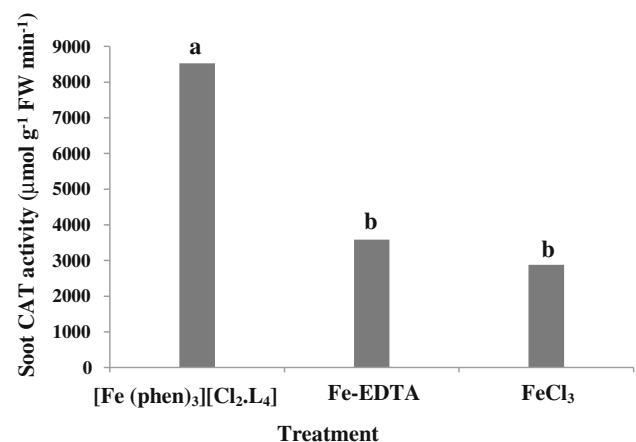


**Fig. 10** Root N concentration of tomato plant grown in nutrient solution containing [Fe(phen)$_3$][Cl$_2$·L$_4$], Fe-EDTA, FeCl$_3$. *Bars* having different letters are significantly different at the 5 % level by LSD

concentration of the EDTA complex, which has six chelate rings, is much higher than a corresponding complex with three bidentate nitrogen donor ligands. Thus, the phenomenon of the chelate effect is a firmly established empirical fact and complex [Fe(EDTA)]$^{3+}$ is very Stable therefore complex [Fe(phen)$_3$]$^{2+}$ can release Fe easier than [Fe(EDTA)]$^{3+}$ (Steed and Atwood 2009).

Improved Fe nutritional status of tomato plants supplied with [Fe(phen)$_3$][Cl$_2$·L$_4$] chelate compared with Fe-EDTA supplied plants could also be related to improved N nutritional status. Urea group can be absorbed by the plant due to [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment let to increased root N concentration.

The effect of Fe treatment on root and shoot Fe content was not significant, it is because of increasing plant functionality due to consumption [Fe(phen)$_3$][Cl$_2$·L$_4$] treatment.(dilution effect) but Fe concentration of root and shoot significantly.



**Fig. 11** Shoot CAT activity of tomato plant grown in nutrient solution containing [Fe(phen)$_3$][Cl$_2$·L$_4$], Fe-EDTA, FeCl$_3$. *Bars* having different letters are significantly different at the 5 % level by LSD
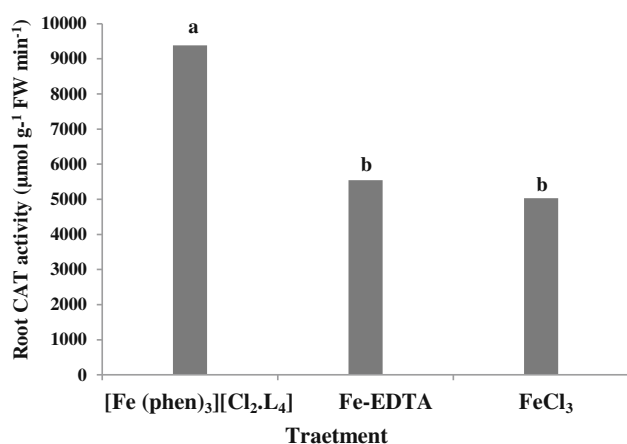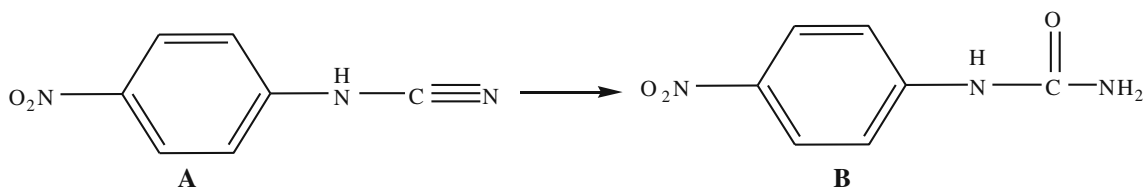
**Fig. 12** Root CAT activity of tomato plant grown in nutrient solution containing $[Fe(phen)_3][Cl_2 \cdot L_4]$, Fe-EDTA, $FeCl_3$. *Bars* having different letters are significantly different at the 5 % level by LSD

Therefore this complex ($[Fe(phen)_3][Cl_2 \cdot L_4]$) can be introduced as a Fe supply for tomato. Furthermore, $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment let to tomato shoot heightening.

Tomato plants supplied with $[Fe(phen)_3][Cl_2 \cdot L_4]$ chelates accumulated higher amounts of Fe in their shoots compared with those supplied with Fe-EDTA and $FeCl_3$. Phenanthroline can form soluble complexes with Fe and thereby play an important role in maintaining Fe availability for the plant.

Regarding that plant obtains its needed nitrogen as $NO_3^-$ and $NH_4^+$ ions it seems that "B" structure easier than "A" structure can be considered as nutrition resource for the plant. As the result, "B" structure is more proper than "A" structure for the plant.

In $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment, root grew better than in Fe-EDTA and $FeCl_3$ so that root was longer and its mass was greater. In $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment, plant stem was also thicker than stems of plants grown in $FeCl_3$ and Fe-EDTA. Daily study of decrease in food solution shows that plants which have grown in $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment have absorbed more water than plants grown in food solution containing Fe-EDTA and $FeCl_3$. Shoot height was noticeably different in $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment and $FeCl_3$ so that length of shoots in $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment was longer than the others (Fig. 4). Plants treated with $FeCl_3$ had the smallest height because of nitrogen and iron deficiency. Results for the effect of treatments on dry and wet weight of branches and root showed that the highest yield was related to $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment and the lowest yield was observed in $FeCl_3$ treatment. The highest iron concentration of branches was related to $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment and the lowest concentration was observed in $FeCl_3$ treatment. The yield of dry weight of shoot and root was significantly increased by using treatment fertilizer, because of dilution effect, iron concentration of shoot and root did not show any significant difference in three fertilizer treatments. So in order to study iron absorption amount through fertilizer treatment, total absorption amount or iron content was used. In $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment root and branches iron absorption was meaningfully more than $FeCl_3$ and Fe-EDTA treatments. So, because $[Fe(phen)_3][Cl_2 \cdot L_4]$ compound increased iron uptake, this compound can be introduced as a resource for providing needed iron for tomato plant. In compare with $FeCl_3$, $[Fe(phen)_3][Cl_2 \cdot L_4]$ and Fe-



On the other hand, 1,10-phenanethroline ligand react to iron and they form iron phenanthroline 4-nitrophenil urea complex which is considered as an effective chelate of iron in agriculture.

No specific signs relating to iron shortage such as leaves paleness as well as nitrogen shortage such as decrease in the stem and bush growth was observed in none of $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatments. Plant leaves were greener and have more freshness and grew better in $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment.

EDTA treatments increased meaningfully nitrogen concentration of branch but no meaningful difference was observed between $[Fe(phen)_3][Cl_2 \cdot L_4]$ and Fe-EDTA fertilizer treatments. Although $[Fe(phen)_3][Cl_2 \cdot L_4]$ fertilizer treatment has nitrogen element in the form of urea, because added nitrogen to food solution is very little in compare with nitrogen needed by the plant and total nitrogen is provided by nitrate calcium, nitrate potassium and nitrate ammonium so the effect of $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment on nitrogen concentration of branches was not meaningful.

In compare with $FeCl_3$ and Fe-EDTA treatments, $[Fe(phen)_3][Cl_2 \cdot L_4]$ fertilizer treatment increased meaningfully nitrogen concentration of the root. Since in compare with $FeCl_3$, $[Fe(phen)_3][Cl_2 \cdot L_4]$ and Fe-EDTA fertilizer treatments increased nitrogen absorption of the branches meaningfully but no meaningful differences was observed between $[Fe(phen)_3][Cl_2 \cdot L_4]$ and Fe-EDTA treatments. Although plant root could absorb urea group in $[Fe(phen)_3][Cl_2 \cdot L_4]$ fertilizer treatment, it is possible that the plant could not transform this compound to the branch so no meaningful differences were observed between nitrogen of the branches in $[Fe(phen)_3][Cl_2 \cdot L_4]$ and Fe-EDTA treatments. In $[Fe(phen)_3][Cl_2 \cdot L_4]$ treatment, nitrogen absorption of the root was meaningfully more than Fe-EDTA and $FeCl_3$ treatments. Probably urea group in $[Fe(phen)_3][Cl_2 \cdot L_4]$ fertilizer treatment could be absorbed by the plant and it caused the increased nitrogen absorption of the whole root in compare with two other treatments.

Regarding to the significant increase in the yield of wet and dry weight of root and shoot and iron and nitrogen absorption and catalase enzyme activity it seems that synthesized complex has an important role in increase of iron and nitrogen absorption as well as the improvement of plant growth.

In aerobic systems whose pH is in physiological scope, the concentration of $Fe^{+3}$ and $Fe^{+2}$ is less than 10 molar. So $Fe^{+3}$ chelates and sometimes $Fe^{+2}$ are dominant forms of iron in soil solution or food solution. Generally speaking bi-capacity iron is the main form of absorbable iron of the plant and it prefers on triple-capacity iron but it depends on plant species. In Fe-EDTA compound, iron oxidation number is +3, and in $[Fe(phen)_3][Cl_2 \cdot L_4]$ compound iron is in the form of 2+. Since the main form of iron absorption in the plant is $Fe^{2+}$, $[Fe(phen)_3][Cl_2 \cdot L_4]$ compound could be more effective in iron absorption by the plant. In complexes which have chelate ligand more numbers of chelate loops (mordant numbers) more stable is the complex. Regarding that EDTA ligand is hexadentate and phenanthroline ligand is bidentate, it is expected that Fe-EDTA complex is more stable than $[Fe(phen)_3][Cl_2 \cdot L_4]$ and to release the iron harder. So $[Fe(phen)_3][Cl_2 \cdot L_4]$ compound that is less stable than Fe-EDTA could increase the availability of iron to the plant. $FeCl_3$ is less stable because of having unidentate ligand and it is possible to analyze quickly and so iron separates from ligand. $[Fe(phen)_3][Cl_2 \cdot L_4]$ complex has created more stability because of bidentate phen in compare with $FeCl_3$. Food solution has no buffer feature like soil, pH changes is high so if Fe cannot be stable in form of complex it would quickly sediment in the form of iron hydroxyl and plant cannot reach to it. Complexes should be so stable that they could keep iron available for the plant during the growth period while it could release the iron in the surface of the root because ion absorption of iron by the plant is harder than molecular complex. According to Ghasemi's paper amino acid-metal complexes are created from alpha amino acids reaction to bi-capacity or multi-capacity metal ions and formation of heterocyclic loops structure. Amino acids have two free heads N, O for connecting and linking with metals and are considered as bidentate ligand like a phenanetrolin ligand. Amino chelates made in Ghasemi's paper have a pentagonal loop which is created by the reaction between two oxygen atoms of carboxylate group and nitrogen amino acid to iron ion (Ghasemi et al. 2012). Based on results from FT-IR spectrums amino acids act as bidentate ligands and compose iron chelates. Research results showed that feeding of iron amino chelates will cause meaningful increase in iron absorption of root and branches in tomato item. In addition to increasing iron absorption iron amino chelates can have an important role in improvement of plant nutrition by increasing activity of enzymes which contain iron.

## Supporting information

Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary materials (CCDC No. 997210).

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