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Research article

The valorization of iron ore Jerissa in agronomy as iron chelate/complex (EDTA/Fe (S), HBED/Fe (S) and D/Fe (S)) for alleviate iron deficiency in calcareous soil

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Abstract

Iron is one of the essential micronutrients for plants, but it can't absorb in calcareous soils due to its low solubility. This condition requires the treatment by iron based chelates or complexes. In this work, it has been evaluated the efficacy to applicate iron ore Jerissa as an iron fertilizer in alkaline soils for alleviating iron chlorosis and to improve the ferric state of the plant. Three product EDTA/Fe (S), HBED/Fe (S) and Digestat/Fe (S) were prepared with natural iron and applied in soil to study their potentiality on the correction of iron deficiency in Tomato plants. The trial was setup in randomized block design with three replications for each treatment and three plants per pot in order to supply sufficient dry matter. Different physical and chemical analyses were used for characterized iron ore Jerissa. The SPAD chlorophyll index, iron content in plants and iron in soils were determined at the end of the trial. The three treatments have increased the iron content in the plants and correct iron deficiency. Iron ore Jerissa with different complexing agents could be useful to prevent iron chlorosis and reducing soil pH.

Key words: Valorization, Siderite, Limestone soil, ferric chlorosis, iron fertilizer, Tunisia.

1. Introduction

Tunisia is a small country with a total land area of 163,610 Km^2, from which 50 % is arable land. Tunisia is a country dependent on agriculture in their economy (11, 6% of the GDP). Then, Due to the aridity of the climate and the calcareous nature of the geological substratum that characterize almost area in Tunisia, the soils are in most limestone with 45% (Mtimet, 2016). Approximately half of the cultivated area (5 M hectare) has be planted in calcareous soil or irrigated by water rich in calcium carbonate (pH superior to 7.5). Therefore, limestone soils cover more than 30% of the earth's surface (Marschner, 1995).

In this work, we treated one of a major nutritional problem limiting agricultural production in Tunisia and in many other countries in the world as Spain, Italy and turkey, the problem of iron (Fe) deficiency in calcareous or alkaline soils. Iron chlorosis is a nutritional disorder limiting agricultural production and causes severe economic losses. It is not caused by an absence of iron in the soil but by a very low iron availability in calcareous soils (Álvarez-Fernández et al., 2006a; Rombolà et al., 2006). Their fertilization with the iron chelates or complex thus became essential to have a satisfactory rate of return. In this study, we have proposed as a solution to treat this problem the use of iron ore Jerissa with some chelate ligands (EDTA, EDDHA and HBED).

Iron ore Jerissa (Siderite) in Tunisia could be an important and profitable practice in agriculture field such as a mineral amendment or fertilizer for plants in alkaline soils. This iron ore is encountered as a deposit of iron carbonate and Hematite/Goethite in northwestern of Tunisia (Kef) where it is generally used as a raw material in the industrial field such as manufacturing steel (Mahjoubi., 1978; Aissaouia et al., 1988; Mlayah et al., 2011).

Given the importance of iron as micronutrient for achieving high productivity of calcareous agricultural land, efforts are made to improve it by the application of iron fertilizers. Fertilization with iron chelate is the most effective agriculture practice to correct iron deficiency in alkaline conditions. Studies over the last twenty years have shown the usefulness of some iron fertilizers to prevent ferric chlorosis in plants. Iron deficiency can be corrected by application of iron chelates (Hernández Apaolaza et al., 1997; Álvarez-Fernández et al., 2004; Lucena, 2006).

Iron chelates are synthetic organic compounds that contain Fe in a complex form, protect against the reaction in soil and maintain iron in the soil solution (being available to the plants). Additional, plants can absorb soluble chelate as complete molecules and then metabolize the metal (Hagin and Tucker., 1982). The effectiveness of iron chelates varies greatly, depending on soil pH (Lucena, 2006).

EDTA (iron ethylene diamine tetraacetate) has six electron donor groups in its structure: two amines and four carboxylic acids. The chelates formed by EDTA, despite having the same number of bonds with the metal, as the o, o-EDDHA and the absence of phenolic groups give it less stability. EDDHA (ethylendiamine diohydroxyphenylacetic) acid) is prepared by a Mannich-like reaction between phenol, ethylenediamine and glyoxylic acid (Petree et al.,1978). The o,oEDDHA positional isomer is found to form the most stable complexes with Fe (Hernández-Apaolaza et al., 1997; Yunta et al., 2003a) and presents two regioisomers with different agronomic efficacy (Cerdán et al., 2006). Fe/ EDDHA products are employed to treat Fe chlorosis in crops grown on calcareous soils (Ahrland et al., 1990; Gomez-Gallego et al.,2005, 2006; Yunta et al., 2003a).

Then, N, N´-bis (2-hydroxybenzyl) ethylenediamine-N, N´-diacetic acid (HBED) is a great iron chelating agent (Brittenham, 1992; Bergeron et al., 2002). Its structure is identical to that of o,oEDDHA and forms a very stable Fe chelate (Chaney, 1988) . Nawrocki et al. (2009) suggest a process for the synthesis of HBED that yields a treatment with high chelated Fe content of about 9%. This propriety makes the chelate more effective and environment friendly for use as iron fertilizer when compared to other treatments.

Some environmentally friendly ligands have been used to prepared iron complexes as organic acids lignosulfonates, humic and fulvic acids, gluconic acids and flavonoids (Villen et al., 2007). Those natural and organic agents present lower stability in soil than chelates (Lucena, 2009). Many authors were explaining the beneficial effects of humic substances by their capacity of complexing iron in the soil. Stevenson (1994) noted that humic material is the most stable part of the organic matter in the soil. Humic acid is an essential role in agricultural processes; it increases the ability of cation exchange and increases fertility (Rodríguez, 2010). Therefore, it has many benefits; improves the soil structure by the formation of organometallic complex, improves the development of roots and the life microorganisms in the soil, regulates soil pH, retain the mineral elements incorporated in the soil and reduce their loss by leaching.

The aim of this study was to valorize the natural iron ore of Jerissa ($FeCO_3$) in agronomy as iron fertilizer. Specifically, the study of the efficacy of synthetic products prepared with natural iron ore and complexing agents by their application in calcareous soil cultivate with Tomato to correct iron deficiency was realized. The efficiency of the three products (HBED/Fe (S), EDTA/Fe (S) and Digestat/Fe (S) compared with commercial chelates (EDDHA/Fe (C) and HBED/Fe (C)) as Fe fertilizers have been assessed by soils and foliar analysis of Tomato grown on calcareous soil (El Manar).

2. Material and methods

2.1. Treatments and experimental design

The experiment was consisted of three iron treatments prepared by natural siderite (S) of Jerissa (EDTA/Fe (S), HBED/Fe (S) and Digestat/Fe (S)), two commercial (C) chelates (EDDHA/Fe(C) and HBED/Fe(C)) and one control without iron (Control -) in order to apply it in alkaline soil to correct iron deficiency in Tomato plants (Table 1).

Table 1. Different iron treatments used in the Fe chlorosis correction experiment.

Treatment	**Agents chelating/ complexing**	**Iron**	**Molarity**	**pH**	**Volume**
HBED/Fe (S)	HBED	Siderite	0.001M	4.92	100ml
EDTA/Fe (S)	EDTA	Siderite	0.001M	5.1	100ml
D/Fe (S)	Digestat	Siderite	0.001M	8.1	100ml
Without iron (Control -)	-	-	-	-	-
EDDHA/Fe (C)	EDDHA	Fe	0.001M	7.5	100ml
HBED/Fe (C)	HBED	Fe	0.001M	8	100ml

Chelates and complexes solutions were prepared with equal molarity 0.001M and 60 µmol Fe/pot (3.35 mg Fe per pot). During the preparation of treatments solution, EDTA/Fe (S) and HBED/Fe (S) the pH was maintained between 5.0 and 7.0 to facilitate the preparation of the complex, and finally it was adjusted to 5.0. The pH of Digestat/Fe (S) was regulated to eight. A randomized complete block design using three replicate pots per treatment were employed with a three plants per pot. Treatments were applied when plants showed chlorosis symptoms and were daily irrigated up to 80 % saturation (Nadal et al., 2009) with nutritive solution. Two measurements by Regular Soil and Plant Analyzer Development (SPAD) every week were effected.

Tomato plants were used in this experiment since they are considered susceptible to chlorosis and are considered as a model for crops treated with iron chelates.

2.2. Chemical and physical characteristics of the soil

The soil was taken from land located in north of Tunisia (El Manar City). The chemical properties of soil El Manar were analyzed as follows: pH water = 8.02, EC =16.7µS/Cm, $CaCO_3$ = 43.63 % and a soltanpour extractable iron content = 0.1mg/Kg. The particle size analysis and X-ray diffractometric were showed that the soil is a sandy loam (Figure 1).

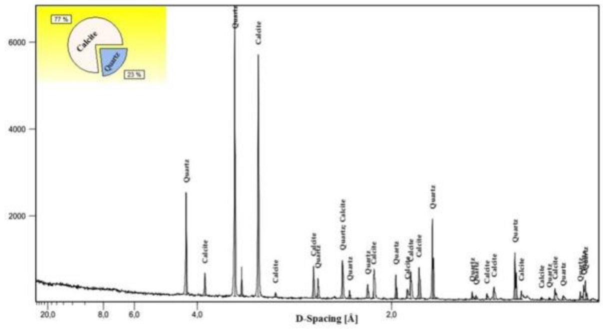


Fig. 1. X-ray diffractometric analysis of soil El Manar.

2.3. Localization and characteristics of the iron ore Jerissa

The iron ore was from Jerissa located in northwest Tunisia, 220 km SW of the city of Tunis. Geochemical analysis was performed at ICP for minor elements (ppm) and by atomic absorption for major elements (%) (Table 2). They were supplemented by a mineralogical study based on X-ray diffractometric analysis using the powder method (Figure 2A) and the analysis of FTIR spectra on a Bruker IFS66vd spectrometer (Germany) using KBr pellet method in the 4000-500 cm^{-1} region at a resolution of 4 cm^{-1} in the transmittance mode (Figure 2B). Iron ore Jerissa is composed by siderite as major elements (52 %) and calcite ($CaCO_3$), Quartz (SiO_2), Clay, Zn, Cu and Organic Matter. The FTIR spectra of siderite sample was showed, a broad band at 3407 cm^{-1} due to the vibration of hydroxyl groups, bands in 1805 and 1623 cm^{-1} assigned to carboxylate anion were observed. In addition, bands at 2923-2856 and 2513 cm^{-1} were associated with C-H stretching vibration (Tonković, 1983) and the vibrations of Fe-O were presented in the region 1000-600 cm^{-1}. The main chemical properties of these ore are pH water: 8.09, EC: 451µS/Cm and $CaCO_3$: 20.3 %.

Table 2. Chemical composition of iron ore Jerissa by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Major elements (%)		**Trace's elements (ppm)**	
Fe_2O_3	53.06	Cu	13
SiO_2	2.51	Mn	5470
Al_2O_3	0.09	Zn	93
MgO	2.37	Ni	3
CaO	9.02	Pb	9
K_2O	0.30	Co	29
Na_2O	0.08	Ti	8

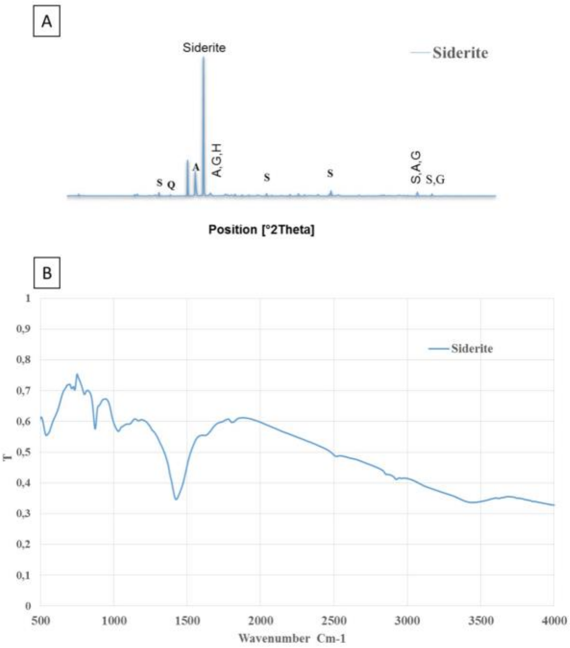


Figure 2: A: X-ray diffractometric analysis of iron ore Jerissa with S: Siderite; G: Goethite; H: Hematite; Q: Quartz; C: Calcite; A: Ankerite and B: Infrared spectra between 500 and 4000 cm ^{1}, the sample composed of siderite, calcite, Hematite, and goethite.

2.4. Origin and characteristics of Digestat (Humus substance)

Digestat (D) was provided by the national waste management agency (ANGED) from Thibar farm. The wastes are of agricultural origin and consist mainly of excrement Animals. The Digestat is a solid or pasty liquid residue composed of organic elements and of minerals. This product is derived from the methanisation, which is an anaerobic digestion process.

The chemical characteristic of Digestat were summarized in Table 3.

Table 3: The chemical characteristics of Digestat.

	pH	EC µs/cm	OC (%)	OM (%)	ON level (‰)
Digestat	9.1	3940	28.29	48.8	0.82

2.5. Plants and Soil analysis

2.5.1. Plant analysis

SPAD readings with a chlorophyll meter (Minolta SPAD) were taken two times per week during the experiments in three leaves. After 8 weeks of treatment, plants were harvested. The leaves, stem and roots were separated, washed with 0.1 M HCl and distilled water (Álvarez-Fernández et al., 2001), subsequently weighed (fresh weight) and dried in an oven at 80 ° C for 3 days, followed by grinding and weighing. 0.25 g of the plant material underwent acid digestion (4 ml H_2O, 1.5 ml HNO and 1 ml H_2O_2) by autoclaving at 480 °C for 2 h (Jones, 2001) followed by filtration of the solutions. Fe concentration was determined in leaves, stems, and roots by atomic absorption spectrometry (AAS) (Perkin-Elmer Analyst 800).

2.5.2. Soil analysis

The soils were recovered for the measurement of the following parameters: pH, soluble and available iron. The complete pot contents were immersed in 1 litre-distilled water and shaken until total disaggregation of the substrate. 40 milliliters of the soil–water mix was centrifuged and the supernatant filtrated. Soluble iron was determined in these solutions by AAS. The solid in the centrifuge tube was extracted with 25 ml of Soltanpour and Schwab, (1977) extractant and filtered with 0.45 µm Millipore membranes. The extraction was repeated three times in total, the extracts joined, and volume made up to 100 ml. HNO_3 was added to remove excess bicarbonate and analysis of iron content by AAS.

2.6. Statistical analysis

All statistical analyses were conducted with SPSS 24.0 statistical software. All the data were analysed from each treatment with three replicates.

3. Results and discussion

3.1. Response of Tomato plants to different treatments of iron chelates EDTA/Fe(S), HBED/Fe(S)) and iron complex D/Fe (S)

It is well known that, firstly Fe plays a major role in the growth and development of plants and second, Tomato responds relatively well to ferric fertilization. As shown in Figure 3, it was observed that iron treatments applied in calcareous soil had significantly impact on shoot dry matter weight (DW) after 8 weeks of treatment. The adding of iron chelate (EDTA/Fe (S), HBED/Fe (S)) and iron complex D/Fe (S) were contributed to increase the shoot dry weight than negative control plants. The great yield in dry weight of aerial biomass was obtained by the HBED/Fe (S) treatment (3.97g DW). Further, EDTA/Fe (S) showed a medium result on the yield of Tomato plants grown in limestone soil compared to control (-) by 1.97 g DW. D/Fe (S) showed a lower yield than the two iron chelate (EDTA/Fe (S) and HBED/Fe (S) but remained well compared to the negative control (without iron).

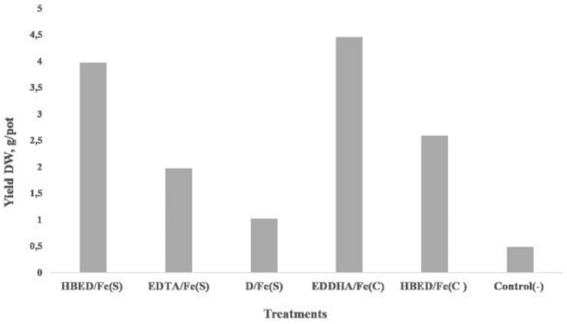


Fig. 3. Dry weight (DW) yield in g per pot of the aerial biomass of Tomato according to the different treatments (HBED/Fe (S), EDTA/Fe (S), D/Fe (S), EDDHA/Fe (C) and HBED/Fe (C)).

Moreover, the results in the Figure 4 presented the length of plants treated with iron treatments that could explain the growth rate of plants and confirm the results obtained by shoot dry matter. Noting that plants untreated were showed a small length with yellow leaves that is meaning the bad growth of plants with iron chlorosis. Then, the best length was recorded in the plants treated with HBED/Fe (S) (41.33 cm) in comparison with EDTA/Fe (S) and D/Fe (S) treatment.

In addition, visual differences between treated and untreated (Control (-)) plants were very clear and providing the same information indicated by the SPAD chlorophyll index. Control (-) plants showed slight chlorosis symptoms and low SPAD values (Figure 5). The chlorophyll value, which is related to the efficacy of the iron chelate/Complex applied in the soil, was higher when plants were treated with HBED/Fe (C), EDDHA/Fe(C), HBED/Fe (S) EDTA/Fe(S) and D/Fe(S) than control (-). HBED/Fe (S) was work as commercial chelates (HBED/Fe (C), EDDHA/Fe(C)), almost the same curve from the second reading SPAD to eighth SPAD measurement.

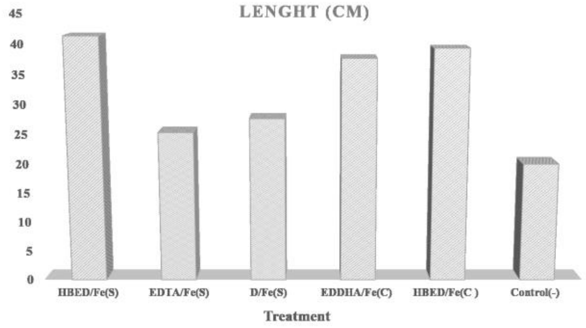


Fig. 4. Effect of the different iron treatments (HBED/Fe (S), EDTA/Fe (S), D/Fe (S), EDDHA/Fe (C) and HBED/Fe (C)) on the length of plants.

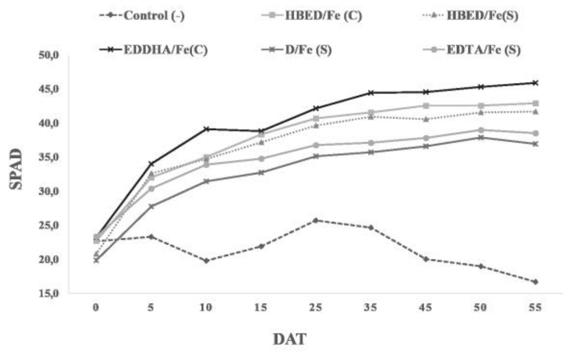
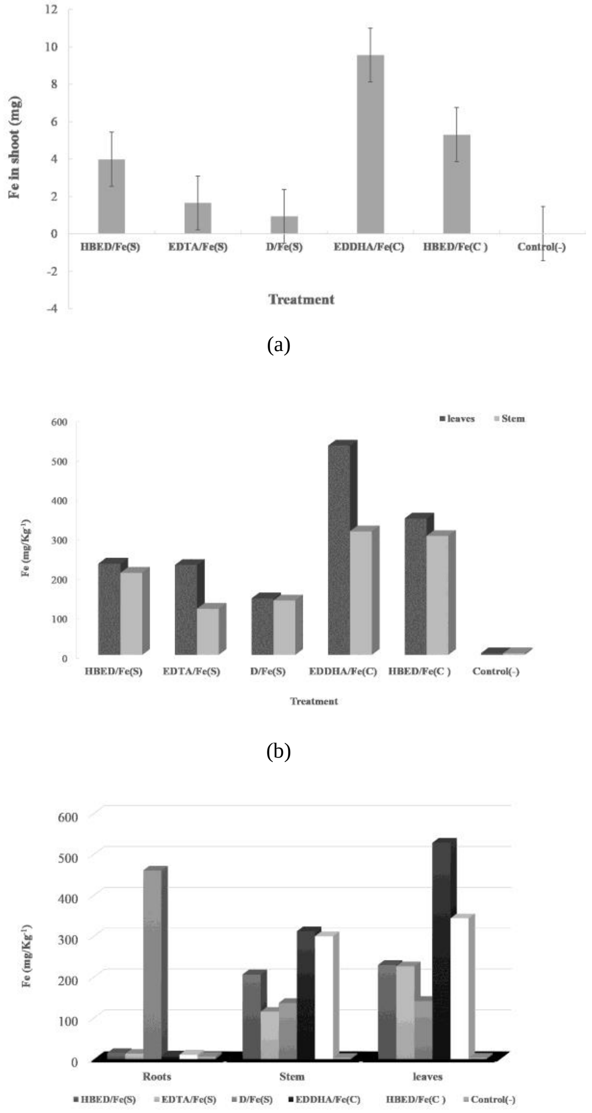


Fig. 5. SPAD index for the leaves of Tomato plants grown in calcareous soils treated with different treatments of iron chelate/Complex carriers at different times.

Results in Table 4 were demonstrated that the three iron products imposed a good effect on iron concentration in shoot of Tomato plants. However, the uptake of iron by the roots after their interaction in soil and the iron content in the shoots were studied by comparison between the three iron treatments with the two commercial iron chelates (see Figure 6 a, b, and c). Based on foliar analysis the three treatments prepared by iron Jerissa showed positive results in Fe amount compared to the negative control. Fe amount in dry aerial biomass of plants grown on untreated soil (0.013 mg Fe) was very lower than those plants treated by iron products HBED/Fe (S), EDTA/Fe (S) and D/Fe (S).

The plants treated with commercial iron chelate EDDHA/Fe (C) used as a positive control presented the most important iron nutrition than plants treated with HBED/Fe (C) and the other three treatments with siderite. Almost all the amount of iron added to the soil goes up to stem and leaves; this latter is explained by the great dry matter yield value recorded in the plants treated with EDDHA/Fe (C) (Figure 4) and the high stability, durability of EDDHA agent. The efficacy of commercial iron chelates containing EDDHA has been widely demonstrated since the 1950s (Wallace et al., 1955; Hill-Cottingham and Lloyd-Jones, 1958; Barak and Chen., 1987; Lucena et al., 2006; Schenkeveld et al., 2010).



(a)

(b)

(c)

Fig. 6. (a): Iron amount (mg) on the shoot after treatment application. Error bars denote standard error (SE); (b): Iron concentration (mg/Kg^{-1} DW) in leaf and stem after treatment application in calcareous soil; (c) Effect of the different iron treatments on the Fe concentration (mg/Kg^{-1} DW) in roots stems and leaves in the calcareous soil.

As expected, the three iron treatments assayed were able to alleviate the Fe chlorosis in the plants in high pH conditions, with significant differences were found among the treatments. The study of iron concentration results in different parts of Tomato plant (leaf, stem, and root) was showed that plants treated with HBED/Fe (S) contains more Fe than those treated with EDTA/Fe (S) and D/Fe (S).

HBED/Fe (S) was increased the Fe content in the shoot and improved iron nutrition in plants. Leaves and stem of plants treated with HBED/F (S) were recorded 230.45 mg/Kg^{-1} and 207 mg/Kg^{-1} Fe respectively. However, a small Fe amount in the roots was observed. In this context, the product HBED/Fe (S) provides similar amount of iron in plants than HBED/Fe (C) commercial product, which confirmed the efficacy of siderite Jerissa with HBED to provide Fe to chlorotic Tomato plants in alkaline conditions (Figure 7). This result is consistent with the results of Nwrocki et al., (2009) who found that HBED-based iron chelates increased the content of this element in leaves, stems, and roots.

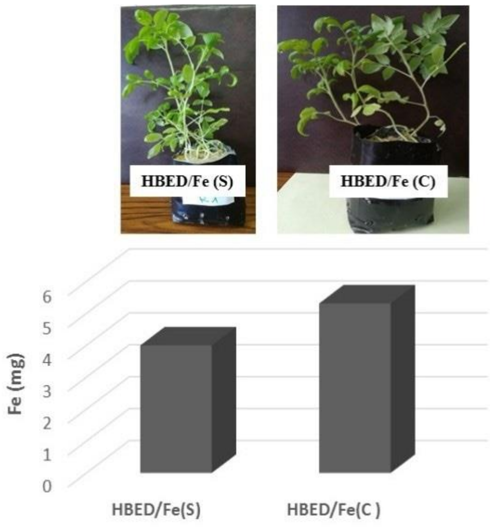


Fig. 7. Comparison between iron content (mg) in shoot of plants treated with (HBED/Fe (S) and shoot of plants treated by commercial iron chelate (HBED/Fe (C).

Then, this finding agrees with Chaney (1988) and Nadal et al., (2009) concluded that HBED/Fe supplied sufficient iron for the growth of soybean plants in hydroponic cultures at pH 7.5. However, López-Rayo et al., (2009) demonstrates that HBED/Fe could be a good fertilizer for correcting Fe chlorosis in a single application in soils with high lime content, because of its high stability and low reactivity with the soil mineral phases.

Regarding EDTA/Fe (S), we observed a medium iron content in plants treated with EDTA/Fe (S), when it was compared to the commercial chelate EDDHA/Fe (C) and HBED/Fe (C). EDTA/Fe (S) showed a better Fe concentration in stem and leaves than control negative plants. These results are consistent with the work of Schenkeveld, (2010) based on the stability of Fe chelates in solution. He concluded that EDDHA stabilities with Fe were greater than EDTA/Fe. In addition, the latter is confirmed by the study of Sandra Lopez et al., (2012); EDTA is a non-phenolic chelating agent able of protecting Fe from dissolution or precipitation in the soil despite its low stability compared with other more stable agents as EDDHA and HBED. Consequently, EDTA can formed chelate with iron of Jerissa but with a low stability then it able to solve the Fe deficiency in plants.

The iron complex used in the soil experiment D/Fe (S) improve the ferric status of plants very slowly than the two iron chelates (EDTA/Fe (S) and HBED/Fe (S)). The leaves were recorded 142.67 mg/Kg^{-1} Fe and the largest Fe amount remains in the roots level. According to Rodriguez et al (2010), organic materials can improve the physical nature of the rooting medium and provide plant nutrients in a slow-release form, facilitating vegetation establishment. Nevertheless, when D/Fe (S) treatment was compared with control (-) considered as a good iron complex; Digestat complexing agent was facilitated the mobilization of siderite in soil (at pH 7) and to supply iron to plants but this latter needs a lot of time for transferring the iron from the roots to the leaves. Rodriguez et al. (2009, 2010) have studied in several works the effectiveness of various iron complexes on plants grown in hydroponic culture or in soil. If we compare the three treatments between them, we find this order: HBED/Fe (S)> EDTA/Fe (S) > D/Fe (S). The positive response of Tomato plants to the contribution of iron ore treatment would be due to the beneficial effects of Fe element with the other metallic and organic elements on the growth rate of plants. Thus, the results obtained by Fe foliar analysis demonstrates the possibility of use natural siderite of Jerissa in agriculture as an iron fertilizer (HBED/Fe (S), EDTA/Fe (S) and D/Fe (S) to correct ferric chlorosis in plants grown on calcareous soils.

3.2. Soluble and available iron in the soil

The soil has a sandy texture rich in calcium carbonate (43.6%) and having an initial pH of 8.06. These physicochemical properties are favorable to the appearance of ferric chlorosis (Mengel et al., 2001). Each pot in our experiment was amended by the same amount of iron (60 µmol/pot) with one of the 5 treatments prepared (EDDHA/Fe (C), HBED/Fe (C), HBED/Fe (S), D/Fe (S), and EDTA/Fe (S)) except for the negative control pots (Without iron) and these were carried out for 8 weeks. The soluble and the available fraction of iron in different pots were studied for understand the interaction between treatments and soil. Concentration of Fe in the soluble fractions of the soils are presented in Figure 8. Pots treated with EDTA/Fe (S) showed a less concentration of soluble iron than the other pots treated. Then, no significant differences could be observed among the iron treatments EDDHA/Fe(C), HBED/Fe(C) and HBED/Fe (S) regarding the Fe soluble in soil. Iron in the available fraction, significant differences could be recorded in pots treated and control (-Fe) pots. The results illustrated in Figure 8 were shown iron concentration of available fraction between 0.66 and 2.73 mg/Kg^{-1} Fe.

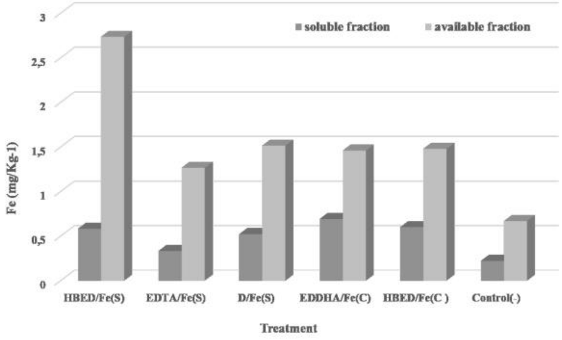


Fig. 8. Fe concentration in the soluble and available fractions of the soils after the harvest of plant experiment.

4. Conclusion

The effectiveness of the application of iron ore with HBED, EDTA, and Digestat has been proven in this work to alleviate iron chlorosis in Tomato plants and to maintain iron soluble in soil conditions. Indeed, efficient use of the fertilizer involving the optimization of the benefits in terms of crop yield and absorption of Fe by plants. The difference in content of Fe in the plant depends partially or completely on the chelating agent in interaction with the soil. All results indicate that iron fertilizers (chelate or complex) prepared with iron ore Jerissa can be a good agricultural practice to correct Fe deficiency in corps. Further HBED/Fe (S), EDTA/Fe (S) were improved Fe nutrition in plants faster than D/Fe (S) due to its high stability in soil and its long lasting effect providing Fe to plants. Digestat with iron ore Jerissa is apparently less efficient than iron chelates in soil. But it is capable to correct iron deficiency in long time and it provide iron available in the soil roots interface that allows a slow uptake of iron by the plants.

This work about raw iron proves to be an interesting topic in the agriculture field from the study of valorization on the one hand of local and natural iron ores and on the other hand to diversify agricultural inputs to improve the economy of countries.

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