

Research article

## The use of natural iron ore Tamra as ferric treatment to correct iron chlorosis in Tomato grown on calcareous soil

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### Abstract

Iron deficiency is a nutritional disorder limiting agricultural production in Tunisia and other Mediterranean countries. Studies about iron fertilization have gained importance in agronomy to improve the productivity. Then, agricultural areas with pH  $\geq 7.5$  affected by iron deficiencies need to be treated. The application of iron chelate /complex, capable of maintain this micronutrient in solution even in soils with high pH, is the most practice to remedy iron chlorosis. The aim of this work was to evaluate the application of iron ore Tamra as iron chelate/complex in calcareous soil El Manar and to study their efficacy for treated iron chlorosis in Tomato. Experimental trials of tests iron ore Tamra as fertilizers was conducted in laboratory during 65 days in random block with three replicates for each treatment. The all of iron treatments (EDTA/Fe (T), Digestat /Fe (T) and EDDHA/Fe) were equal in iron dose (50 $\mu$ mol Fe) and were applied after to the set in of iron chlorosis in plants. The effect of iron ore Tamra to correct iron deficiency has been examined in soil and plants by AAS analysis. The results of applying EDTA/Fe (T) and Digestat/Fe (T) showed positive results and correct the ferric chlorosis with different content but rest lower than the results obtained by EDDHA / Fe. The use of raw iron from Tamra has a good effect on Tomato plants grown in alkaline soil, so this latter confirm the potential valorization as iron fertilizers in agronomy. It can bring positive economic and social effects in Tunisia.

**Key words:** Calcareous soil, Digestat, EDTA, Fertilizer, Iron ore Tamra, Tunisia.

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### 1. Introduction

The Tamra mine, located in the Nefza mining district (NW Tunisia), exploits a 50 m-thick of Mio-Pliocene heavily Fe-mineralized sediments with evidences of other metals (Mn, Pb, Zn) (Decree 2008). The Tamra mine studied in this paper is known since Roman times (Berthon 1922). It has been actively exploited since 1884 and iron ore is nowadays used in the local cement industry. In 1950, the estimated tonnage of the Tamra mine was 1.6 Mt at an average grade of 50 % Fe (Gottis 1952).

In agronomy, iron (Fe) is an essential micronutrient for its role in the formation of chlorophyll and in various enzymatic processes (Marschner 1995). A low bioavailability of Fe may lead to Fe chlorosis in crops. Fe chlorosis is a nutritional disorder characterized by a significant decrease of chlorophyll in the leaves. Kirkby and Römheld 2004; Tagliavini

and Rombola 2001 noted the typical symptoms of iron deficiency in plants are the yellowing of young leaves. Kirkby and Römheld 2004 explained that yellowing due to iron chlorosis was caused by several factors: high calcium and bicarbonate content in soil, increased phosphate fertilization and soil hardness. Iron deficiency is characteristic of Tomato plants growing in calcareous soils, where the lack of Fe shortens the growth of plants, decreasing yields and resulting in poor quantity and quality fruit. Most alkaline soils are found in areas of the reference soil groups calcisols and solonchaks, which cover nearly 10 million km<sup>2</sup> of the earth's surface (FAO/AGL 2000). Tunisia is one of countries open to Mediterranean so superior to 50 % of soil in Tunisia are calcareous (Mtimet 2016). In general, there are two solutions of correcting Fe deficiency. First way is the gentic improvement of corps, second way is the

use of iron fertilizers (inorganic salts, lignosulfonates, chelater...).

Amino polycarboxylic acids, such as ethylenediamine tetraacetic acid (EDTA) or ethylenediamine-2-hydroxyphenylacetic acid (EDDHA), are currently the main synthetic chelating agents used to tackle iron chlorosis. The best common treatment to correct Fe chlorosis in plants is the application of synthetic Fe chelates (Chen and Barak 1982). Fe/EDDHA is the most effective Fe fertilizers on alkaline and neutral soils (Lucena et al., 1992b; Reed et al. 1988). Fe/EDDHA is very stable in a wide pH range (Yunta et al., 2003a ; Yunta, et al. 2003b). Iron chelates are the best solutions for correct Fe chlorosis, but they are very expensive.

Then, there are many studies about beneficial effects of humic substances were explained by their capacity of complexing iron in the soil and treated Fe chlorosis. Stevenson (1994) noted that humic material is the most stable part of the organic matter of the soil. Yılmaz (2007) explained that acids Humic contribute to soil pH modification, which increases the availability of trace elements of the plant. Sanchez et al., (2006) examined the grapes grown in Spain for their response to organic compounds in improving iron uptake by the plant. He found that the use of humic and a combination of amino acids with chelates iron has been fed iron and the effect of the humic was greater than the effect of amino acids in iron absorption.

The aim of this research was to apply natural iron ore Tamra in agronomy as iron treatment for treated iron deficiency in alkaline soil. To examine the effect of different iron treatments in calcareous soils cultivate with Tomato, a group of analysis were done. The efficacies of iron products prepared with iron ore and the agents chelate/complex were compared with commercial chelate EDDHA/Fe (positive control) and with negative control (without iron).

**2. Materials and methods**

*2.1. Soil, iron ore Tamra and Digestat*

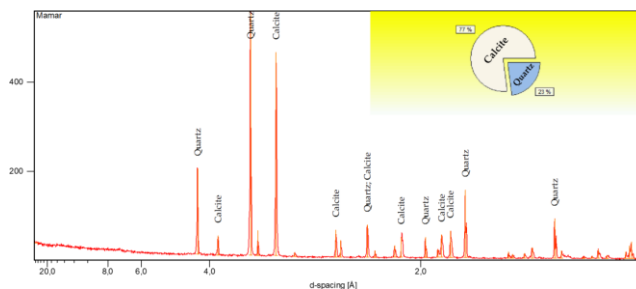
Soil was collected from north Tunisia, located in El Manar site from the top layer (0 – 20 cm). In crops grown at this site, Fe chlorosis was manifest because rich in CaCO<sub>3</sub>. Pre-treatment consisted of drying and sieving (2 mm).

The characteristics of the El Manar soil are given in Table 1 and Figure 1. We note that the soil el Manar is very poor in organic matter and rich in CaCO<sub>3</sub> (43.63%). It consists essentially of sand (80%) with a

pH: 8.02 and electrical conductivity (EC): 16.7µS/Cm. The application of iron ore will therefore improve the iron situation of the plants in order to guarantee good growth.

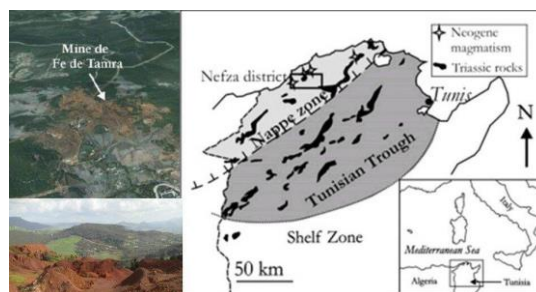
**Table 1.** Characteristics of soil from El Manar city

| Parameters | pH   | EC (µS / Cm) | CaCO <sub>3</sub> % | Organic matter % |
|------------|------|--------------|---------------------|------------------|
| Values     | 8.02 | 16.7         | 43.63               | 0.02             |

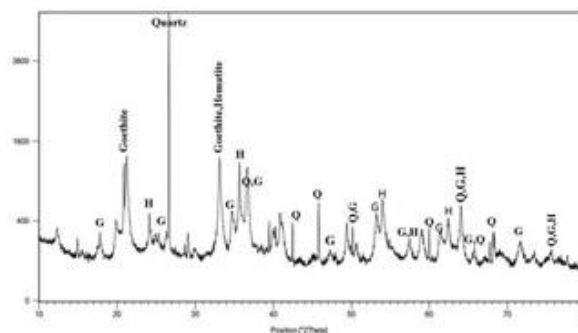


**Fig. 1.** X-ray diffractometric analysis of calcareous soil from El Manar city

Iron ore Tamra (T) was sampled from the Tamra iron mine located in the NW of Tunisia (Nefza region) (Figure 2). An economic deposit of lateritic Fe oxides (Mio-Pliocene). This ore is composed by Goethite - Hematite and Limonite as major elements (Figure 3 and 4). The main chemical properties of ore are pH: 5.01; EC: 136 µS / Cm; CaCO<sub>3</sub>: 4.27% and Fe %: 39%.



**Fig. 2.** Location map of the Tamra mine in northern Tunisia (modified from Rouvier et al., 1985; Savelli, 2002).



**Fig. 3.** XR analysis of iron ore Tamra that composed by G: goethite; H: hematite; Q: Quartz

Digestat (D): In this work, we tested synthesized Digestat (humus substance) produced naturally by animal waste from the Thibar farm (stable organic matter), for its suitability to mend iron chlorosis in Tomato. The Digestat is a liquid residue composed of organic elements and of minerals (See Table 2).

**Table 2:** Chemical characteristics of Digestat.

| Parameters | pH   | EC ( $\mu\text{S}/\text{Cm}$ ) | OM (%) | OC (%) | ON (%) |
|------------|------|--------------------------------|--------|--------|--------|
| Values     | 6.01 | 39.40                          | 48.8   | 28.29  | 8.2    |

## 2.2. Experimental solutions

Three iron solutions were used in the interaction pots experiment (Table 3) : (1) The EDDHA/Fe solution was prepared from commercial product as positive control; (2) EDTA/Fe (T) solution was prepared through the addition amount of natural Fe (iron ore Tamra) to EDTA solution (0.001M), equal to the molar of EDDHA/Fe. The pH was raised to 6 ( $\pm 0.5$ ) and the solution was left 24H in rotate shake, and (3) The experimental solution from humus substance (Digestat) and iron Tamra (D/Fe (T)) was prepared as EDTA/Fe (T) but the pH adjusted at 8 then was shaken for 24H. The all of experimental solutions had a same quantity of iron (50  $\mu\text{mol}$  Fe). The three iron products were applied in the calcareous soil (Soil El Manar) for correct iron chlorosis in Tomato plants.

**Table 3.** Iron solutions

| Solutions             | Agent    | Iron                               | Origin        | Molarity | Volume |
|-----------------------|----------|------------------------------------|---------------|----------|--------|
| EDTA /Fe(T)           | EDTA     | Hematite/<br>Goethite/<br>Limonite | Tamra<br>mine | 0.001M   | 100ml  |
|                       |          | Chelate/<br>complexing             |               |          |        |
| D/Fe(T)               | Digestat | Hematite/<br>Goethite/<br>Limonite | Tamra<br>mine | 0.001M   | 100ml  |
| EDDHA /Fe (Control +) | EDDHA    | Fe                                 | Com.*         | 0.001M   | 100ml  |

\* Commercial

## 2.2. Soil preparation and Tomato plantation

Three seeds per pot were putted. Pots filled with 1 kg soil then were placed in a laboratory. Pots were daily irrigated up to 80% saturation (Nadal et al., 2009) with nutritive solution without iron. Treatments were applied 7 days after plants showed deficiency symptoms. Three treatments with Fe were evaluated and three replicate pots per treatment were used.

## 2.3. Sampling and measurement

Plant material was sampled after 35 days of the treatment application with EDTA/Fe (T), EDDHA/Fe and D/Fe (T). The different parts of each plant (aerial part and roots) were separated, washed with 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 (dry weight). Total Fe was determined in leaves, stems and roots after dry mineralization by AAS.

The soluble and available Fe fractions were determined in the soils. The complete pot contents were submerged in 11 distilled water and shaken for 15 min. 40 ml of the soil-water mix was centrifuged (5000 tr/5min) and the supernatant filtrated with 0.45 $\mu\text{m}$  cellulose membrane.  $\text{HNO}_3$  (65%, Merck) was added to the supernatant to obtain a concentration of 1%. Soluble Fe were determined in these extracts by AAS. The remaining solid in the centrifuge tube was extracted with 25 ml of Soltanpour and Schwab (1977) extractant (DTPA + ammonium bicarbonate, pH = 7.6) and then filtered. The extraction was repeated three times in total, the extracts joined, and volume made up to 100 ml. Enough  $\text{HNO}_3$  was added to eliminate excess bicarbonate and to allow the acid media for analytical determinations by AAS.

## 2.4. Statistical analysis

The experimental results found were statistically analyzed in order to be able to interpret and compare the effect of the different treatments compared to the negative control for the Tomato plants, the analysis of the soil parameters as well as the plants were carried out by the Excel and were determined at a 95% confidence interval.

## 3. Results

### 3.1. Iron in the soils

The soil has a sandy texture rich in calcium carbonate (43.6%) and having an initial pH of 8.02. These physicochemical properties are appropriate to the apparition of ferric chlorosis (Mengel et al. 2001). After the treatments of the plants with the different iron chelating EDTA/Fe (T), EDDHA/Fe, and iron complexing D/Fe (T), the soil underwent a decrease of pH from 8 to 7. Which could be to the correction of the high rate of limestone, and to an increase of the

electrical conductivity in relation with the addition of the nutritive solution (responsible for the elevation of the ionic concentration) (Figure 4).

The soluble fraction of iron extracted after stirring, centrifugation and filtration was analyzed by AAS. Results of Fe soluble in soil are presented in Figure 5. The amount of Fe in the soluble fraction is higher in the pots treated with EDDHA/Fe. However, with EDTA / Fe (T) and D / Fe(T) (0.26 and 0.24 mg/L<sup>-1</sup> respectively), the Fe soluble for the plants is important than in the control (-). Thus, it appears that the chelating agent (EDTA) and the complexing agent (Digestat) mobilized natural iron (Tamra) in the calcareous soil El Manar.

The results illustrated in Figure 6 show the amount of iron in the available fraction in the different treated pots with a concentration not exceeding 1 mg /L<sup>-1</sup>. The quantity of available iron in the pots treated by iron complex D/Fe (T) recorded the highest iron value compared to other soils. The content of available iron (mg/L<sup>-1</sup>) in EDTA/Fe (T) treated soils is most important to the amount of iron available recorded in the pots treated with commercial iron chelate (EDDHA/Fe). The study of soil-iron chelate interactions was showed that iron concentrations in the soluble fraction are higher in pots treated with EDTA/Fe (T) however, D/Fe (T) recorded the most value in available iron.

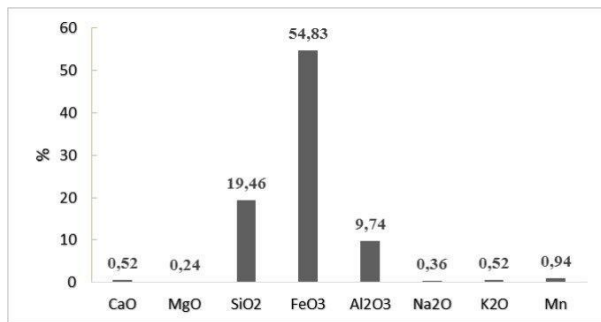


Fig. 4. Diagram of major elements (%) of iron ore Tamra by AAS analysis.

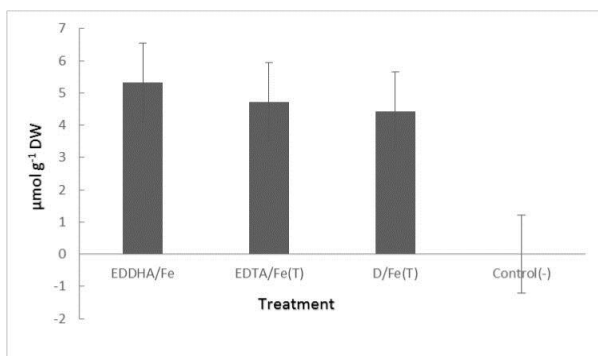


Fig. 5. Soluble fraction of iron in the soil treated by the iron treatments after the harvest of pot trial.

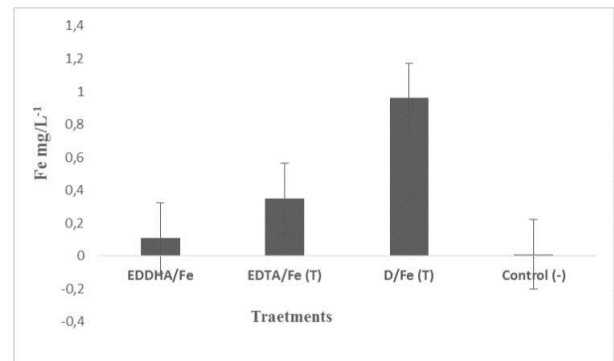


Fig. 6. Available iron fraction in soils treated by different iron treatments in the end of experiment.

### 3.2. Effect of iron chelate / complex in the correction of iron chlorosis in the plants

The growth of the plant can be defined as all the changes it undergoes during its development. Noting that the plants sown in the pots before treatment showed yellowing of the leaves, indicative of a ferric chlorosis.

After treatment with EDTA/Fe (T), D/Fe (T) and EDDHA/Fe the plants showed a change from yellow to dark green. The better plants growth was recorded in pots treated with D/Fe (T), EDTA/Fe (T) and EDDHA/Fe compared to untreated plants (control -) (Figure 7). Similarly, plants treated with EDDHA/Fe and D/Fe (T) branched first with green leaves indicating an increase in the content of chlorophyll in the leaves. While untreated plants show yellowing dominance of young leaves and weak branching, they are indicative of low leaf chlorophyll activity.

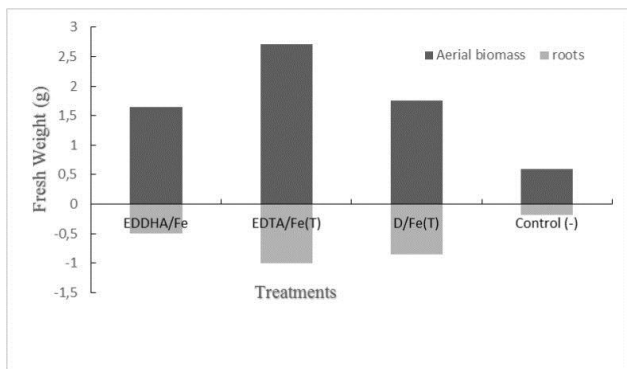
A comparative analysis between the plants subjected to the iron treatments and the two control (control (-) and EDDHA/Fe plants) presented in Figure 8. The plants treated with EDTA/Fe(T) showed the highest fresh weights than EDDHA/Fe, and D/Fe (T).

After drying the samples of plants, the results revealed the same findings for the fresh weight with a higher weight for the EDTA/Fe (T) treatment than the control (-) plants have the lowest values in dry weight. The pots treated with D/ Fe (T) was recorded an important dry weight than EDDHA/Fe.

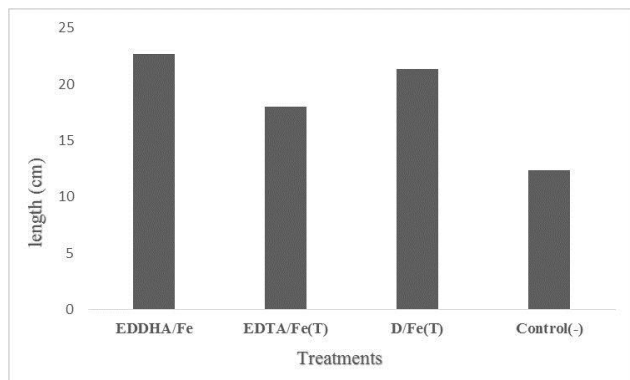
The results shown in the Figure 9 reveal that the growth rate of plants represents by their length is more important for plants that have undergone all three treatments. Noting that plants untreated with small size show leaves yellowing. The best length recorded in the plants treated with EDDHA/Fe and Digestat/Fe (T) (22 cm and 21 cm, respectively).



**Fig. 7.** Comparison between the plants before (A) and after (B) iron treatments.



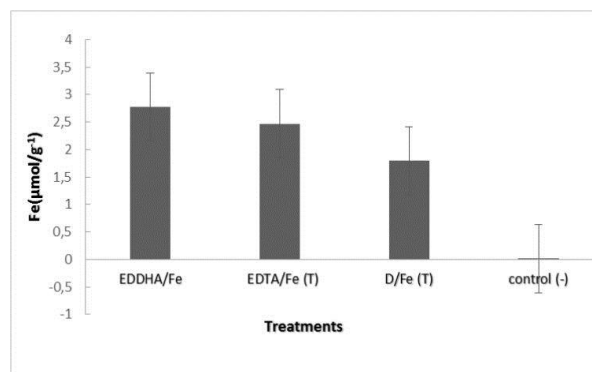
**Fig. 8.** Fresh weight (FW) yield in g per pot of the aerial biomass and roots of Tomato plants according to the iron treatments (EDTA/Fe (T), D/Fe (T), EDDHA/Fe).



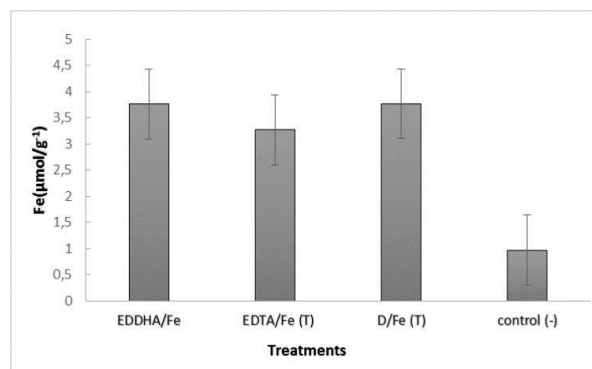
**Fig. 9.** Effect of the iron treatments EDTA/Fe (T), D/Fe (T), and EDDHA/Fe on the length of Tomato plants cultivated in calcareous soil.

The concentration of iron in the roots can tell us the amount of iron absorbed and that will feed the aerial part of the plant. The results of iron analysis in the roots of plants treated with the iron chelate/complex revealed that EDTA/Fe (T) showed high values compared to D/Fe(T) (Figure 10). The amount of iron in the roots of the untreated plants was almost null in comparison with the other treatments. This variation is in ascending order: control (-) < D/Fe(T) < EDTA/Fe(T) < EDDHA/Fe. Thus, it appears that the EDTA and Digestat are responsible for increasing the

mobility of natural iron in the soil. The plants treated with EDDHA/Fe, EDTA/Fe and D/Fe (T) presented almost the same content of Fe in the aerial parts of plants.



**Fig. 10.** Effect of the different Fe treatments (EDTA/Fe (T), D/Fe (T) and EDDHA/Fe) on the Fe concentration (µmol/g⁻¹) in roots cultivated in calcareous soil.



**Fig. 11.** Fe concentration (µmol/g⁻¹ DW) in the shoots of Tomato plants cultivated in the calcareous soil

### 3. Discussion

Iron deficiency is a nutritional disorder in plants that reduces quantitative and qualitative yields and causes severe economic losses. It occurs worldwide, mainly in plants grown on calcareous soils, due to a limited bioavailability of iron, related to the low solubility of iron at a high pH between 7 and 8.5. Iron chelate or complex fertilizers are among the most effective solutions for preventing and correcting Fe deficiency in plants grown in alkaline soil.

The fundamental goal of this work has been to search for the application of iron ore Tamra to correct ferric chlorosis using chelating agents such as EDTA and Digestat. Two products prepared with iron ore Tamra and applied on a calcareous soil cultivated by the Tomato plant were realized. Then a comparative study was done with a commercial chelate (EDDHA/Fe) for understand their efficacies. The efficacy of commercial iron products containing EDDHA has been widely demonstrated since the 1950s (Wallace et al., 1955;

Barak and Chen, 1987; Lucena et al., 2006).

This work indicated that natural iron (from mine of Tamra) with the two ligands supplied an effective percent of iron to Tomato plants cultivated in calcareous soil. The agents EDTA, Digestat and EDDHA are beneficial in mobilization of iron in soil and subsequent the movement of iron from the roots to the aerial part of plant.

After treatment, the plants treated with EDTA/Fe (T) showed a good result in iron state at pH above 7, when we compared to the commercial EDDHA/Fe chelate. These results are consistent with the work of Schenkeveld W.D, 2010 based on the stability of Fe chelates in solution. He concluded that EDDHA stabilities with Fe were greater than EDTA. The results obtained is in agreement with EDTA/Fe (T) was lower than EDDHA/Fe. The addition of EDTA/Fe (T) in the soil has increased the percentage of soluble iron in the solution of soil so indirectly improves the iron state in the plant that is to say the correction of chlorosis ferric in a very short period. Sandra Lopez et al., 2015, confirm the latter. EDTA is a non-phenolic chelating agent capable of protecting the iron element from dissolution or precipitation in the soil despite its low stability.

According to Tordoff et al (2000), organic materials have the ability to improve the physical nature of the rooting medium and to provide plant nutrients in a slow-release form, facilitating vegetation establishment. In this work, we also used the Digestat as a complexing agent in calcareous soil. The formation of an iron complex between the Digestat and Tamra iron is very easy and very effective for the mobilization of iron in the soil. Most results show that the effect of iron complex D/Fe (T) is similar to iron chelates (EDTA / Fe (T) and EDDHA / Fe). Rodriguez-Lucena et al. (2009, 2010 and 2011) have studied in several studies the effectiveness of various iron complexes (lignosulfonates of Fe), both in hydroponic culture and in foliar application on plants grown in hydroponic culture or in soil.

The results obtained confirm the possibilities of upgrading Tamra iron ore in agriculture as a fertilizer of alkaline soil to treated ferric chlorosis in plants. The application of the Tamra mine ore with EDTA, Digestat in the calcareous soil was confirmed despite the low dose and molarity (0.001M) used and the short duration of treatment.

#### 4. Conclusion

The present work proposes tests of plants treatment by iron ore to evaluate the effectiveness of EDTA and Digestat with iron ore from Tamra for provide the iron needed for Tomato plants growing on calcareous soil. Five weeks of treatment and monitoring of plant growth were performed. Iron deficiency was induced on the sandy soil (untreated pots) which had lower organic matter and very rich in calcite. In alkaline soil, after treatment by EDTA/Fe (T) and D/Fe (T),

the increase of iron content in plants was observed. Then, results obtained indicate that the absorption of Fe is similar in plants treated with EDTA/Fe (T) and D/Fe (T). The EDTA/Fe (T) chelate should be a good iron-based fertilizer capable of maintaining soil-soluble iron because of high stability. Digestat (organic matter) could be well iron ligands in basic conditions as EDTA and EDDHA. Besides, results indicate that EDDHA/Fe has a faster effect than D/Fe (T) seems to have a more lasting effect. This is in good agreement with the greater stability of EDDHA/Fe. The evident increase in yield and iron nutritional value of plants confirms the usefulness of iron ore Tamra with EDTA and Digestat application on alkaline soils.

Finally, studies on Tamra iron ore show interesting results that encourage us to use natural resources in agronomy. It plays an important role in agriculture as a fertilizer. It has a positive effect on tomato culture and alkaline soil. It can bring positive economic and social effects in Tunisia. However, it is important to improve the agronomic efficiency of these products so that they are more lucrative economically.

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