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The effects of two Fe-EDDHA chelated fertilizers on dry matter production and Fe uptake of tomato seedlings and Fe forms of a calcareous soil

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Abstract

The present study was carried out to investigate the effects of two different ratios of Fe-EDDHA chelated fertilizers, (F1:4.8% and F2:6%) having the same amount of 6% soluble Fe content, on dry matter production and Fe uptake of tomato seedlings at different growth periods and Fe forms of a calcareous soil. The experiment was conducted in a factorial experimental design using Fe-EDDHA chelated fertilizers and the plant growth periods (10, 20, 30 and 40 days after seedling) with three replicates under the greenhouse conditions. The results indicated that the dry matter content, Fe uptake, chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid contents in plants generally increased over the control with increasing the growth periods. The plant dry matter contents were higher in F1 than F2 fertilization. The plant Fe uptakes in F1 treatment during the growth periods were also higher than that in F2 treatment. The carotenoid content and the chlorophyll formations in terms of both chlorophyll-a, chlorophyll-b were higher in F2 fertilization at the 20th day and higher in F1 fertilization at the 40th day. The DTPA-Fe and exchangeable-Fe contents in soil samples generally decreased while the organically bounded-Fe content in soil samples increased with increasing growth periods. It can be suggested that 4,8% of Fe-EDDHA fertilizer is more effective on Fe uptake when compared with 6% of Fe-EDDHA chelated Fe fertilizer. Therefore, F1 fertilizer can be used when chlorosis is seen on plants in calcareous soils. On the other hand, F2 fertilizer can be used if long-term Fe fertilization is desired. The differences in effectiveness between Fe-EDDHA chelated fertilizers having the same amount of water-soluble Fe content may be occurred due to differences in their chelating formulas.

Keywords: Tomato, Fe-EDDHA, Fe forms, seedlings.

Introduction

Iron is one of the basic nutritional elements for plant growth and development and affects both quality and yield parameters in plant production. Iron has a role of catalyst in chlorophyll formation. Therefore, plants cannot synthesize chlorophyll in iron deficient conditions, and yellowing (chlorosis) is observed in the veins between the leaves. When the plant is exposed to iron deficiency during the growing period, even if a short time, plant grows slowly, yield decreases, and the plant becomes more sensitive to stress conditions (Sainju et al, 2002; Fernández and Ebert, 2005). In recent years, several studies have shown that iron deficiency can cause yield losses in various plants (Takahashi, 2001; Jin et al., 2009; Ravet et al., 2009, 2012).

Iron deficiency in plants can be caused by the lack of iron in the growing media and the iron in the environment in a form that the plant cannot benefit from. Iron deficiency is seen in soils with high pH and lime content, especially in arid and semi-arid climates. The bicarbonate ion in calcareous soils prevents the movement of the accumulated iron from the roots to the leaves which directly affects its availability by

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buffering the soil pH (Elkins and Fichtner, 2012). The crucial factors decreases the effectiveness of iron such as; high pH, oxidation-reduction reactions, the richness of phosphate and carbonate in the environment, oxygen deficiency in the root area, antagonistic relationship due to high concentrations of manganese, zinc, or copper (Fernández and Ebert, 2005; Chohura et al., 2007).

To eliminate iron deficiency in plants, the most useful iron source is iron-chelate complexes. Chelated fertilizers are well soluble in water, have low dissociation constant, and show a stable structure (Wreesmann, 1996). These ferrous fertilizers are gradually transferred to the soil solution, or they can be kept in the form of organo-mineral complexes. Besides the effectiveness of the fertilizers used causally related to the chelating agent, the other most common chelating agents include EDTA, DTPA, and EDDHA (Lucena, 2003). Especially when soil pH level is higher than 7.2, many chelated iron fertilizers become ineffective. However, EDDHA chelated iron fertilizers are not affected by this situation due to the stable structure of this chelate. It prevents iron from precipitating even when soil pH rises above 9 (Fageria et al., 1990; Forner-Gina and Ancillo, 2011). Gülser et al. (2019) reported that different iron sources and application doses significantly affected plant growth criteria in soybean seedlings. They found that the highest shoot development was determined in 15 ppm nano-Fe application compared with the $FeSO_4.7H_2O$ and Fe-EDDHA treatments, and nano-Fe applications were more effective on seedling growth.

Among many vegetables, tomato is considered as one of the most available source of carotenoids. Carotenoids are pigments found in plants that give the colors between light yellow and red. It contains antioxidant properties, which are effective in preventing or delaying cancer (Stahl and Sies, 2005; Krumbein et al., 2012). Wala et al. (2022) reported that supraoptimal Fe-HBED supplementation significantly increased in xanthophylls and β-carotene contents in tomato plant which is very desirable for food quality.

Many researchers indicated that the relative efficiency of the different Fe chelates to remediate the Fe deficiency in plants has not been explored in depth, mainly due to difficulties in evaluation originating in the lack of purity of commercial products used in the studies (Lucena 2003; Álvarez-Fernández et al., 2005). Schenkeveld et al. (2010) reported that understanding the behavior of the FeEDDHA components in the soil-plant system as a function of time and dosage is important to relate this behavior to Fe uptake by plants. The aim of this study was to investigate the effects of two different ratios of EDDHA Fe chelated fertilizers (4.8% Fe-EDDHA and 6.0% Fe-EDDHA) on DTPA extractable, exchangeable and organically bounded-Fe contents in a calcareous soil, and also dry matter production, Fe uptake, chlorophyll and carotenoid contents of tomato plant seedlings at different growth periods.

Material and Methods

The experiment was carried out fertilizing tomato sedlings with two different Fe-EDDHA chealted sources in the greenhouse of Soil Science and Plant Nutrition Department in Agricultural Faculty of Ondokuz Mayıs University, Samsun-Turkey. After the soil sample was sieved through a 4 mm sieve, 4.5 kg of soil was weighed into plastic pots without drainage holes, and a tomato seedling was planted in each pot on March 2019. The experiment was conducted with fertilization treatments (C: control without fertilization and two different ratios of EDDHA chelated Fe fertilizers (F1: 4.8% Fe-EDDHA (Sidero) and F2: 6.0% Fe-EDDHA (Fe-Sequestrene), containing the same amount of water-soluble Fe content (6%)) and four different plant growth periods (10, 20, 30 and 40 days) in a factorial experimental design with three replicates. The Fe fertilization was applied as 13.6 mg Fe/kg soil for each pot. During the study, the pots were weighed daily, and soil moisture level was kept at field capacity. The tomato plants in pots were harvested after the 10, 20, 30 and 40 days after seedling. Soil particle size distribution was determined according to Bouyoucos hydrometer method (Demiralay, 1993), soil reaction (pH) and electrical conductivity (EC) values in 1:1 soil:water suspension, lime content by Scheibler calcimeter method, organic matter (OM) content by 'Walkley-Black' method and exchangeable cations (Ca, Mg, K, Na) by 1 N ammonia acetate extraction method, total N by Kjeldahl method, available phosphorus by Olsen Method (Kacar, 1994), and DTPA extractable Fe, Cu, Mn and Zn contents using atomic absorption spectrophotometer (Lindsay and Norwell, 1978). The basic properties of the soil used in this experiment are given in Table 1.

Table 1. Some chemical and physical properties of the soil.

Texture	OM	$CaCO_3$	pH	EC	Total N	P	Ca	Mg	Na	K	Fe	Cu	Mn	Zn
	%		(1:1)	µS/cm	%	ppm	me/100g				ppm			
Sandy loam	0,9	14,5	7,8	212,7	0,063	12,2	20,3	5,0	0,28	0,25	8,0	0,26	0,5	0,27

The Fe fractions in soil samples were determined as follows;

i) DTPA exchangeable Fe 1:2 (w/v) soil:solution mixture of DTPA (Lindsay and Norvell, 1978),

ii) Exchangeable-Fe 1:4 (w/v) soil:solution mixture of 1M Mg $(NO_3)_2$ and

iii) Organically bounded-Fe 1:2 (w/v) soil:solution mixture was extracted with 0.7M NaOCl (Shuman, 1985) and iron contents were determined by atomic absorption spectrophotometer.

In plant analyses, 0.2 g fresh leaf sample was taken after harvesting and the chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid contents were determined according to Witham et al. (1971). After drying the plant samples at 65°C with aeration until reaching a constant weight, 0.5g dried plant sample was weighed, and dry ashing was carried out in a furnace at 550°C for 4-8 hours. The ash was dissolved in hydrochloric acid (HCl) and the iron content was determined using atomic absorption spectrophotometer (Jones et al., 1991).

The variance analysis of the data was determined according to Yurtseven (1984) in a factorial experimental design, and LSD test was used to compare the mean values of the results.

Results and Discussion

The Fe fertilization had significant effects on some plant properties and Fe fractions in soil at different growth periods (Table 2). The dry matter amounts of tomato seedlings increased by the Fe fertilization (Figure 1A). Both Fe fertilizer applications increased the dry matter ammounts over the control. While the dry matter amount was the lowest in the F1 application (0.79 g) at the 10th day, the highest dry matter amount (5.97 g) was also obtained in the F1 application at the 40th day. Chohura (2007) examined the effect of different chelated irons on the yield and quality of tomato plants and reported that there was a difference between iron chelates and increased the tomato yield. Similarly, many researchers indicated that Fe fertilization increased the tomato growth and dry matter production (Karaman et al., 2012; El-Desouky et al., 2021).

Table 2. The effects of Fe-EDDHA fertilizers (F), harvest periods and their interactions on some plant and soil properties

LSD	Dry matter	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Carotenoid	Fe uptake	DTPA-Fe	Exc.-Fe	Org.Fe
	g/plant	mg/g fresh matter				mg/g plant	mg/kg soil		
F	0,410*	0,137**	0,068**	0,855**	0,029**	0,127**	1,098**	0,592**	0,690**
HP	0,473**	0,158**	0,079**	0,987**	0,033**	0,147*	1,268**	0,684**	0,797**
F*HP	0,820*	0,275**	0,136**	1,710**	0,058**	0,255**	2,197**	1,185**	ns

**;significant at 0.01 level, *;significant at 0.05 level, ns; non-significant, F; Fertilizers, HP: Harvest period.
Exc.Fe : Exchangeable-Fe ; Org.Fe : Organically bounded-Fe

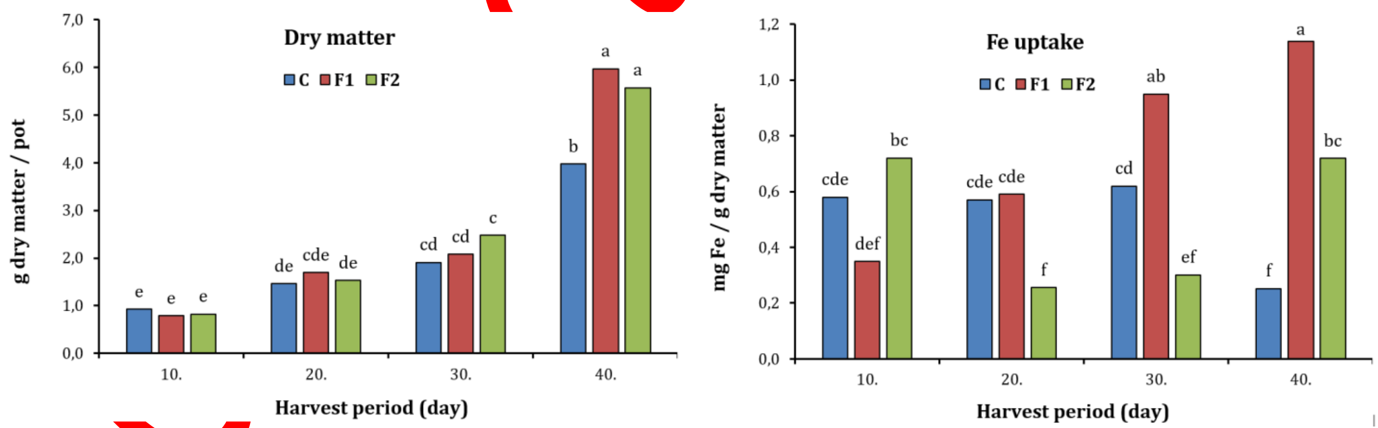

Figure 1. The effects of Fe fertilizations on the amount of dry matter (A) and the iron uptake (B) of the tomato plant at different growth periods. C: Control, F1: 4.8% Fe-EDDHA, F2: 6.0% Fe-EDDHA

Fe uptake values by the plants significantly influenced by the fertilization, period and interaction between fertilization and harvest period, statistically (Table 2). At the end of the 10th day, the highest iron intake occurred in the F2 application containing 6% chelated iron. While there was no statistical difference between the C and F1 treatment, the lowest iron uptake was in the F2 treatment at the end of the 20th day. The highest iron uptake was observed in the F1 application on the 30th and 40th days. In general, iron uptake of tomato plants increased with chelated iron fertilization applied to the soil compared to the control. This can be explained by the fact that EDDHA chelate is effective in most soils, as stated in previous studies (Sekhon, 2003; Gil-Ortiz and Bautista-Carrascosa, 2004).

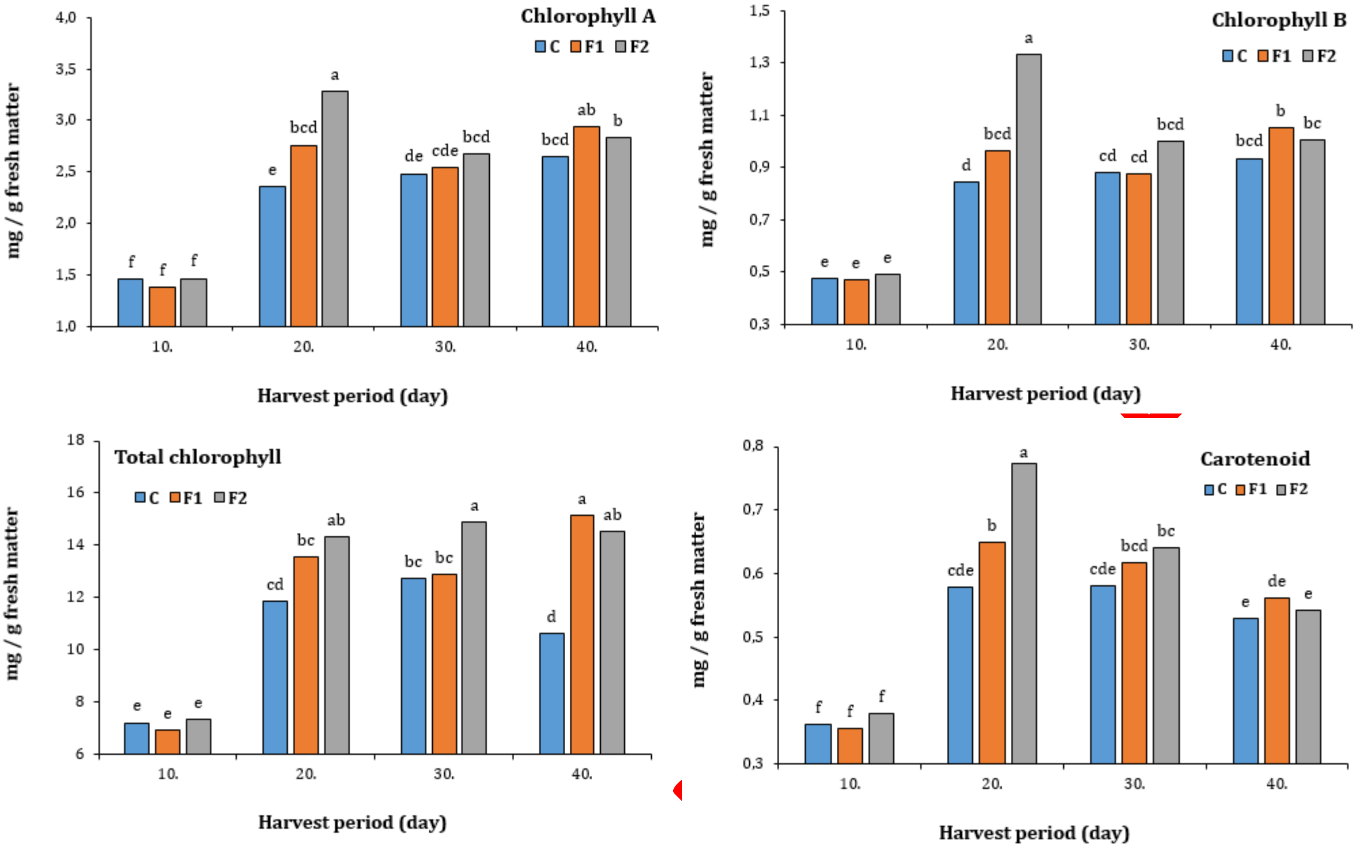

Figure 2. The effects of Fe fertilizations on the amount of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids of the tomato plant at different growth periods. C: Control, F1: 4.8% Fe-EDDHA, F2: 6.0% Fe-EDDHA

The effects of different Fe fertilizations on chlorophyll-a and chlorophyll-b contents related with growing periods were statistically significant (Figure 2 A and B). The lowest amount of chlorophyll-a was determined as [1.37 mg (g fresh plant)$^{-1}$] in F1 application from the plants harvested on the 10th day, while the highest chlorophyll-a amount was determined as [3.29 mg (g fresh plant)$^{-1}$] in F2 application from the plants harvested on the 20th day. The lowest chlorophyll-b amount was determined as [0.47 mg (g fresh plant)$^{-1}$] in control and F1 applications in the plants harvested on the 10th day. In comparison, when the plants were harvested on 20th day, the highest chlorophyll-b content was recorded as [1.33 mg (g fresh plant)$^{-1}$] in F2 applications.

The total chlorophyll content of tomato plants were also significantly influenced by the Fe fertilizations and growing period (Figure 2 C). While the lowest total chlorophyll amount was determined as [6.95 mg (g fresh plant)$^{-1}$] in the plant harvested on the 10th day of the F1 application, the highest one was determined as [15.15 mg (g fresh plant)$^{-1}$] on the 40th day of the F1 application. In both Fe fertilization treatments, chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid amounts were increased compared with the control treatment. The reason behind F2 application increased more than F1 application could be explained by the fact that the amount of chelated iron content of F2 fertilizer (6%) was higher than F1 fertilizer (4.8%). Erdal et al., 2013 found that although there was increase in the amount of chlorophyll-a in the plant, it was statistically insignificant as they applied different doses of ferrous fertilizers to the bean plant. However, they recorded statistically significant increases in chlorophyll-b and total chlorophyll values. Leaf chlorophyll content is the method best suited to assess plant Fe status (Abadía et al., 2004). Terry and Low (1982) reported that chlorophyll content is quantitatively related to the bound Fe content of the chloroplast lamellae, and Fe deficiency may reduce chlorophyll and lamellar Fe contents.

The effects of different Fe fertilizations on carotenoids contents related with growing periods were statistically significant (Figure 2 D). Carotenoids have important functional roles in plant physiology such as the protection of the photosynthetic systems against light energy excess through dissipating actions (Pogson and Rissler, 2000), energy transfer to chlorophyll related to the activity of the light-harvesting complex involving carotenoids (Ronen et al., 1999). The lowest amount of carotenoid [0.31 mg (g fresh plant)$^{-1}$] was obtained on the 10th day of harvesting from both control and F1 treatments. The highest carotenoid [0.72 mg (g fresh plant)$^{-1}$] was determined in plants harvested on the 20th day of F2 application. Borowski and Michalek (2011) stated that foliar application of iron salt increased carotenoid contents of French bean.

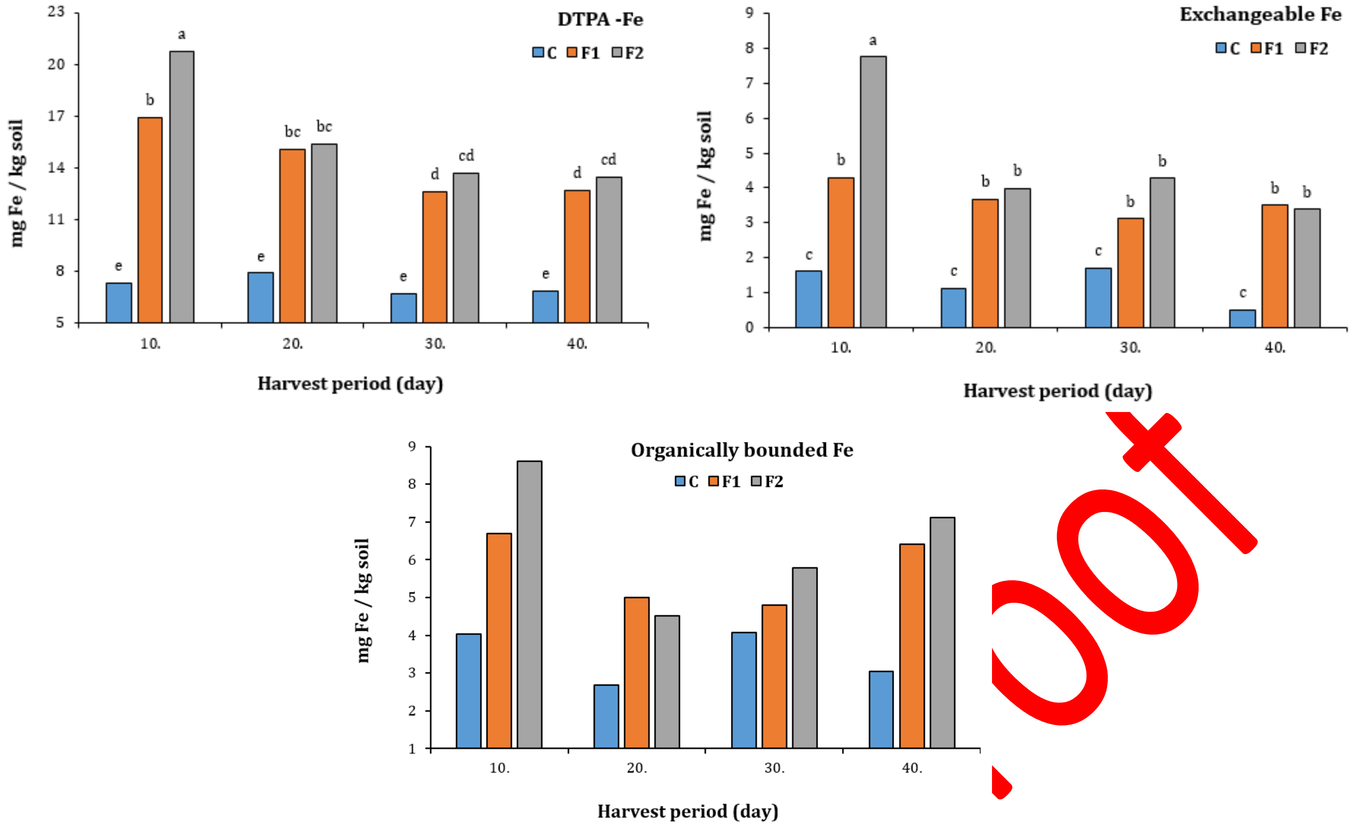

Figure 3. The effects of Fe fertilizations on DTPA extractable, exchangeable and organically bound forms of Fe contents in the soil sampled at different growth periods. C: Control, F1: 4.8% Fe-EDDHA, F2: 6.0% Fe-EDDHA.

In this study, the amount of DTPA extractable, exchangeable, and organically bounded-Fe amounts of soils were significantly influenced by the Fe fertilization treatments at the different growth periods (Figure 3). The Fe contents of soil samples in terms of all three forms (DTPA-Fe, exchangeable-Fe and organically bounded-Fe) were generally ordered as follow F2>F1>C for all growth periods. The lowest DTPA-Fe was found as [6.73 mg (kg soil)$^{-1}$] in the control soil sampled on the 10th day and the highest amount [20.77 mg (kg soil)$^{-1}$] was found in F2 application soil sampled on the 10th day. It had been seen that DTPA-Fe and exchangeable-Fe contents of soils decreased with increasing the growth periods.

The effects of Fe applications and sampling times on the exchangeable-Fe contents of soil samples were statistically significant. While the lowest exchangeable-Fe content [0.51 mg (kg soil)$^{-1}$)] was determined in the soil sampled on the 40th day of control application, the highest exchangeable-Fe content [7.76 mg (kg soil)$^{-1}$)] was determined in the soil sampled on the 10th day of the F2 application. Similar to DTPA-Fe contents, the F2 treatment increased the exchangeable-Fe contents in the soil samples greater than F1 treatment.

The organically bound Fe contents in all soil samples decreased after the 10th day, but these Fe contents in Fe fertilization treatments, especially in F2, increased from 20th to 40th day. While the lowest amount of organically bounded-Fe [2.68 mg (kg soil)$^{-1}$] was determined in the control soil at the 20th day, the highest amount was determined as [8.61 mg (kg soil)$^{-1}$] in the F2 application at the 10th day.

DTPA-Fe and exchangeable-Fe contents in soil samples were reduced after 10th day. This result can be explained by the use of DTPA- Fe and exchangeable-Fe in the soil by plants. Levesque and Mathur (1986) described that the most common plant available ferrous metal forms, as in other metals, are water-soluble and exchangeable-Fe forms. In this study DTPA-Fe and exchangeable-Fe contents in soil samples reduced with increasing plant Fe uptake and probably changing these forms to organically bounded-Fe forms during the growth periods. The organically bounded-Fe contents in soils reduced after the 10th days, but they gradually increased for F1 and F2 fertilizations until the 40th day. Schenkeveld et al. (2010) determined that FeEDDHA concentration in soil declined strongly within the first week with the Fe uptake by soybean plant and removal of FeEDDHA from the soil system displayed a similar trend with the Fe uptake by the plants in vegetative stages (3rd and 4th week) and the pods filling with seeds (6th week).

Conclusion

In this study, applications of two different Fe-EDDHA chelated fertilizers (F1:4.8% and F2:6%) having the 6% soluble Fe content were compared each other at different growth periods of tomato seedlings. The dry matter production of tomato seedlings increased with the F1 fertilization more than F2 fertilization, but this increase was not statistically significant. The plant Fe uptakes in F1 treatment during the growth periods, except 10th day, were generally higher than that in F1 treatment. The carotenoid content and the chlorophyll formations in terms of both chlorophyll-a, chlorophyll-b in the F2 fertilization were higher than the F1 fertilization and control treatment at the 20th day. However, these parameters were the highest in F1 fertilization at the 40th day.

While the DTPA-Fe and exchangeable-Fe contents in soil samples generally decreased, the organically bounded-Fe content in soil samples increased with increasing the growth periods. The all Fe forms determined in this study reduced after the 10th day, but organically bounded Fe content in soil samples gradually increased for F1 and F2 fertilizations during the following growth periods. Therefore, it can be concluded that when chlorosis is seen on plant leaves in calcareous soils, 4,8% of Fe-EDDHA chelated iron fertilizer (F1) can be used due to high Fe uptake by plant. If a long-term Fe fertilization is desired, 6% of Fe-EDDHA chelated iron fertilizer (F2) can be applied. The differences in soil and plant systems between F1 and F2 fertilizer applications may be occurred due to differences in their chelating formulas.

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