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# A Preliminary Study on the Effect of Chelated Iron on Humic Substances on Growth, Yield and Nutritional Status of Potato Plants Grown in Sandy Soil

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**Abstract:** A field experiment was conducted at the research farm of the National Research Center in the Nubaria region, with the aim of studying the effect of different chelating iron compounds, whether manufactured in the laboratory or commercially and comparing that with the control treatment (without iron spray) in order to study the possibility of replacing cheap local compounds with high-priced commercial counterparts, as well as to determine the importance of iron as an important nutrient for potato plants grown in sandy soil. For this purpose, humic and fulvic acid were extracted from compost in the traditional way and then iron was chelated on each of them, where the iron concentration in them was (6%). Two rates of iron spraying on potato plants were used (3 and 4 ml per liter) and compared with the control treatment (without iron spray). It is clear from the obtained results the importance of iron fertilization on potato plants in sandy lands, where it is evident as the control treatment gave the lowest values in both growth and yield of potatoes as well as the content of leaves from chlorophyll and the content of tubers from starch and protein compared to all other treatments, which were used chelated iron compounds. Also, the chelation of iron on fulvic acid gave the highest results, especially at a concentration of 4 ml per liter compared to iron chelated on humic and commercial acid. Therefore, attention should be paid to the importance of organic acids with low molecular weights, which play a key role in chelating iron and facilitating its absorption within plants, thus increasing the effect of Iron inside plant tissues. The use of plant residues found in the surrounding environment and converting them into mature fertilizer and the extraction of organic acids, with which iron can be chelated in a way that facilitates the absorption of that element within plants and thus treatment of iron deficiency in sandy soils, which enhances the growth of potato plants and improves their productivity. Also, it is possible to use locally made chelating compounds at a very low cost as an alternative to high-priced commercial vehicles, which helps to reduce the production cost of crops and achieve a high financial return.

**Key words:** Fe-Fulvate · Fe-Humate · Fe-Commercial · Potato plants · Growth · Yield · Nutrients content

## INTRODUCTION

Iron (Fe) is an essential microelement for the growth andlife of plants. Iron chlorosis is a widespread disease thataffects plant growth and reduces the yield of many crops [1]. Iron deficiency results in a decrease of chlorophyll inplant leaves, leading to a progressive yellowing of plants, starting from the younger leaves and proceeding to theolder parts of plants.The causes of plant iron deficiency can be different, ranging from nutritional disorder to infections caused bymicroorganisms. Soil pH plays a key role in iron supply toplants [2], since iron solubility is enhanced in acidic soilsand depressed in alkaline soils. In high pH calcareoussoils, iron availability can be dramatically reduced [3], and iron chlorosis is widely diffused [4], because solubleFe may be as low as $10^{-10}$ M, while a normal requirementfor plant growth is = $10^{-7}$ M [5].

Fertilization with synthetic chelates is the most commonpractice to solve iron chlorosis and ethylenediamine-*N*, *N'*-bis (*o*-hydroxyphenyl) acetic acid (EDDHA) is one of the most efficient iron chelating

**Corresponding Author:** Abd El-Rheem Kh. M., Soils and Water Use Dept., National Research Centre, El-Dokki, Giza, Egypt agents [6]. However, a natural plant survival process is the exudation in the rhizosphere of a variety of natural organic ligands, which can form soluble complexes with $Fe^{3+}$, thus favoringthe mobilization of Fe from soil oxides/hydroxides [7, 8] or Fe–humates [9-11]. Microbial siderophores [12], plant root exudates including phytosiderophores [13], organic acids [14, 15] and phenolic compounds [16, 17] belong to the natural organic ligands that facilitate ironuptake by plants. It is reported that more than 95% of thetotal plant-available Fe in the soil solution may be representedby this organic Fe pool [18].

Humic substances, which include humic acid and fulvic acid, are among the most complexand biologically active organic matter compounds in the soil and are known to stimulate both plantsand microbial activities through a number of mechanisms (e.g. through humic extracts of leonardite, compost or other organic fertilizer) [19]. Humic substances do not only have a positive impact on soil physicochemicalproperties and soil microbial community structure and activity, resulting in availability of highernutrient content for plant growth, furthermore but it was also observed that they positively influenceroot growth, especially lateral root emergence and root hair initiation, involved in plant nutrient [20].

An important Fe source in soil is represented by theinsoluble Fe complexes with humic substances. Iron complexation by humic substances is attributed tothe oxygen-containing functional groups (carboxylic, phenolic and carbonyl) and, to a lesser extent, nitrogenorsulfur-containing functions [21]. This iron can bemade soluble by displacing humic molecules through aligand exchange mechanism. In fact, the stability constantsof Fe complexes with organic acids [22] and with siderophores[23] are generally much larger than those calculated for iron–humate complexes [24].

Potato (*Solanum tuberosum,* L.) is one of the important vegetables in Egypt for both local consumption and exportation [25]. Potato is a major source of inexpensive energy; it contains high levels of carbohydrates, the predominant form of this carbohydrate is starch and amounts of vitamins B and C [26]. Potato, after rice, wheat and corn is the most common foodstuff consumed in the world. Accordingly, the problems related to human diet micronutrient deficiency can be solved economically and sustainability by increasing microelements contents in potato tuber and improving their bioavailability.

The paper aimed to study a comparison of chelated iron compounds on both fulvic acid and humic acid and manufactured in the laboratory with a commercial chelated iron compound, as well as with the control treatment (without iron spray) and its effect on the growth, yield and nutritional status of potato plants grown in sandy soil.

## MATERIALS AND METHODS

Field trial was conducted at the Agricultural Research Station, National Research Centre, El-Nubaria district, Egypt (latitude of 30°30N and longitude of 30°20E) during the winter season of 2020, in a sandy soil (Entisol-Typic Torripsamments). This work aimed to study the effect of Fe- humate and Fe-fulvate on growth, yield and nutritional status of potato plants.

Iron humate and fulvate were prepared in the laboratory and compared with a commercial iron compound with the same concentration for the three types, where the concentration was 3 and 4 ml $L^{-1}$.

**Preparation of Fe- Humate and Fe-Fulvate:** Residues of medicinal and aromatic plants were used to make mature compost and the Table (1) is some analysis of the resulting compost using the standard procedures outlined by Cottenie [27].

Extraction of humic and fulvic acid (humic substances) from compost was based on traditional extraction of humic substances (International humic acid substances society, IHSS- $N_2$ method).

The standardized International Humic Substances Society method is most widely applied for humic substances extraction [28]. In this classical method, the compost is dried at 40°C, homogenized and mechanically ground using a ball mill. Humic acids extraction is carried out using 0.2 *M* NaOH under $N_2$ conditions, then acidified by HCl and centrifuged to isolate fulvic acid. The precipitated humic acid fraction was redissolved using NaOH and centrifuged at under $N_2$. Humic acid was precipitated using HCl centrifuged under $N_2$ conditions.

After obtaining both humic and fulvic acid, a quantity of ferrous sulfate is dissolved until the iron concentration inside the compound reaches 6%, taking into account that the pH of the two compounds is adjusted up to 6.5. In order to compare the efficiency of the two compounds that were manufactured in the laboratory, it was necessary to compare them with a commercial chelated iron compound with the same iron concentration inside it. All iron compounds were sprayed a month after planting and this was repeated twice.

Table 1: Some chemical analyses of the resulting compost

| Analysis | Unite | Compost |
|---|---|---|
| pH | - | 8.14 |
| EC | dS m$^{-1}$ | 4.20 |
| Total Nitrogen | % | 1.40 |
| Organic matter | % | 43.6 |
| Organic carbon | % | 25.3 |
| Ash | % | 56.4 |
| C/N ratio | - | 1:18.1 |
| P | % | 0.61 |
| K | % | 0.98 |
| Fe | ppm | 320 |
| Zn | ppm | 70.2 |
| Mn | ppm | 90.2 |

Table 2: Some physical and chemical properties of the experimental soil at the beginning of the experiment

| Soil properties | Values |
|---|---|
| Soil physical properties<br>Particle size distribution (%) | |
| Sand | 86.6 |
| Silt | 8.76 |
| Clay | 4.69 |
| Soil texture | Sandy |
| Soil chemical properties | |
| Calcium carbonate (%) | 5.36 |
| pH | 8.4 |
| EC (dS m$^{-1}$) | 0.31 |
| Soluble cations (meq. L$^{-1}$) | |
| Ca$^{++}$ | 1.15 |
| Mg$^{++}$ | 0.74 |
| Na$^{+}$ | 1.00 |
| K$^{+}$ | 0.21 |
| Soluble anions (meq. L$^{-1}$) | |
| $CO_3^{2-}$ | - |
| $HCO_3^{-}$ | 0.10 |
| Cl$^{-}$ | 1.20 |
| $SO_4^{2-}$ | 1.80 |
| Available nutrients (mg kg$^{-1}$) | |
| N | 28.1 |
| P | 5.40 |
| K | 160 |
| Fe | 3.16 |
| Mn | 0.87 |
| Zn | 1.90 |

Surface soil samples (0–30 cm) were collected from the experimental field, the collected samples were air-dried, crushed and passed through a 2-mm sieve and preserved for analysis. To judge soil characteristics perfectly the following ideal methods were used: Particle size distribution for soil was carried out using the pipette method as described by Dewis and Fertias [29]. Field capacity of the soil was determined using the methods described by Richards [30]. Total carbonate was estimated gasometrically using Collins Calcimeter and calculated as calcium carbonate according to Dewis and Fertias [29]. Soil reaction (pH) was measured in saturated soil paste using combined electrode pH meter as mentioned by Richards [30]. Total soluble salts were determined by measuring the electrical conductivity in the extraction of saturated soil paste in dS m$^{-1}$ as explained by Jackson [31]. Amounts of water soluble cations (Ca$^{2+}$, Mg$^{2+}$, Na$^{+}$ and K$^{+}$) and anions ($CO_3^{2-}$, $HCO_3^{-}$ and Cl$^{-}$) were determined in the extraction of saturated soil paste by the methods described by Hesse [32], whereas ($SO_4^{2-}$) ions were calculated as the difference between total cations and anions. Soluble Ca$^{2+}$ and Mg$^{2+}$ were determined by titration with standardized versenate solution. Soluble Na+ and K+ ions were determined by using flame photometer. Soluble $CO_3^{2-}$ and $HCO_3^{-}$ ions were determined by titration with standardized $H_2SO_4$solution. Soluble Cl$^{-}$ ions were determined by titration with standardized silver nitrate solution. Soil available nitrogen was extracted using KCl (2.0 M) and determined by using macro-Kjeldahl method according to Hesse [32]. Soil available phosphorus was extracted with $NaHCO_3$ (0.5 M) at pH 8.5 and determined colorimetrically after treating with ammonium molybdate and stannous chloride at a wavelength of 660 nm, according to Jackson (1967). Available potassium was determined by extracting soil with ammonium acetate (1.0 M) at pH 7.0 using flame photometer as described by Hesse [32]. Available iron, zinc and manganese were extracted using DTPA method [33] and measured using atomic absorption spectrophotometer PerkinElmer model 5000. Some physical and chemical properties of the experimented soil are listed in Table (2).

Standard agricultural practices for sweet potato production were carried out according to the recommendations of the Egyptian Ministry of Agriculture (475 kg N, 85 kg P and 200 kg K ha$^{-1}$). Mineral fertilizers N, P and K were added in the recommended amounts by the Ministry of Agriculture. A random sample of four plants was taken from each experimental unit to determine the growth parameters, *i.e.* (plant height "cm" and tuber diameter).

At harvesting time (115 days from planting), a representative sample of 10 healthy tubers from each experimental plot was selected from the larger sizes to obtain the quality of tuber as follows:

- Tuber yield.
- Total Starch content.
- Crude protein percentage "multiplying total nitrogen percentage by 6.25 to give the crude protein content"
- N, P , K, Fe, Zn and Mn content.

**Biochemical Determination:**

- Total chlorophyll were determined in representative fresh leaves samples (at vegetative growth) according to Moran; R. [34].
- Soluble starch content was determined following Malik and Srivastava [35].

**Nutritional Status:** To analyze macro- and micronutrients in potato tubers, samples were taken from each plot, dried at 70° and grounded using stainless steel equipments. From each sample 0.2 g was digested using 5 $cm^3$ from the mixture of sulfuric ($H_2SO_4$) and perchloric ($HClO_4$) acids (1:1) as described by Peterburgski [36] to determine NPK concentrations. Total nitrogen content was estimated by modified Kjeldahl's methods Motsara and Roy [37]. Phosphorus was determined calorimetrically by $NH_4$-Metavanidate method Motsara and Roy [37]. Potassium was flame-photometrically estimated [38].

## RESULTS AND DISCUSSION

Data in Table (3) showed the effect of chelated iron, whether on fulvic or humic acid, on growth and yield characteristics of potato plants and comparing that with commercial chelated iron.

In general, the results indicate the importance of iron fertilization to potato plants grown in sandy soil, as fertilization with iron compounds all affected the growth and yield of potatoes significantly compared to the control (without adding iron). Also, spraying any source of chelated iron with a concentration of 4 ml per liter gives the best results, especially when spraying with Fe-fulvate.

Iron is the third most limiting nutrient for plant growth and metabolism, primarily due to the low solubility of the oxidized ferric form in aerobic environments [39]. Iron deficiency is a common nutritional disorder in many crop plants, resulting in poor yields and reduced nutritional quality. In plants, iron is involved in chlorophyll synthesis and it is essential for the maintenance of chloroplast structure and function. Being the fourth most abundant element in the lithosphere, iron is generally present at high quantities in soils; however, its bioavailability in aerobic and neutral pH environments is limited. In aerobic soils, iron is predominantly found in the $Fe^{3+}$ form, mainly as a constituent of oxyhydroxide polymers with extremely low solubility [40]. In most cases, this form does not sufficiently meet plant needs. The visual symptoms of inadequate iron nutrition in higher plants are interveinal chlorosis of young leaves and stunted root growth [41]. Iron predominantly exists as $Fe^{3+}$ chelate forms in the soil and plants ultimately cannot absorb it under various physiological conditions such as high soil pH in alkaline soils. Thus, plants growing in high-pH soils are not very efficient at developing and stabilizing chlorophyll, resulting in the yellowing of leaves, poor growth and reduced yield [42].

Potato plants were not significantly affected by the use of chelating compounds manufactured in the laboratory compared to the commercial chelated compound, but sometimes it surpassed it in some growth and yield traits. Thus, we can say that the compounds that were manufactured in the lab (cheap price) can be used in complete safety instead of the chelated compound of commercial iron (high price).

The capacity of humic substances to complex metals and affect the mechanisms of nutrient acquisition and plant metabolism provide evidence for a multifaceted role of these organic fractions on Fe nutrition [43].

Humic substances are able to form stable complexes with metal micronutrients, due to the presence in their structure of oxygen-, nitrogen- and sulfur-containing functional groups. This, in turn, would help maintaining micronutrients in solution and/or in bioavailable forms at pH values found in most soils [44]. In the case of Fe, highly stable humic substances complexes mainly involve O-containing groups (carboxylic and phenolic groups) [44]. More recently it was shown that carboxylic acids in aliphatic domains are also involved in Fe(III)-HS complexation [45].

It is also clear from the results the importance of iron chelation on fulvic acid, which gave the highest results of potato growth and yield compared to the rest of the compounds, which indicates the importance of iron chelation on fulvic acid because of its high ability to chelate and as a result of its small partial weight compared to humic acid, this helps a lot to absorb Iron easily inside the tissues of the plant and then clearly affects the growth of the plant and also the potato crop.

The results in Table (4) indicated the effect of different concentrations of chelated iron on different chelating materials on chlorophyll content in leaves and tuber content of carbohydrates and protein. It is clear from the results to the importance of iron in the formation of chlorophyll inside the leaves, as it was found that in the treatment that did not add iron (control) the total content of the leaves of chlorophyll was affected compared to the rest of the other treatments to which iron was added in different chelated forms. The chelated iron on fulvic acid treatments were superior compared to the

Table 3: Effect of chelating iron compounds on potato plants growth and yield parameters

| Treatments | Concentration ml $L^{-1}$ | Plant length cm | No. leaves | Leaves dry weight g | No. Tubers | Tubers weight kg | Tuber length cm | Tuber diameter cm | Total yield ton $fed^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| Fe- Fulvate | 3 | 68.2 | 65.2 | 10.8 | 12.1 | 1.03 | 8.80 | 5.81 | 17.9 |
| | 4 | 85.4 | 70.4 | 14.8 | 15.2 | 1.47 | 9.81 | 7.02 | 18.7 |
| Fe - Humate | 3 | 64.3 | 62.2 | 10.5 | 11.2 | 0.92 | 7.92 | 5.33 | 17.7 |
| | 4 | 76.1 | 65.1 | 13.5 | 14.2 | 1.21 | 8.84 | 6.34 | 17.9 |
| Fe - Commercial | 3 | 63.2 | 63.4 | 10.9 | 12.4 | 1.02 | 8.62 | 5.91 | 17.2 |
| | 4 | 79.2 | 69.5 | 13.9 | 14.3 | 1.39 | 9.24 | 6.82 | 18.1 |
| Control | | 60.0 | 59.2 | 9.50 | 10.2 | 0.85 | 7.10 | 5.01 | 17.0 |
| $LSD_{0.05}$ | | 4.0 | 4.04 | 0.52 | 1.2 | 0.16 | 0.50 | 0.62 | 0.60 |

Table 4: Effect of chelating iron compounds on leaves chlorophyll content and tuber starch and protein content of potato plants

| | | | Starch | Protein |
|---|---|---|---|---|
| Treatments | Concentration ml $L^{-1}$ | Total chlorophyll 100 mg $g^{-1}$ | -------------------- % | ------------------ |
| Fe- Fulvate | 3 | 64.0 | 13.2 | 10.4 |
| | 4 | 68.4 | 13.7 | 11.1 |
| Fe - Humate | 3 | 60.2 | 13.1 | 10.3 |
| | 4 | 65.3 | 13.5 | 10.9 |
| Fe - Commercial | 3 | 60.1 | 13.4 | 10.5 |
| | 4 | 64.7 | 13.6 | 10.9 |
| Control | | 48.5 | 12.9 | 9.40 |
| $LSD_{0.05}$ | | 8.44 | 0.30 | 0.11 |

Table 5: Effect of chelating iron compounds on nutrients content in potato tubers

| | | N | P | K | Fe | Zn | Mn |
|---|---|---|---|---|---|---|---|
| Treatments | Concentration ml $L^{-1}$ | ---------------------- | % | -------------------- | ------------------ | mg $kg^{-1}$ | -------------- |
| Fe- Fulvate | 3 | 1.22 | 0.28 | 2.61 | 43.2 | 25.2 | 20.2 |
| | 4 | 1.24 | 0.29 | 2.65 | 47.5 | 26.1 | 21.2 |
| Fe - Humate | 3 | 1.23 | 0.27 | 2.63 | 44.2 | 25.6 | 19.8 |
| | 4 | 1.24 | 0.28 | 2.64 | 46.9 | 25.9 | 20.8 |
| Fe - Commercial | 3 | 1.24 | 0.28 | 2.62 | 43.2 | 24.5 | 19.8 |
| | 4 | 1.25 | 0.29 | 2.65 | 46.9 | 26.2 | 20.8 |
| Control | | 1.23 | 0.26 | 2.63 | 26.1 | 24.2 | 18.0 |
| $LSD_{0.05}$ | | 0.11 | 0.02 | 0.11 | 3.11 | 2.20 | 2.20 |

chelated iron treatments on humic acid or commercial iron, especially when spraying Fe-fulvate at a rate of 4 ml $L^{-1}$. Iron was important in the carbohydrate and protein content of the tubers, although it was not significantly affected by the type of material chelated by iron and the concentration of 4 ml per liter was the best treatment in all chelated compounds compared to the control.

Iron plays a significant role in various physiological and biochemical pathways in plants. It serves as a component of many vital enzymes such as cytochromes of the electron transport chain and it is thus required for a wide range of biological functions [46]. In plants, iron plays a vital role in photosynthetic electron transport (iron in pheophytin, ferredoxin and cytochromes) and functions as a cofactor for a variety of enzymes, such as iron in coproporphyrinogen III oxidase functioning in chlorophyll biosynthesis pathway [47].

The results in Table (4) indicated to the effect of different concentrations of chelated iron on different chelating materials on tubers content of macro nutrients (N, P and K %) and micronutrients (Fe, Zn and Mn mg $kg^{-1}$). The effect of chelated iron compounds increases the content of elements, whether macro or micro and we note that spraying chelated iron compounds correct iron deficiency and thus the laboratory-manufactured chelating materials (fulvic acid and humic acid) ability of chelation iron with them and increase the absorption of iron by potato plants and thus can. It replaces the high-priced commercial iron-containing compounds in the treatment of iron deficiency in sandy soils.

Humic substances application led to increase micronutrients concentration in potato tubers. Humic substances structure presents a variety of potential sites for binding of trace metals. Binding could be occurred through: (1) a water bridge; (2) electrostatic attraction to a charged COO- group; (3) formation of coordinate linkages and ring structures; and (4) formation of chelate structures, such as those with $COO^-$ and phenolic $OH^-$ site combinations [48].

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