INVESTIGATE DIFFERENT LEVELS OF IRON CHELATE FERTILIZER AND FOLIAR SPRAYING TIME ON THE ECONOMIC YIELD OF SAFFRON (*CROCUS SATIVUS* L.)*

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Saffron is a sterile triploid plant, and iron chelate plays an important role in its growth and development. The experiment was conducted in Kermanshah, Iran, from 2015 to 2018 to evaluate the influence of iron chelate fertilizer on saffron. The experiment was laid out in a split-split plot (dummy plot technique) arrangement in a randomized complete block with three replications. Treatments included foliar application of fertilizer in the early, mid, and late vegetative growth stages of saffron growth as the main plot, seven concentrations of iron chelate solution (0, 500, 1000, 1500, 2000, 2500, and 3000 mg L^{-1}) as sub-plots, and three harvest times as sub-sub-plots. The fresh and dry weights of corms, stigmas, and petals, number of daughter corms and flowers, length of stigma, and corm diameter were investigated. Iron chelate content up to 2500 mg L^{-1} caused an increase in the number of flowers, length of stigma, and fresh and dry weights of petals and stigmas compared to the control. However, the application of 3000 mg L^{-1} fertilizer reduced the number of flowers, length of stigma, and fresh and dry weight of petals and stigmas compared to the control. Overall, the results showed that a supply of 2000 mg L^{-1} fertilizer in mid-vegetative growth and the third year of harvest resulted in the highest performance.

corm, flower, foliar application, iron chelate, saffron

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INTRODUCTION

Saffron (*Crocus sativus* L.), a sterile triploid geophyte in the family Iridaceae, reproduces only by use of replacement corms created by the mother corm following blooming (Ramezani et al., 2022). Saffron is a spice derived from the dried stigmas of the saffron crocus (*Crocus sativus*). The saffron annual climate cycle follows a warm-cold-warm pattern, with new corm growth typically starting shortly after fall flowering, during the cooler season. Flowering usually occurs in summer or warmer periods (Douglas et al., 2014). Changes in saffron yield are closely linked to variations in flower and stigma production. Factors such as nutrient absorption, crop density, mother corm weight, and fertilizer applications significantly influence flower and stigma yield (Colla, Rouphael, 2009). Iran dominates global saffron production, accounting for approximately 90% of the market, with India following closely behind in 2023. Iranian saffron is renowned for its exceptional quality. India, producing around 19 tons annually, also stands as one of the world's major saffron producers (source: https://agromedbotanic.com/).

To determine saffron yield, it is essential to provide mineral nourishment (Aboueshaghi et al., 2023). According to Asil's (2023) findings, corms treated with 2 g of iron chelate per corm weighed the heaviest (366.57 g), while the highest number of corms was obtained with 4 g of iron chelate per corm (73.00 g). Plant growth, development, and the production of high-quality products rely on iron. Moreover,

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Table 1. Monsoon rainfall data (mm) in Kermanshah in the growing season 2015 - 2018

Year	2015-2016	2016-2017	2017-2018
October	203.8	1	33.8
November	48.1	14.7	10.2
December	25.6	75.4	27.2
January	54.4	808	95.1
February	96.2	68	30.1
March	161.3	132.5	63.4
April	54.6	22.1	169
May	0.3	0	5.2
June	0	0	0
July	0	0	0
August	0	0	0
September	0	0	29.1
Total	644.3	1121.7	463.1

iron is an essential micronutrient for plants, playing a crucial role in the functioning of vital plant enzymes (Asil, 2023).

Crops are distinguished by growth stages that require a certain supply of all or some nutrients to produce their best yields. The recommended time for foliar sprays typically corresponds to individual plant growth stages where the need for certain nutrients is more necessary. Foliar application at an early growth stage is advised for some crops and some nutrients. It is vital to find a balance between early treatment and enabling the crop to develop a leaf area large enough for the absorption of significant amounts of nutrients (Alexander, 1986).

A crucial micronutrient that plants must have in large amounts is iron. Plants can mobilize and absorb iron from the soil through physiological and morphological changes in low concentrations to make sure it is adequate for essential cellular functions (Hindt, Guerinot, 2012). Due to their solubility and efficiency of translocation into plant leaves, chelated forms of iron are preferred to inorganic forms. While some chelates degrade quickly, which may limit their capacity to address deficiencies, the overall stability of chelates is a good indicator of their usefulness. (Schenkeveld et al., 2012). In severe deficiency situations, the use of iron chelates may raise the rate of iron as well as lengthen the time during which iron is accessible to the growing plants (Rasmussen, 2016).

To address iron deficiency in highly calcareous soils, significant amounts of chelated iron need to be added, which can be costly in certain cases. Applying chelates with a high-quality surfactant directly on plant leaves can improve iron absorption compared to soil application, offering a more cost-effective solution (Abadia et al., 2011). Synthetic Fe chelates bind iron to a chelating agent, increasing iron solubility over a wide pH range, and making them ideal fertilizers. These fertilizers are typically used on high-value crops due to their effectiveness, despite being expensive. Polyaminocarboxylate chelating compounds have also gained attention for their potential negative environmental impact, as they can influence metal availability and mobility and persist in soil solutions (Abadia et al., 2011).

The economic yield of saffron flowers is often calculated on the weight of saffron flowers or saffron weight harvested per unit area. Saffron includes both the dried stigma and the cream, so production is sometimes calculated based on the weight of both parts per unit area (Ranjbar et al., 2016). Until now, there has been no study on determining the best time to spray iron chelate with the appropriate concentration during three years of consecutive cultivation of saffron on economic yield. The aims of this research were to assess the relationships between the timing of foliar sprays and foliar application of different concentrations of iron chelate fertilizer during three years of consecutive cultivation of saffron on economic yield and corm characteristics.

MATERIALS AND METHODS

The experiment was conducted for three years (2015-2018) on clay loam soil structure at the Campus of Agricultural and Natural Resources Research Farm of Razi University, Kermanshah, Iran (34° 21′ N, 47° 9′ E), with a typical continental climate characterized by cold and mild temperatures, with 450 mm of average annual rainfall. Table 1 shows the data of monsoon rainfall

Table 2. Analysis of variance for characteristics measured in Saffron corm

S.O.V	df	mean of square			
		Fresh weight of corms	The dry weight of corms	Number of daughter corms	Corm diameter
Block	2	10853.37**	996.97**	306.96**	1.74**
Spraying times (S)	2	20113.62**	2599.08**	31.53*	0.03 ns
Error a	4	417.69	38.99	2.62	0.08
The concentration of iron (C)	6	10733.00**	1172.93**	180.75**	0.98**
S*C	10	1663.17**	336.86**	25.62**	0.27*
Error b	32	184.86	22.27	8.43	0.12
Harvested times (H)	2	241327.34**	20887.29**	2115.63**	19.04**
S*H	4	2003.04**	370.29**	44.82**	0.16ns
C*H	12	2747.15**	345.23**	19.68*	0.61**
C*H*S	20	1482.23**	156.13**	31.68**	0.23 *
Error	76	67.24	21.87	9.85	0.12
C.V(%)		6.00	11.86	15. 20	16.51

in Kermanshah in 2015 - 2018. The soil contained 1.34 mg kg $^{-1}$ Cu, 1.4 mg kg $^{-1}$ Zn, 3.9 mg kg $^{-1}$ Fe, 4.6 mg kg $^{-1}$ Mn, 520 mg kg $^{-1}$ K, and 5.8 mg kg $^{-1}$ P, with 1.25 percent of Organic carbon.

This experiment was performed based on a split-split plot design (dummy plot technique) in a completely randomized block design with three replicates. Iron chelate fertilizer foliar spraying times based on the different phenological stages of saffron growth, including early (after flowering), mid, and late vegetative growth was considered as the main plot, seven concentrations of iron chelate solution (0, 500, 1000, 1500, 2000, 2500, and 3000 mg L^{-1}) were as sub-plot and three harvested times (Destructive harvesting) were as sub-sub-plot. The 6% iron chelate fertilizer produced by Kiasabz Ashian Kosha Company was used. To prepare the solutions, a stock solution of iron with a concentration of 10000 mg L^{-1} (170 grams per liter) was first prepared. Then, the studied concentrations were prepared by diluting this stock in a suitable proportion with distilled water.

During the preceding fall, the experimental area was prepared by 30 cm deep ploughing, disk harrowing, and levelling. Each plot consisted of five rows, and seven corms (Zaveh ecotype of Kashmar, Iran) on each row (i.e. each plot consisted of 35 corms), with 20 cm inter-row spacing. All corms were planted on 12 October 2015. All of the plots were kept weed-free by mechanical weeding. In addition, starting irrigation after planting, and supplementary irrigation of saffron was done at 24-day intervals (Sepaskhah, Kamgar-Haghighi, 2009).

Observations and data were gathered from four square decimeters of each plot for each replication during three years, for fresh and dry weight of corms, stigmas, and petals, the number of daughter corms and

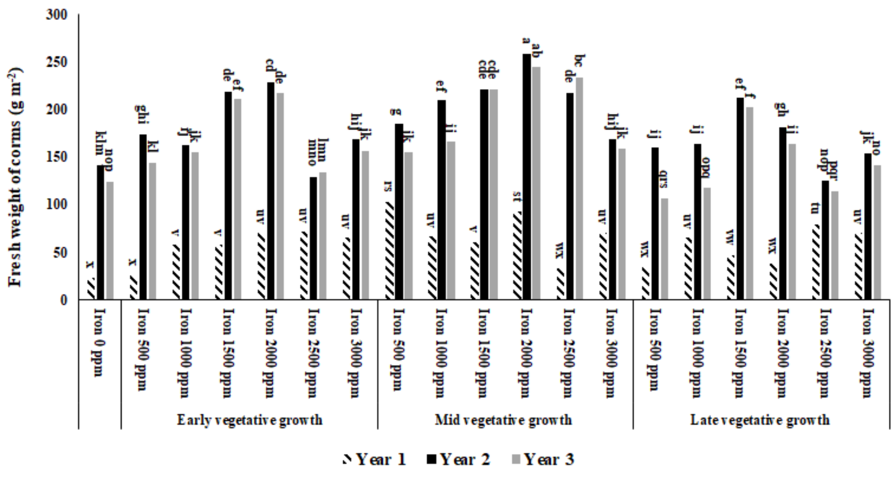

Fig. 1. Interaction effect of spraying times × concentrations of iron chelate (mg L^{-1}) × harvested times on fresh weight of corms

flowers, length of stigma and corm diameter traits. Sampling was done for flower-related traits during flowering in November of each year and corm-related traits in April of each year after the shoots had dried.

Data was checked for normalcy once it was recorded. Then SAS statistical analysis program performed variance analyses (v9.3 package, the SAS Institute, Cary, USA). With a 0.05 probability threshold, Duncan's multiple range test was used to compare treatment means.

RESULTS

The fresh and dry weight of corms

Results showed that there was a significant difference between treatments and all of their interactions in the fresh and dry weight of corms ($P < 0.01$; Table 2). The highest fresh weight of corms (258.34 g m^{-2}) was observed in 2000 mg L^{-1} of iron chelate, mid-vegetative growth in the second year of harvest time with no significant difference with the third year of harvest time. The dry weight of corms (92.42 g m^{-2}) was higher in 2000 mg L^{-1} of iron chelate, mid-vegetative growth in the second year of harvest time treatment (Fig. 1 and 2). On the other hand, the lowest fresh and dry weight of corms was observed in the control conditions in the first year of harvest time (23.54 and 6.46 g m^{-2} respectively) as well as 500 mg L^{-1} of iron chelate in early vegetative growth in the first year of harvest time (Fig. 1 and 2).

Number of daughter corms

According to the analysis of variance (Table 2), the effects of spraying times, concentrations of iron and harvested times were significant at 5, 1, and 1% probability levels, respectively. Also, the interaction effect of spraying times × concentrations of iron × harvested times was significant at a 1% probability level. The highest number of daughter corms were achieved in 2000 mg L^{-1} of iron chelate, mid-vegetative growth in the third year of harvest time treatment (33.83 per m^2) with no significant

Fig. 2. Interaction effect of spraying times × concentrations of iron chelate (mg L^{-1}) × harvested times on dry weight of corms

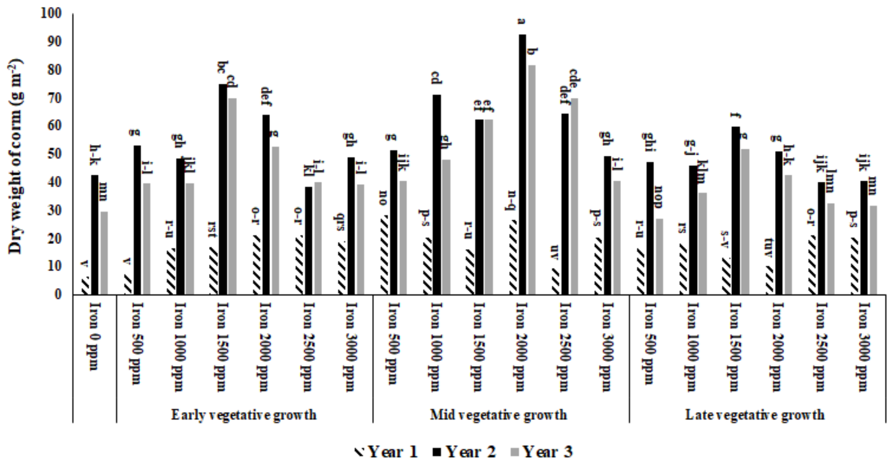

Fig. 3. Interaction effect of spraying times × concentrations of iron chelate (mg L^{-1}) × harvested times on a number of daughter corms

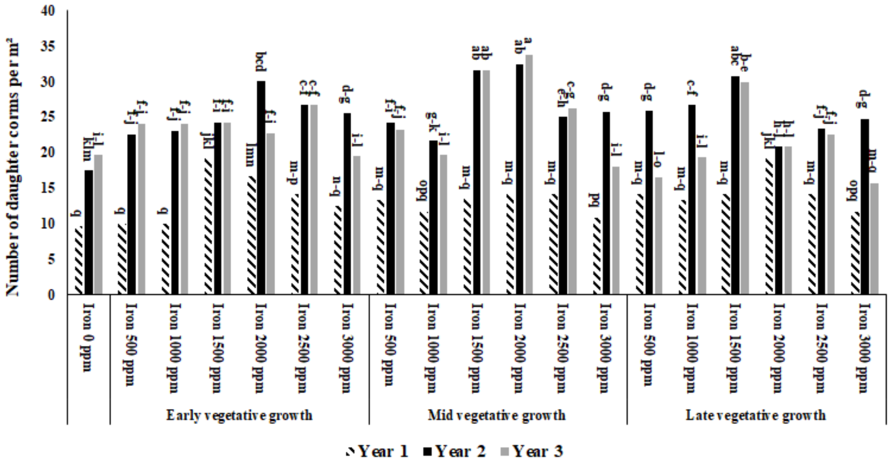

Table 3. Analysis of variance for characteristics measured in Saffron flower

S.O.V	df	mean of square					
		Fresh weight of petals	The dry weight of petals	Fresh weight of stigmas	The dry weight of stigmas	Number of flowers	Length 7of stigma
Block	2	1084.89**	1.94^{ns}	17.09**	0.52**	1498.99**	2.48**
Spraying times (S)	2	915.13**	40.82**	5.17**	0.24*	4950.76**	5.91**
Error a	4	0.44	0.49	0.24	0.01	65.05	0.007
The concentration of iron (C)	6	2423.54**	46.69**	18.78**	0.59**	27703.53**	1.33**
S*C	10	122.55**	12.65**	1.82**	0.03**	696.59**	0.90**
Error b	32	13.69	0.67	0.16	0.004	131.06	0.06
Harvested times (H)	2	1370.45**	60.19**	13.78**	0.44**	53283.31**	3.19**
S*H	4	6.01^{ns}	3.70**	0.18^{ns}	0.006^{ns}	644.80**	0.04^{ns}
C*H	12	15.41*	1.04^{ns}	0.51**	0.02**	787.47**	0.09**
C*H*S	20	22.92**	1.32*	0.07^{ns}	0.003^{ns}	415.56**	0.03*
Error	76	7.60	0.69	0.14	0.003	119.90	0.02
C.V (%)		8.62	20.22	19.00	16.71	9.78	4.58

difference with the second year of harvest time, as well as 1500 mg L^{-1} of iron chelate in mid vegetative growth in second and third years of harvest time, and the lowest number of daughter corms, were recorded in no sprayed iron chelate (control) in the first year (9.66 per m^2) as well as 500 and 1000 mg L^{-1} of iron chelate in early vegetative growth in the first year of harvest time (Fig. 3). Daughter corm numbers increased significantly (250%) by using a 2000 mg L^{-1} concentration of foliar fertilizer in mid vegetative growth in the third year of harvest time.

Corm diameter

There was no significant difference between the spraying times and the interaction between spraying times and harvested times for Corm diameter (Table 2). On the other hand, the effects of concentrations of iron

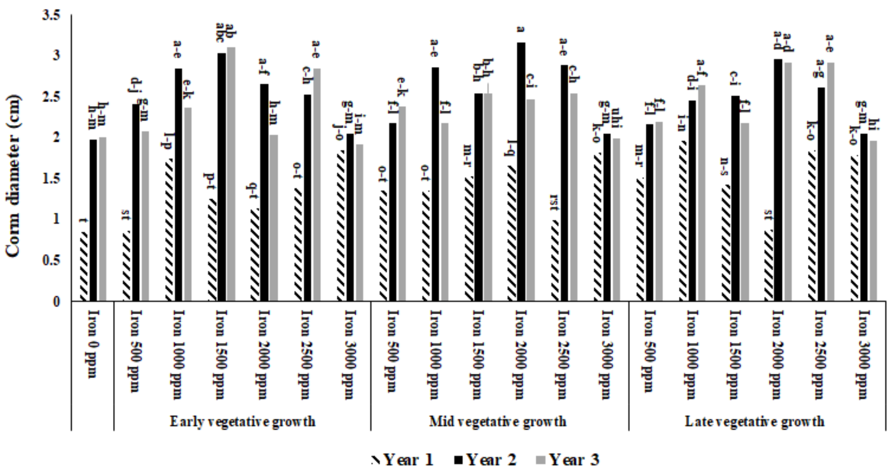

Fig. 4. Interaction effect of spraying times × concentrations of iron chelate (mg L^{-1}) × harvested times on corm diameter

and harvested times were significant ($p < 0.01$), and the interaction of spraying times × concentrations of iron × harvested times was significant at 5% probability (Table 2). The highest and the lowest corm diameter were obtained in 2000 mg L^{-1} of iron chelate, mid-vegetative growth in the second year of harvest time treatment (3.16 cm) and in control in the first year (0.84 cm), respectively (Fig. 4).

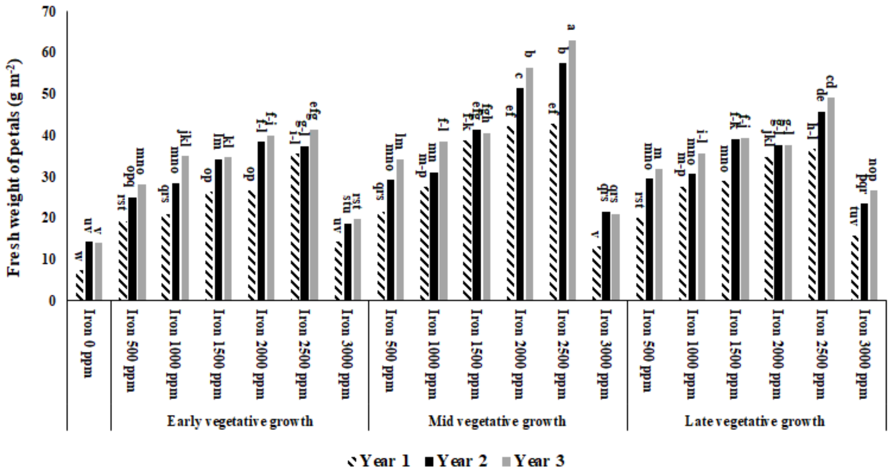

Fig. 5. Interaction effect of spraying times × concentrations of iron chelate (mg L^{-1}) × harvested times on fresh weight of petals

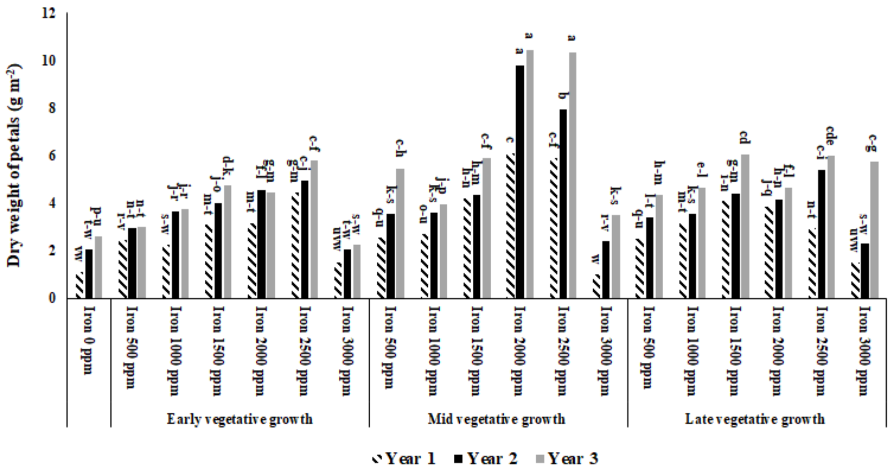

Fig. 6. Interaction effect of spraying times × concentrations of iron chelate (mg L^{-1}) × harvested times on dry weight of petals

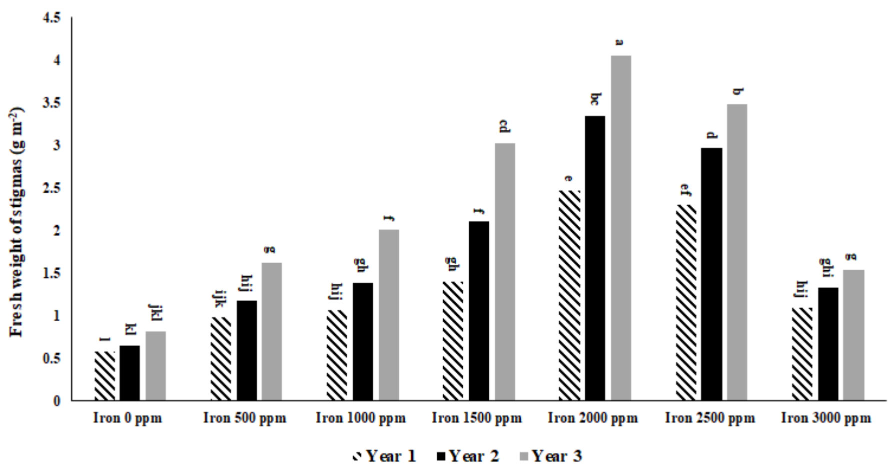

Fig. 7. Interaction effect of concentrations of iron chelate (mg L^{-1}) × harvested times on fresh weight of stigmas

The fresh and dry weight of the petals

Results showed that there was a significant difference between treatments and all of the interaction effects in the fresh and dry weight of petals ($p < 0.01$) (except spraying times × harvested times in the fresh weight of petals and concentrations of iron × harvested times in the dry weight of petals). However, the effect of concentrations of iron × harvested times on the fresh weight of petals and triple interaction effects in the dry weight of petals were significant at 5% probability (Table 3).

The highest fresh weight of petals (63.02 g m^{-2}) was observed in 2500 mg L^{-1} of iron chelate, mid-vegetative growth in the third year of harvest time treatment (Fig. 5) and the highest dry weight of petals (10.45 g m^{-2}) was obtained in 2000 mg L^{-1} of iron chelate, mid vegetative growth in the third year of harvest time treatment with no significant difference with 2500 mg L^{-1} of iron chelate in this condition (Fig. 6). On the other hand, the lowest fresh weight of petals (7.35 g m^{-2}) was recorded in a control condition in the first year. The lowest dry weight of petals (1.02 g m^{-2}) was observed in 3000 mg L^{-1} of iron chelate, mid vegetative growth in the first year of harvest time treatment with no significant difference with other 3000 mg L^{-1} of iron chelate and control in the first year (Fig. 5 and 6).

The fresh and dry weight of stigmas

The effects of spraying times, concentrations of iron, harvested times, and concentrations of iron × harvested times significantly affected the fresh and dry weight of stigmas ($p < 0.01$; Table 3). Although the dry weight of stigmas was significantly affected by spraying times ($p < 0.05$). Based on the results of comparing means of concentrations of iron × harvested times, the highest fresh and dry weight of stigmas were related to 2000 mg L^{-1} of iron chelate in the third year (4.06 and 0.66 g m^{-2} respectively), while the control had the lowest fresh and dry weight of stigmas (Fig. 7 and 8).

Number of flowers

The analysis of variance showed a significant ($p < 0.01$) difference in the number of flowers among all of the treatments, and their interactions (Table 3). The results of the mean comparison revealed that with increasing levels of harvested times, the number of flowers increased (Fig. 9). The highest number of flowers belonged to 2500 mg L^{-1} of iron chelate, mid-vegetative growth in the third year of harvest time treatment (218.33 per m^{-2}) and the lowest number of flowers belonged to control in the first year (24.16 per m^{-2}; Fig. 9).

Length of stigma

Results indicated that there was a significant ($p < 0.01$) difference between spraying times, concentrations of iron, harvested times, spraying times × concentrations of iron, and concentrations of iron × harvested times for the length of stigma (Table 3). There was a significant difference between spraying times × concentrations of iron × harvested times interaction ($p < 0.05$) (Table 3). The highest length of stigma was observed in 2000 mg L^{-1} of iron chelate, mid-vegetative growth in the third year of harvest time treatment (4.66 cm), and the lowest length of stigma was reported in control in the first year (1.82 cm; Fig. 10).

DISCUSSION

Although micronutrients are only found in trace concentrations in soils and plants, they can play an important role in the growth and development of plants (Hodges, 2010). With a total content ranging from 20 to 40 g kg^{-1}, iron is a relatively common element in many agricultural soils. In aerobic circumstances with neutral and alkaline pH, iron is extremely insoluble. It exists either in divalent or trivalent status, while in the divalent status and to a lesser extent in the form of iron chelates (Hodges, 2010). It has been demonstrated that the application of iron fertilizer in sunflowers decreased the adverse effects of stress and improved the NPK absorption, which led to plant growth and yield (Delgado, Sánchez-Raya, 2007). The foliar application of iron to wheat had an impact on the yield components, according to another study, and it also boosted grain yield (Nazran et al., 2010).

A previous study on saffron indicated that weight, diameter, and the number of daughter corms improved with an increase in foliar fertilizer concentration of Dalfard 15® up to 15%; however, an increase in the concentration of up to 20% only improved those variables in the mother corm class below 5 g (Khorramdel et al., 2015). The most significant factor affecting saffron's economic yield is the stigma's dry weight. According to Khorramdel et al. (2015), foliar fertilizer at a 15% concentration increased stigma formation (Khorramdel et al., 2015). Akbarian et al. (2012) studied elements (potassium, zinc and iron) and dosage (0, 1 and 3 litres per hectare) in well-irrigated conditions and concluded when iron, zinc, and potassium were applied topically, by increasing their dosages, the yield of saffron was increased (Akbarian et al., 2012).

Common iron chelate and Nano chelate increase the dry yield of stigma so that the dried yield of stigma (saffron yield) has been significantly influenced by fertilizer treatments that are consistent with traits of the wet yield of flower and wet yield of stigma, indicating the correlation of this trait with the mentioned traits (Farahani and Shahverdi, 2015). Poor farm in terms of iron content on one side, the efficacy of nano-chelate in a slow release on the other can result in the growth of yield which is indicative of the effectiveness of iron found in this plant, like other plants, and its involvement in the process of

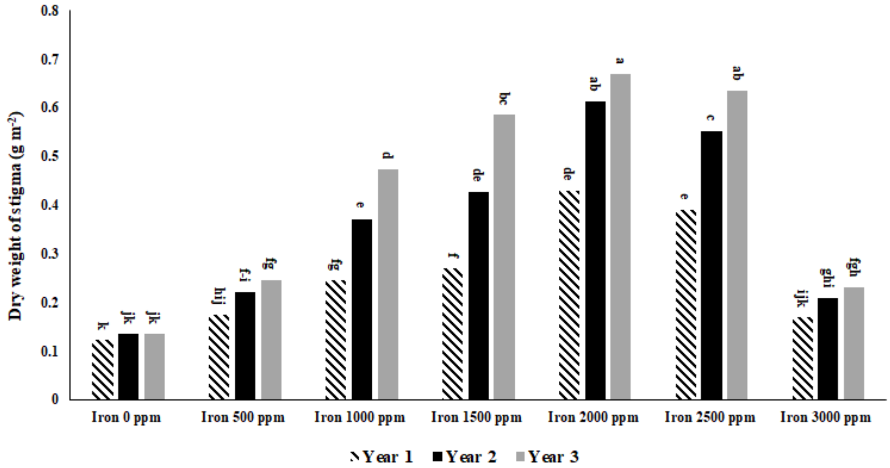

Fig. 8. Interaction effect of concentrations of iron chelate (mg L^{-1}) × harvested times on dry weight of stigmas

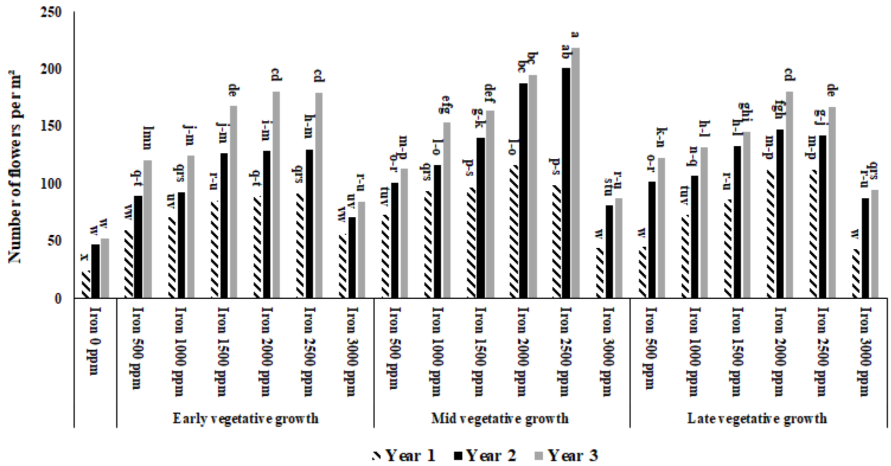

Fig. 9. Interaction effect of spraying times × concentrations of iron chelate (mg L^{-1}) × harvested times on a number of flowers

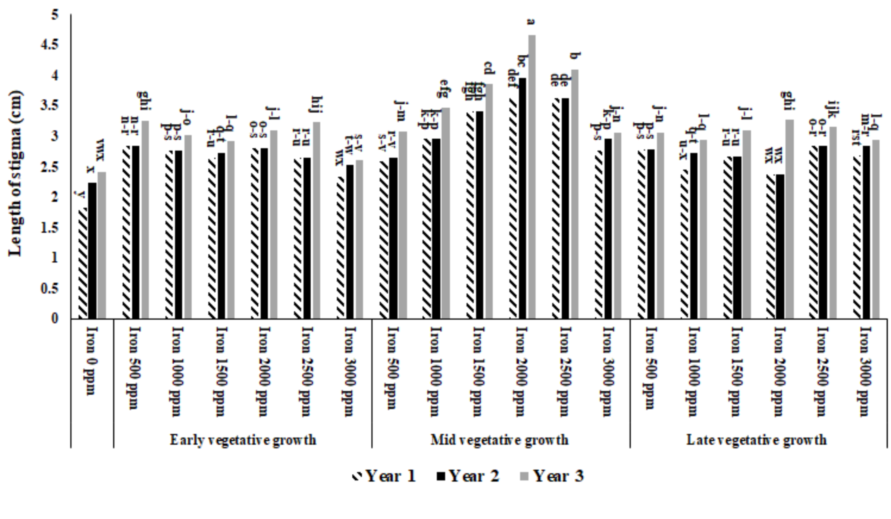

Fig. 10. Interaction effect of spraying times × concentrations of iron chelate (mg L^{-1}) × harvested times on length of stigma

respiration and anabolic (Zuo, Zhang, 2011). However, the data demonstrate that an excessive rise in iron has a negative impact on stigma yield and that treatment with more iron fertilizer, which likely gave the plant access to more iron, has decreased the stigma yield. The results of our experiment showed that the application of 3000 mg L^{-1} of iron decreased all of the traits. The findings demonstrate that the increase in iron fertilizer might negatively impact yield (Asil, 2023), which is consistent with our results. In general, continued use of chemical fertilizers, such as those applied by soil application or even foliar spraying, may have negative long-term impacts on the ecosystem and saffron yield potential (Khorramdel et al., 2015).

Iron chelates or fertilizers have been applied to seeds, soil, and leaves for many years with various degrees of success. Variations in the severity of chlorosis, the type of soil, plant genetics, and low treatment rates can all contribute to these discrepancies. (Wiersma, 2005). In significant deficiency situations, the use of iron chelate may raise the rate of iron as well as lengthen the time during which iron is accessible to the growing plants (Wiersma, 2005).

Iron plays a crucial role in the synthesis of essential components within plant cells, such as cytochromes, phytofurthins, and ferredoxins. These components act as electron transporters during photosynthesis, supporting growth by enhancing plant height and leaf area (Incesu et al., 2015). Additionally, iron is vital for the biological processes that facilitate chlorophyll synthesis, as it constitutes approximately 29 to 35 percent of the iron content in green leaves (Al-Tameemi, 2019). Iron also aids in chlorophyll production by activating enzymes like coproporphyrinogen oxidase, necessary for the sixth stage of porphyrin metabolism, and á-aminolevulinic acid, a key component in chlorophyll synthesis (Barker, Stratton, 2015). Furthermore, iron is essential for photosynthesis, carbohydrate accumulation (especially in the presence of zinc), and the reduction of stomatal resistance. This reduction enhances stomatal conductance, allowing plants to access sufficient water and carbon dioxide for photosynthesis and nutrient absorption from the soil, thereby promoting growth and increasing shoot weight (Kamiab, Zamanibahramabadi, 2016). Iron also plays a crucial role in the formation of leghemoglobin, a protein involved in oxygen transport during nitrogen fixation, and participates in the activity of various enzymes like cytochromoxides, nitrogenase, catalase, peptidase, proteinase, and dehydrogenase. Moreover, iron acts as an active transporter system that aids nutrient translocation from sources (such as leaves) to sinks. It significantly influences the nitrogenase enzyme involved in nitrogen fixation in nitrogen-fixing plants, leading to enhanced flower primordia production. This process allows for increased nutrient supply to the flowers, resulting in larger flower sizes (Al-Tameemi, 2019).

The researcher found that iron fertilizer efficiently enhances the number of flowers and that the number of flowers in saffron was strongly related to the cultivated corm yield (Farahani, Shahverdi, 2015); therefore, unlike this plant's low fertilizer requirement, 16–80% of the variation in flower yield is based on soil factors (Colla, Rouphael, 2009). According to Farahani and Shahverdi 's findings, the maximum mean of the number of flowers across experiment treatments for 10 kg treatments of iron fertilizer was found, while the minimum average weight was discovered for the control treatment. Wet stigma yield is one of the most crucial components of yield because the economic value of saffron depends on it (Farahani, Shahverdi, 2015). Based on a study on the flower number characteristics, there is a strong association between the number of flowers and the stigma yield. Therefore, producing more flowers leads to producing more stigma (Farahani, Shahverdi, 2015).

Our findings indicate that iron-chelate treatment had an impact on the fresh and dry yield of the flower and that treatment of 2000 mg L^{-1} iron fertilizer boosted the fresh and dry yield of the flower. In a study, Farahani, Shahverdi (2015) revealed that appropriate nutrition has an impact on an enhancement in fresh flower yield. The impact of iron fertilizer on this trait suggests that this element is useful in the flower production process and can make a significant difference between different treatments (Farahani, Shahverdi, 2015). Our results are to the findings of Ibrahim and Abd Elkawy on the Valencia orange tree and Farahani, Shahverdi (2015) on Saffron (Farahani, Shahverdi, 2015; Ibrahim, Abd Elkawy, 2018). They discovered that foliar applications of an iron compound increased yield. Furthermore, in a study on kiwi trees, the amount of chlorophyll in the leaves increased quickly as a result of iron chelate, which showed noticeable increases in fruit size and tree yield. (Loupassaki et al., 1995). In some respiratory and photosynthetic enzyme systems, iron catalyzes the synthesis of chlorophyll. Iron deficiency prevents the development of vegetative growth and flower buds, which reduces the number of buds and fruit sets in citrus on calcareous soils (Zekri, Obreza, 2003).

CONCLUSIONS

Regarding the results of this study, different levels of foliar fertilizer, spraying times, harvested times, and their interactions had significant impacts on economic yield and other characteristics of saffron. The best conditions for all the studied parameters were a concentration of 2000 mg L^{-1} foliar fertilizer and mid-vegetative growth in the third year of harvest. By extending the times of harvest compared to the first year, there was a considerable increase in all corm traits and yield components (except the dry and fresh weight of stigmas) in the absence of foliar fertilizer.

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