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EFFECTS OF CHELATED IRON (EDDHA-FE) TREATMENTS ON CORM AND STIGMA QUALITY IN SAFFRON (*CROCUS SATIVUS* L.)

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Abstract

Effects of different doses of chelated iron (EDDHA-Fe) on the yield and quality of saffron leaf, corm and stigma were investivated. The highest weight of harvested corms was obtained when treated with 2 g of iron chelate per corm, 366.57 g, and the highest number of corm was obtained when treated with 4 g of iron chelate per corm, 73.00 g. When the effects of the iron chelate treatments on the quality of the stigma studied, it was found that the treatment with 2 g of iron chelate per corm gave a yield of dry stigma 754.00 mg and the highest average amount of safranal of 1386.50 mg/kg. The treatment with iron chelate had a positive effect in increasing the yield of the corm, the yield of dry stigma, and the quality of stigma saffron.

Introduction

Saffron (*Crocus sativus* L.) is a sterile triploid plant and is grows only vegetatively with its corms (Koocheki and Seyyedi 2015). Therefore, the selection of efficient corms is the most important factor in saffron production. Çavuşoğlu (2010) reported that the size of corms has a positive effect on saffron flowering. Saffron is grown for spice production. However, the production of corms and leaves is an important secondary source of seed income (Shajari *et al.* 2021). Saffron and its main constituents are traditionally used as pharmaceutical agents. Quality control is very important in the food industry and especially in spices, due to consumer demands and regulatory requirements. The quality of saffron is chemically determined by three important secondary metabolites; crocin, picrocrocin and safranal are responsible for colour and bitter taste. These metabolites are essential for the quality of bile (Asil 2021b).

The nano-Fe particles, iron chelates (Fe-EDDHA and Fe-EDTA), which exhibit high absorption are the most widely used. In addition, nanoparticles have been reported to reduce soil salinity, drought, and heavy metal toxicity. Therefore, a new approach to synthesize more efficient iron chelates seems to be necessary for the delivery of iron to plants (Fakharzadeh *et al.* 2020, Gürgan and Adiloğl 2021). Iron plays an important role in plant growth, development and extraction of quality products. In addition, iron is an essential micronutrient that plants need because of its role in the activity of important enzymes in plants. Although iron is a relatively abundant element, it is mostly useless for plants in soils of neutral to basic pH, and iron levels can reach toxic levels in soils of industrial and agricultural activities, especially in acidic soils. In addition, it has recently been reported that nano-Fe particles are more effective than Fe chelates in improving plant growth, photosynthesis and yield.

Iron chlorosis is one of the most important nutritional problems limiting agricultural production in many countries of the world. It is a nutritional disorder that limits agricultural production and causes severe economic losses. The main reason for this problem is the lack of iron (Fe) in calcareous or alkaline soils. Especially in many countries such as Iran, Tunisia, Spain, Italy, and Turkey, this is one of the most important problems. Complex fertilizers or iron chelates are used as a solution to this problem (Baradar *et al.* 2015).

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Thus the aim of the present study was to investigate the effects of different doses of iron chelates (EDDHA-Fe) on the yield and quality of the leaves, corm and stigma.

Materials and Methods

The present study was conducted from October 2019 to May 2021 in Hassa district of Hatay province. In the study, an average weight of 6 g (5 to 7 g) for the small size of saffron corms was weighed and used for the experiments. Ornamental plants Rose, Gladiolus, Gerbera, Carnation, etc. as per recommendation (3 g - 4 g per meter row are similar to the saffron plant (Asil 2021b), 6% Metallic Iron (EDDHA Na Fe) was preferred in the treatment. Safranal ≥ 90% stabilized, (W338907-Sample-K), crocetin dialdehyde (18804-10 MG) and crocin (17304-1G) standards were purchased from Sigma Aldrich and used as received.

Two separate experiments were conducted: application to corm and leaves. Also, 4 different doses (0, 2, 4 and 8 g/m) of iron chelate treatment doses were used for each application. When the corms were applied, four different doses were added to separate containers of 500 ml of water, each. Then, iron chelate and aqueous corms were added and kept for half an hour. In foliar application, after the plants reached a leaf length of 10 cm, four different doses were added to 500 ml of water and applied to the leaves by spraying. The experiment was established according to the randomized parcel trial pattern (Asil *et al.* 2022) with 3 repetitions in two different environments with the same characteristics and application of corm and foliar. İn each plot 10 corms were planted to a depth of 10 cm and the first irrigation was applied immediately after planting. No further irrigation was applied under field conditions. Weed control was performed manually. No treatment or fertilizers were used. Statistical analysis was performed using the computer program MSTAT-C and differences between means were determined using Duncan's test.

The 0.40 mg/ml safranal, crocin and crocetin standard solutions were prepared in ethanol, diluted with 10 - 2.000 ng/ml concentrations and stored at 4°C. The extractions of saffron stigma samples were accomplished using the ultrasonic-assisted solvent extraction method. Stigma (40 mg) was ground and put in a flask. Ethanol (10 ml) was then added to the flask. The obtained mixture was then sonicated in an ultrasonic bath for 15 min. After sonication, the obtained extract was centrifuged for 3 min at 5000 rpm. This process was repeated three times and obtained supernatants were collected in a different flask. The solvent mixture was evaporated with a final volume of 1ml and obtained extracts were stored at the +4^{o}C in the dark for GC-MS FID analyses.

GC-MS analysis was carried out using Hewlett-Packard 6890 series GC-MS analyzer in Hatay Mustafa Kemal University Central Research Laboratory. The column of the device is HP-88 fused silica column (100 m 0.25 mm i.d. film thickness 0.25 µm) and the detector is Hewlett-Packard mass selective detector 6890. GC-MS analysis was performed according to the procedure specified in the literature. The oven was heated to 90°C and waited for 1 min at this temperature. Then, the temperature was increased by 15 degrees per min to 175°C and kept for 15 min, and then the temperature increased by 5 degrees per min to 225°C and waited for 5 min. Finally, the temperature was increased by 10 degrees per minute to 255°C and waited for 10 minutes. For flame formation, 60 ml/min H2 (UHP grade), 400 ml/min air (zero grade), Helium (99.99%) as a carrier gas, and 10 ml/min flow rate were used as a gas mixture. The injector temperature was kept at 200^{o}C (Asil 2021a, Turkmen 2021).

Soil samples were air-dried and sifted with a 2 mm sieve (Table 1). The average total rainfall in the first year of the study was 1120 mm, and in the second year 569 mm.

Table 1. Data of the experiment soil.

Soil properties	Metot	Unit	Value
pH	Potentiometric	(1:2.5 soil: water)	7.75
Conductivity	Potentiometric	µS cm^{-1}	104.00
$CaCO_3$	Calcimetric measurement	%	0.13
Organic matter	Walkey-Black	%	0.78
Available P_2O_5	Spectrophotometric (Flame S.)	kg/d	66.05
Exchangeable K_2O	Spectrophotometric (Flame S.)	kg/d	25.8
Available Ca	Spectrophotometric (Flame S.)	mg/kg	1783.0
Available Fe	Spectrophotometric (F-AAS)	mg/kg	12.07
Available Na	Spectrophotometric (F-AAS)	mg/kg	29.0
Available Cu	Spectrophotometric (F-AAS)	mg/kg	0.58
Available Mn	Spectrophotometric (F-AAS)	mg/kg	19.32
Available Zn	Spectrophotometric (F-AAS)	mg/kg	1.10
Available Mg	Spectrophotometric (F-AAS)	mg/kg	704.00
Texture class loamy soils	Water and saturation	%	49.50

Results and Discussion

The chemical and physical properties of the soil at the experimental site showed that the soil has a slightly acidic pH and does not contain organic matter. The soil has a high content of iron, phosphorus, magnesium, sodium, and sufficient content of copper, zinc, calcium, manganese and low potassium content. The soil has a loamy texture, which is desirable for agricultural activities and water and air balance of the soil.

The analysis of variance (ANOVA) of the parameters for the effects of the applications of chelated iron in the cultivation of saffron (*Crocus sativus* L) is presented in Table 2. The F values of the studied traits (Application (A), Treatment (B) and Application x Treatment (AxB)) were found to be statistically significant.

Effects of chelated iron (EDDHA-Fe) treatments on the corm and leaf of saffron were different for both. Plant mean values for harvested corm weight (between 5 and 10 g and above 10 g) was found to be statistically significant according to the analysis of variance (Table 2).

When the effect of iron chelate treatments on corm and leaf at different doses on the corm weight was investigated, the highest total corm weight was obtained in the corm application with an average of 321.80 g per treatment. The harvested corms were classified into three different groups according to their weight class (less than 5 g, between 5-10 g and more than 10 g). In this classification, when the corm under 5 g was evaluated, the highest corm weight was 105.05 g in foliar application. Corm weights between 5-10 g averaged 130.06 g, and corm weight greater than 10 g averaged 99.99 g in the foliar treatment. When the effects of different dose treatments on the total corm weights were examined, it was found that the highest average of 322.17 g in terms of total corm weight was found in the 2 g dose treatment, the highest mean of 115.67 g in the 0 dose at the corm weight was less than 5 g and the highest mean of 135.35 g in the corm weights between 5-10 g and in the 2 g dose treatment. In addition, for treatment doses greater than 10 g, the highest mean value of 136.27 g was obtained in the 0 dozes treatment. When the effect of iron chelate applications on corm and a leaf in different doses on the number of harvested corms was studied, the highest effect on the total number of corms according to the application was obtained in the application of 68.08 corms on average. When the unit weights of the harvested corms were studied, the highest amount in terms of the application was obtained on corm with an average of 4.76 g, and the highest amount in terms of applied treatment doses was obtained in the treatment with 0 doses with an average of 5.14 g (Table 3).

Table 2. Analysis of variance (ANOVA) of the parameters of the effects of chelated iron on saffron yield and yield characteristics in saffron.

Parameters	F Value (A) (Application)	F Value(B) (Treatment)	F Value (AB) (Application x Treatment)	Coefficient of Variation
Dried stigma yield (g/block)	54.2258 **	14.7633 *	9.0311 *	23.76
Safranal amount (mg/kg)	27.2199 **	3.0395 ***	7.4491 *	46.79
Crocin amount (mg/kg)	4679.1200 *	1931.3128 *	3662.8242 *	3.02
Crocetin amount (mg/kg)	29569.1261 *	37785.6913 *	9430.3322 *	0.79
Total corm weight (g/parcel)	3.0286 ^{ns}	0.7639 ^{ns}	2.1505 ^{ns}	11.75
Corm weight < 5 g (g/parcel)	0.0102 ^{ns}	0.5367 ^{ns}	0.5059 ^{ns}	26.77
Corm weight between 5-10 g (g/parcel)	634.2355 *	10.4026 *	5.2771 **	23.58
Corm weight > 10 g (g/parcel)	3.3273 ^{ns}	4.0649 **	0.5347 ^{ns}	37.41
Total number of corms (pieces/ parcel)	1.9200 ^{ns}	1.3097 ^{ns}	1.0180 ^{ns}	10.45
Harvested unit corm weight (g)	0.0087 ^{ns}	1.6465 ^{ns}	3.3033 **	13.00

*. **.***, ^{ns} . It is significant at 0.01, 0.05 and 0.10 levels. respectively. and there is no statistical difference between the averages shown with the same letter.

The unit weight of the harvested corms is important for the quality of the corms. In this weight scale, the weight of corms over 10 g is first, and then the weight of corms is between 5 and 10 g. Daughter corms are scored for both stigma production and seed corm production. In the present study on different biostimulants in Kırıkhan, Hatay, the total corm weight was 195.0 g when iron chelate was applied to the corm, and the average total corm weight of 257.7 g when iron chelate was applied to the leaf. In addition, analysis of the total number of harvested corms showed 37.3 corms when iron chelate was applied to the corm application, and 42.0 corms when iron chelate was applied to the leaf (Asil 2021b). Iron chelate treatments and dose studies on saffron were found to have a positive effect on saffron corm quality. It was also found that although the amount of iron in the soil appeared sufficient when the experimental soil was analysed, the iron chelate treatment had a positive effect on the quantity and quality of saffron corms.

In the study conducted with different corm weights, the corm weight was planted between 1 and 16 g, and 40 corms were planted from the highest average 16 g corm. In addition, the weight of the corms harvested in the study was determined with an average weight of 3.7 g (Khan *et al.* 2011). The average weight of harvested corms was found to be 4.75 g for an iron chelate application, 4.76 g for a corm application, and 5.42 g for a corm application and a dose treatment of 2 g per unit of corm weight. When compared with the results from the literature study, a higher average corm weight was obtained than with the iron chelate treatment. This is important for the quality of the corm.

Conducted in Tehran on the effect of nano-iron fertilizer on the qualitative and quantitative yield of saffron compared to iron-chelated fertilizer, it revealed that the application of different doses of iron-chelated fertilizer significantly increased the total corm weight and, the number of the daughter's corms (Farahani and Shahverdi 2015). In the study an increase was observed in iron chelate applications on corm weight, the number of corms, daughter corm ratio and unit corm weights compared with the control application as in the literature study. The values obtained regarding the corm weights of saffron in different studies, the present researchers have done in Hatay conditions are as follows; the plot corm weight was between 87.33 and 219.00 g (Yıldırım *et al.* 2017a), the corm weight was between 19 and 219 g 150 m^{-2} (Yıldırım *et al.* 2017b) and in another study, the corm yield per plot was 84-527 g/plot (Hajyzadeh *et al.* 2017) and, this study, corm weights ranged from 277.77 to366.57 g. According to the studies conducted under Hatay conditions and the literature values, these study results were obtained above the average. Thus it may be said that iron chelate treatments have a positive effect on corm weight.

When the effect of saffron iron chelate applications on stigma yield, safranal content, crocin content and, crocetin content was statistically analysed, it was found that the mean values of the plants and the analysis of variance were statistically significant (Table 2).

Table 3. Effect of chelated iron (EDDHA-Fe) treatments on corm quality.

Measured characters	Aplication	Dozes				Average
		0 g	2 g	4 g	8 g	
Total corm weight (g/parcel)	Leaf	312.83 ns	277.77 ns	279.27 ns	297.90 ns	**291.94 ns**
	Corm	312.83 ns	366.57 ns	314.62 ns	293.17 ns	**321.80 ns**
	Average	**312.83 ns**	**322.17 ns**	**296.94 ns**	**295.53 ns**	
Corm weight < 5 g (g/parcel)	Leaf	115.67 ns	113.80 ns	104.90 ns	85.84 ns	**105.05 ns**
	Corm	115.67 ns	93.10 ns	102.37 ns	104.87 ns	**104.00 ns**
	Average	**115.67 ns**	**103.45 ns**	**103.63 ns**	**95.35 ns**	
Corm weight between 5-10 g (g/parcel)	Leaf	60.90 d	80.23cd	87.43 c	119.05 b	**86.90 B**
	Corm	60.90 d	190.47 a	130.03 b	138.83 b	**130.06 A**
	Average	**60.90 c**	**135.35 a**	**108.73 b**	**128.94 ab**	
Corm weight > 10 g (g/parcel)	Leaf	136.27 ns	83.73 ns	86.93 ns	93.01 ns	**99.99 ns**
	Corm	136.27 ns	83.00 ns	82.22 ns	49.47 ns	**87.74 ns**
	Average	**136.27 a**	**83.37 b**	**84.58 b**	**71.24 b**	
Total number of corms (pieces/parcel)	Leaf	61.00 ns	65.33 ns	63.67 ns	58.33 ns	**62.08 ns**
	Corm	61.00 ns	68.00 ns	73.00 ns	70.33 ns	**68.08 ns**
	Average	**61.00 ns**	**66.67 ns**	**68.33 ns**	**64.33 ns**	
Harvested unit corm weight (g)	Leaf	5.14 ns	4.24 ns	4.39 ns	5.23 ns	**4.75 ns**
	Corm	5.14 ns	5.42 ns	4.32 ns	4.16 ns	**4.76 ns**
	Average	**5.14 ns**	**4.83 ns**	**4.36 ns**	**4.70 ns**	

Effects of iron chelate applications on the stigma of corm and leaves, showed that the highest average was 0.500 mg, while the highest average was 0.484 mg and 2 g treatment dose (Table 4).

In the iron chelate treatments on the pharmacologically active ingredients of saffron the highest average value of 829.33 mg/kg was obtained in terms of the amount of safranal from the foliar treatments and, according to the applied treatment doses 1121.00 mg/kg from the 2 g treatment dose. In terms of the amount of crocin the highest average value was obtained in the

98.10 mg/kg treatments. According to the treatments applied, the highest average value of 99.60 mg/kg was obtained from the treatment method applied 8 g. In terms of crocetin amount the highest average was obtained from the treatments of corm with 1.83 mg/kg and, according to the applied treatment doses, the highest was obtained from the treatments of 8 g with 1.14 mg/kg (Table 4). In a study conducted in Ankara conditions, Arslan *et al.* (2007) found the dry stigma yield as 448 g/d. Ipek *et al.* (2009) reported that they obtained the highest dry stigma yield of 583.1 g/d dry stigma from large (8 cm <) corm planting in the second year of the study.

Table 4. Effect of chelated iron (EDDHA-Fe) treatments on stigma quality.

Measured characters	Aplication	Dozes				Average
		0 g	2 g	4 g	8 g	
Stigma yeild (mg/parcel)	Leaf	0.174 d	0.214 d	0.297 c	0.356 c	**0.260 b**
	Corm	0.174 d	0.754 a	0.518 b	0.554 b	**0.500 a**
	Average	**0.174 b**	**0.484 a**	**0.407 a**	**0.455 a**	
Safranal amount (mg/kg)	Leaf	295.50 d	1386.50 a	1022.10 a	613.20 c	**829.33 a**
	Corm	295.50 d	855.50 b	765.00 c	995.50 b	**727.88 b**
	Average	**295.50 c**	**1121.00 a**	**893.55 b**	**804.35 b**	
Crocin amount (mg/kg)	Leaf	36.10 c	32.50 d	36.27 c	5.10 f	**27.49 b**
	Corm	36.10 c	135.20 b	27.00 e	194.10 a	**98.10 a**
	Average	**36.10 c**	**83.85 b**	**31.63 d**	**99.60 a**	
Crocetin amount (mg/kg)	Leaf	2.96 a	0.98 c	0.98 c	0.32 e	**1.31 b**
	Corm	2.96 a	0.43 d	1.97 b	1.95 b	**1.83 a**
	Average	**2.96 a**	**0.71 d**	**1.47 b**	**1.14 c**	

Reseults on the effects of different storage times on the amount of safranal, crocin, and crocetin showed that the amount safranal was between 1924.93 and 6164.79 mg/kg, the amount of crocin was between 55.43 and 296.20 mg/kg and, the amount of crocetin was between 1.67 and 5.13 mg/kg (Asil 2021a). In different locations in Turkey, the amount of safranal varied between 3253.14 and 22532.97 mg/kg, the amount of crocin was between 77.56 and 647.26 mg/kg in Hatay Yayladağı saffron, and the highest amount of crocetin was between 0.20 and 6.73 mg/kg in Ankara Ayas saffron (Asil and Goktürk 2021). In a study conducted in Tehran on the effect of nano-iron fertilizer on the qualitative and quantitative yield of saffron compared to iron-chelated fertilizer. It was reported that the application of different doses of iron-chelated fertilizer increased the dry stigma weight and total flower number by 50% on average (Farahani and Shahverdi 2015). In the present study, it was observed that iron chelate applications have a positive effect on stigma quality and are compatible with literature studies. It was further observed that the application of iron chelate contributes significantly to yield and quality factors even when there is iron deficiency or sufficient iron in the soil. In addition, excessive consumption of chemical fertilizers used in traditional agriculture increases both the accumulation of heavy metals and risky problems such as harmful components and diseases.

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