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The effect of potassium, zinc and iron foliar application on the production of saffron (*Crocus sativa*)

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ABSTRACT

To study the effect of Potassium (K), Zinc (Zn) and Iron (Fe) foliar application on saffron (Crocus sativa L.), two experiments were conducted in 2010 and 2011 in Bam and Gonabad. The experimental design was a randomized complete block with four replications. The type of elements (K, Zn and Fe) and dose of application (0, 1 and 3 lit/ha) were the treatments of these experiments. The experiments were conducted under well-watered condition. Results showed the effects of elements on leaf length, LAI, stigma dry weight, crocin, picrocrocin and safranal were significant. Fe showed higher amounts in mentioned traits than K and Zn. Also the highest amount of these traits were obtained from high dose foliar application (3 lit/ha). On the other hand traits measured in Gonabad experiments were more than Bam. Although combined analysis of variance showed that the difference between years was not significant. Also results showed that although there is the possibility of saffron planting in Bam, but the amount of saffron yield, safranal and picrocrocin in this region was less than Gonabad region. Nevertheless the amount of crocin was not significant in these regions.

Key Words: Saffron, Crocin, Picrocrocin, Safranal, Stigma dry matter

INTRODUCTION

Saffron (*Crocus sativa* L.) is the most expensive spice and 95% of the production is produced in Iran [29, 33, 32]. The researches that have been conducted about the influence of nutrients on saffron quality and quantity, have shown that yield, stigma length, picrocrocin, crocin and safranal were affected by nutrient positively. Agronomic and environmental factors affect quality of saffron [4]. By increasing the altitude, content of phenol compounds and carotenoids increase because UV-radiation is increased [5]. The positive effect of altitude on crocin content has been shown in [4]. The environmental conditions such as temperature influence carotenoids biosynthesis, and quantity of carotenoids increases when temperature decreases [2]. Also phenol compounds increases in high altitude [3]. Crocin, picrocrocin and safranal content in saffron were affected by altitude [1]. By increasing latitude and due to decreasing temperature, amount of crocin and picrocrocin increases [1]. Studies conducted on K nutrition of spice showed that potassium is the second most important nutrient element next to nitrogen for growth and development of spice crops [6]. Potassium is often described as a quality element for crop production. It indirectly improves the utilization of N and protein formation, size, weight, oil content, color etc [6]. Investigation carried out showed that with adequate supply of K, nitrogen utilization by various spice crops are enhanced. Field experiments showed that wherever N:K ratio is 1:2 the yield are maximum in black pepper and for cardamom the N:K ratio is 1:1.5 and for seed spice, the optimum ratio is 1:1 [6]. The effects of K in relation to major, secondary and micro nutrient were studied by relating correlation in different spice. The correlation studies conducted in black pepper showed that soil K was positively correlated with soil Zn ($r=0.47^{**}$) and negatively with soil Fe ($r=-0.39^{**}$). Root K was correlated with root Fe ($r=0.30^{*}$) and with root Cu ($r=0.39^{*}$). The leaf K was also correlated with leaf Ca ($r=0.22^{*}$) and negatively correlated with leaf Mg ($r=-0.21^{*}$) and with leaf Al ($r=0.29^{*}$). Leaf K was positively correlated with soil Al ($r=0.27^{**}$) [6].

Saffron cells can synthesize crocin, crocetin digentiobiosyl ester, in suspension cultures. The crocin family biosynthesis mechanism was studied using high pressure liquid chromatography (H PLC) to determine the glucosyltransferase activity and to develop a method for synthesizing medicine from saffron cells. Previous studies indicated that two glucosyltransferases might be involved in the formation of crocetin glucosyl- and gentiobiosyl-esters. GTase 1 formed an ester bond between crocetin carboxyl groups and glucose moieties while GTase 2 catalyzed the formation of glucosidic bonds with glucosyl ester groups at both ends of the molecule. These enzymes can catalyze the formation of crocetin glucosides in vitro. GTase 1 activity is higher during the first four days of crocin glucosides biosynthesis, but decreases after four days. The formation and accumulation of crocin increased during the first six days and stabilized on the eighth day [12].

The effect of different additives sodium acetate, serine, glycine, polyvinylpyrrolidone (PVP) and activated charcoal on stigma-likestructure (SLS) induction frequency and crocin production from floral organs of *Crocus sativus* L. was studied. Sodium acetate, a potential precursor of crocin biosynthesis, increased induction frequency of petal-originated SLS from 16.0 to 32.7%. Moreover, crocin content in SLS induced on medium supplemented with sodium acetate was increased to 6.00% from 2.21%. PVP prevented browning of explants and slightly increased induction frequency of SLS from petals. The crocin content of SLS was also increased to 5.22% by adding PVP. Although activated charcoal could efficiently reduce browning of explants, dedifferentiation of all explants was inhibited. No obvious stimulation effect had been observed with the addition of serine and glycine [10]. Esmailian reported that the main constituents of saffron to be carotenoids, glycosides, monoterpenes, aldehydes, picrocrocin and antocyanins, flavonoids, vitamins, amino acids,proteins, starch and minerals[44].

MATERIALS AND METHODS

Two field experiments were conducted in Gonabad and Bam, Iran, (34º 21´ N, 58º 41´ E, altitude 1056m, and 29º 6´ N, 58º 21´ E, altitude 1066.9m respectively) during 2010-1011 growing seasons. In each location, soil samples were taken from surface horizon (0- 30 cm) of the soil, air-dried, passed through a 2-mm sieve and analyzed for the following properties. Particle-size distribution determined by hydrometer method [7], soil pH and ECe were measured in saturated paste and saturated extract, respectively, organic compound (OC) were determined by Walkley- Black method [8]. Available Zn and Fe were determined by DTPA extraction [9,43], and phosphorus by sodium bicarbonate extraction [10]. The characteristics of the soil materials were shown in Table 1.

The treatments were compared in a factorial experiment based on Randomized Complete Block Design (RCBD) with three levels foliar application , 3 types of nutrients (Zn, Fe, K) and three levels of doses (control or water sprayed, 1 lit/ha, 3 lit/ha) in four replication. Each plot had 6 rows, 25 cm row spacing and 5 m plot length. Corms were sown on the 25th August 2010 in Gonabad and Bam.

Final plant density was 160000 plant in hectare. Also Foliar application were done at 20th Janury and 20th March with chelated fertilizers in the format of EDTA %15. All of recommended crop

Table 1. The characteristics of the soil in Gonabad and Bam.

Location	EC ds m^{-1}	pH	OC (%)	N (%)	P_2o_5 (mg/100g)	K_2o(mg/100g)	Zn (ppm)	Fe (ppm)
Gonabad	1.27	8.1	0.95	0.051	17.9	31.2	1.08	1.9
Bam	1.04	7.2	0.83	0.042	12.6	21.3	1.3	1.5

production practices were applied uniformly to all treatments. First irrigation was done immediately after sowing and subsequent irrigations were applied to avoid drought stress and soil water in the 1 meter depth was kept above 50% of maximum available water during the all growing season. Weeds were manually controlled in all treatments. Fertilizer recommendations based on soil analysis results (Table 1) were applied at sowing.

Plants were oven dried at 70 °C for 72 hours. Economic yield or Dry Stigma were obtained from means of 10 plants in each plot.

Data were analyzed by Mstat-c software and multiple comparisons was done through least significant range (LSR) test at 1% and 5% probability levels. Also Graphs were performed in Excel software.

RESULTS AND DISCUSSION

Leaf Length

According to analysis of variance (table 2) effects of planting location, elements and dose of application were significant at 1% probability level. Also interaction effect of elements in dose of application was significant at 1% probability level. Mean comparison showed that in terms of impact on leaf length, potassium with 345.2 mm and Zn with 353.2 mm were dominant than Fe element (table 3). The highest and the lowest leaf length were achieved in dose of application with 3 kg/h and no sprayed factor (control) respectively (table 3). Also the result showed that saffron leaf length that planted in Gonabad with 354.1 mm was dominant than Bam location. Although the difference between 2010 and 2011 experiments was not significant.

Evaluation of interaction effect of type of element in dose of application showed that only boosting of Fe dose could increase leaf length (table 3). Studies conducted on nutrient of spices showed that potassium is the second most important nutrient element next to nitrogen for growth and development of spice crop [6]; besides, the growing parts of spice plant contain more K than any other parts [6].

Zinc is a micronutrient needed in small amounts by crop plants, but its importance in crop production has increased in recent years. Zinc is a micronutrient needed in small amounts by crop plants, but its importance in crop production has increased in recent years. It is considered to be the most yield-limiting micronutrient in crop production in various parts of the world [13, 14, 15, 16, 18, 22].

Zinc content of the soils varied significantly depending on type of soil, management practices adopted by the farmers, climatic conditions, crop species planted, and cropping intensity. Zinc content of the lithosphere is approximately 80 mg kg–1, and the common range for soils is 10–300 mg kg^{-1} with an average content of 50 mg kg^{-1} [24].

Presumably the reason of leaf length reaction to Zn application is that, the Zn is necessary for producing chlorophyll and forming carbohydrate, also it is closely involved N-metabolism of the plant.

Table 2.Combined Analysis of variance for quantity and quality characteristics in saffron (the numbers are Sum of Squares)

Sources of variation	d.f.	Leaf length	Safranal	Picrocrocin	Crocin	Stigma dry weight	LAI
Year (Y)	1	13.86^{ns}	0.004^{ns}	0.02^{ns}	0.53**	0.001**	0.05**
Location (L)	1	302.9**	0.12**	0.35*	0.11^{ns}	0.003**	0.11**
YL	1	4.26^{ns}	0.04*	0.02^{ns}	0.25**	0.0002^{ns}	0.002^{ns}
R(LY)	12	162.4	0.137	0.41	0.58	0.0001	0.41
E	2	308.4**	0.81**	4.77**	1.87**	0.003**	0.06**
YE	2	0.73^{ns}	0.07*	0.53*	0.34*	0.0001^{ns}	0.0001^{ns}
LE	2	56.48^{ns}	0.79**	1.09**	0.08^{ns}	0.003**	0.10**
YLE	2	13.07^{ns}	0.08^{ns}	0.589*	0.71**	0.0001^{ns}	0.02^{ns}
D	2	1244.7**	5.65**	23.82**	2.75**	0.151**	1.36**
YD	2	9.77^{ns}	0.04^{ns}	1.66**	0.08^{ns}	0.0001^{ns}	0.02^{ns}
LD	2	19.83^{ns}	0.70**	2.81**	0.11^{ns}	0.001**	0.04*
YLD	2	3.52^{ns}	0.04^{ns}	0.66*	0.13^{ns}	0.0001^{ns}	0.01^{ns}
ED	4	1612.2**	8.68**	5.58**	3.69**	0.148**	1.66**
YED	4	62.71^{ns}	0.02^{ns}	0.93^{ns}	0.27^{ns}	0.0001^{ns}	0.01^{ns}
LED	4	111.71^{ns}	3.219**	6.49**	0.57^{ns}	0.0001 ^{ns}	0.01^{ns}
YLED	4	18.99^{ns}	0.01^{ns}	1.49**	0.38^{ns}	0.0001^{ns}	0.01^{ns}
Error	96	1554.47	0.82	5.43	3.45	0.004	0.65
C.V.(%)		18.37	14.33	16.25	16.91	14.27	12.93

***:p<0.01; *:p<0.05; ns: no significant.*

The abbreviation: Type of element (E), Dose of application (D), Replication (R).

Leaf Area Index (LAI)

Based on the analysis of results (table 2) the effects of elements, dose of application, year and local experiments were significant at 1% probability level. Also interaction effect of elements in dose of application was significant at

1% probability level. Mean comparison showed that Fe and K influenced LAI more than Zn (table 3). Application of 3 kg/h of nutrient was more effective on LAI, and this effect was significant. As a result, most amount of LAI (2.34) obtained from (3kg/h Fe). On the other hand saffron LAI that planted in Gonabad was more than Bam region significantly. Also LAI in 2011 experiment was more than 2010. This result was similar to Amiri and Mesud reports [28, 32]. Nutrients such as K, Fe and Zn are necessary for even growth plants [11]. Crop yields have significantly increased in the last few decades in developed as well as developing countries through the introduction of modern reduction technologies; as a result, supplies of K+ in the soils rapidly depleted [19]. Pretty and Stangel (1985) reported that 17% of the total land area in Africa, 21% of the total land area in Asia, and 29% of the total land area in Latin America are K+ deficient [28]. Hence, application of this element in adequate amount is essential for obtaining optimal crop yields and maintaining soil fertility for sustainable crop production. Also Iron (Fe) plays many important roles in the growth and development of higher plants. Iron has many functions in plants; however, its main role is its participation in many plant metabolic functions, and it is also a component of many enzymes. Uptake of iron by crop plants exceeds uptake of all other essential micronutrients except chlorine. Any factor that decreases the availability of Fe in a soil or competes in the plant absorption process contributes to Fe deficiency [19, 23, 25]. As a result by increasing nutrient, leaf length and LAI was increased, and because of an existence correlation (R^2=0.60) between leaf length and LAI was shown (fig 1), it seems that these nutrients can increase saffron production.

Table3. Mean comparison traits as affected by element, Dose of application, Location and also some of their interactions

Treatments	Leaf Length (mm)	Safranal (%)	Picrocrocin (%)	Stigma dry matter (gr/m^2)	Crocin (mg/gr)	LAI
Elements						
Fe	350.7b	37.87a	65.79b	0.580a	26.00a	2.12a
K	354.2a	37.38c	66.20a	0.577b	25.73b	2.14a
Zn	353.2a	37.46b	66.15a	0.581a	25.79b	2.09b
Dose of application						
0	348.5b	37.73a	65.75c	0.536c	26.03a	1.98c
1	354.4a	37.44b	66.01b	0.577b	25.77b	2.13b
3	355.1a	37.25b	66.56a	0.615a	25.71b	2.21a
Fe0	341.5e	38.24a	65.03f	0.487i	26.50a	1.57d
Fe1	352.7c	37.52bc	65.70e	0.555g	25.76bc	2.18b
Fe3	357.9a	36.94e	66.65a	0.677a	25.73bc	2.34a
K0	355.6abc	37.37d	66.75e	0.572e	25.72c	2.11c
K1	354bc	37.31d	66.22c	0.565f	25.78bc	2.13bc
K3	352.9c	37.47c	66.63a	0.593c	25.70c	2.18b
Zn0	348.5d	37.57b	65.93d	0.549h	25.88b	2.07c
Zn1	356.4ab	37.47c	66.12c	0.610b	25.77bc	2.08c
Zn3	354.6bc	37.34d	66.40b	0.586d	25.72c	2.12bc
Location						
Gonabad	354.1a	37.50a	66.10a	0.581a	25.81a	2.13a
Bam	351.2b	37.44b	66.00b	0.571b	25.87a	2.08b
Year						
2010	353a	37.47a	66.03a	0.573a	25.78b	2.09b
2011	352.4a	37.48a	66.06a	0.579a	25.90a	2.13a

Column means followed by the same letter are not significantly different at 1% probability level

Stigma Dry Matter

According to analysis of variance (table 2) the effects of year, location, elements, dose of application and also interaction of elements in dose of application were significant at 1% probability level. Mean comparison showed that iron and zinc have more influence on stigma dry matter than potassium (table 3). Also with an increase in the dose of application, stigma dry matter increased significantly. Thus maximum stigma dry matter was obtained from Fe (3kg/h) treatment (fig 2). These results were similar to the findings of Rezaian, Jahan, Mesud and Rezvani-Moghaddam [29, 31, 37]. A relatively strong correlation (R^2=0.69) was observed between yield and LAI (fig 3). Also a relatively strong correlation (R^2=0.84) was observed

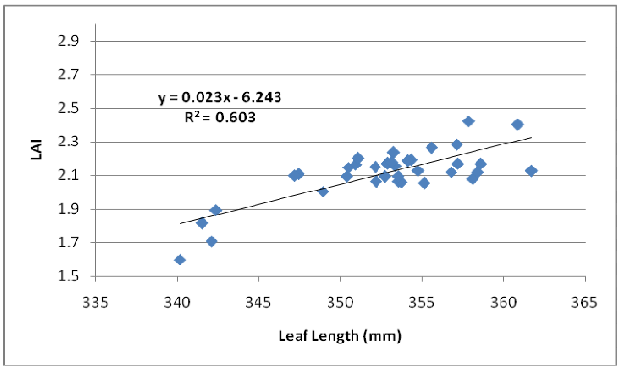

Figure 1. Correlation between leaf length and LAI in saffron

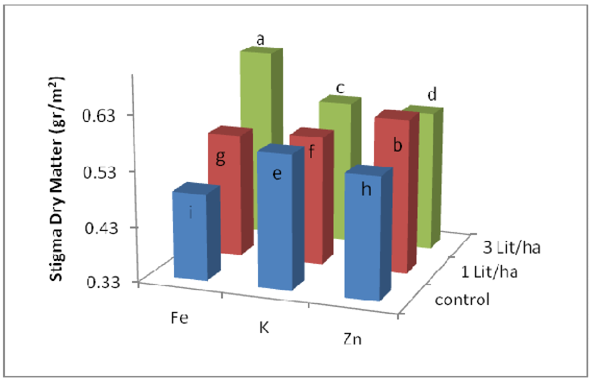

Figure 2. Effect of type of element in dose of application on stigma dry matter

between stigma length and stigma dry matter (fig 4). Thus it seems that the factors that enhance stigma length and LAI, can increase stigma dry matter. Application of Iron, Zinc and Potassium, and increased dose of application of these elements can increase leaf length, leaf number and leaf area index. Taken together, these factors increase the level of photosynthesis. Increasing levels of photosynthesis can increase saffron yield. Yield is a complex plant characteristic and is influenced by many yield components and their interactions [33]. Nutrient concentrations in soil solution have been of interest for many decades as indicators of soil fertility in agriculture [39]. Mineral nutrition refers to the supply, availability, absorption, translocation, and utilization of inorganically formed elements for growth and development of crop plants [25]. Iron is essential for the synthesis of chlorophyll. Chlorophyll synthesis, thylakoid synthesis, and photosynthesis are dependent on the integrity of many iron-containing proteins, including heme and iron sulfur proteins [40]. Also Zinc is necessary for producing chlorophyll and forming carbohydrate. As well as it is required for the synthesis of tryptophane. As tryptophane is also a precursor of indoleacetic acid, the formation of this growth substance is also indirectly influenced by zinc. Likewise zinc has a possible role in plant metabolism involved in starch formation. Also zinc improves root development in crop plants; furthermore, zinc affects carbohydrate metabolism in plants. The reduction of photosynthesis observed in zinc-deficient plants can also be due, in part, to a major decrease in chlorophyll content and the abnormal structure of chloroplasts [41]. Zinc deficiency reduces water use efficiency, which can lead to loss of turgidity and reduced growth [42]. Potassium increases root growth and improves water and nutrient uptake. Also it reduces respiration, preventing energy losses. Potassium aids in photosynthesis and food formation. Likewise it helps translocation of sugars and starch. Although Potassium is not a constituent of chlorophyll, a characteristic symptom of K deficiency is the destruction of chlorophyll. This means that it is suspected that part of the function of K is related to the formation of chlorophyll precursor or to the prevention of the decomposition of chlorophyll [20].

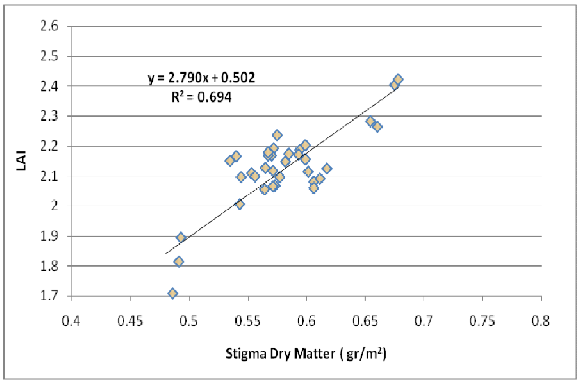

Figure 3. Correlation between LAI and Saffron stigma dry matter

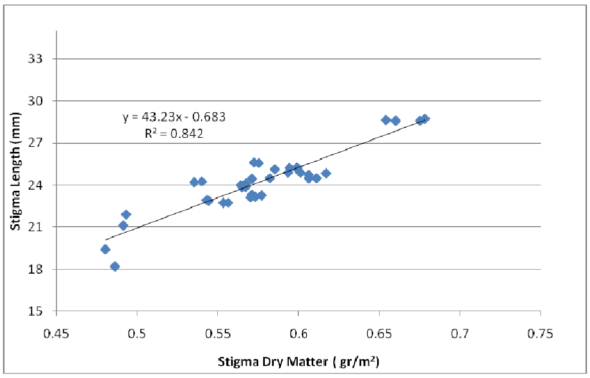

Figure 4. Correlation between stigma dry matter and stigma length

Safranal

Analysis of variance showed (table 2) that elements, dose of application and locality implantation were significant at 1% probability level. Also interaction effect of elements in dose of application was significant at 1% probability level. Mean comparison showed that about safranal, Fe with 37.87% had a higher influence than Zn with 37.46%. It must be said that negative correlation was observed between elements and dose of application. This means that elements application with each concentration reduced safranal(fig 5). On the other hand the effect of year on safranal was not significant, while the effect of implant location was significant at 1% probability level. Content safranal in Gonabad experiment was more than Bam experiment (table 2). According to researchers agronomic and environmental factors affect quality of saffron [4]. By increasing altitude, content of phenol compounds and carotenoids increase [5]. On the other hand because of the biosynthesis of safranal start from cleavage of *zeaxanthin* to produce cyclic carotenoid (safranal and picrocrocin), it seems that decrease in safranal with increase in picrocrocin are due to competition.

Picrocrocin

Based on the analysis result (table 2), effects of elements and dose of application were significant at 1% probability level. Also seeding local was significant at 5% probability level. Further interaction effect of elements in dose of application was significant at 1% probability level. Mean comparison showed that K with 66.20% and Zn with 66.15% were dominant than Fe (table 3). Likewise with increase dose of application, percent of picrocrocin was increased. Studies conducted on K nutrition of spices showed that potassium is the second most important nutrient element next to nitrogen for growth and development of spice crops [6]. The growing parts of spice plants contain more K than any other parts [6]. K+ is required to activate at least 60 different enzymes involved in plant growth also K+ is implicated in increased uptake and transport of Fe in both monocotyledonous and dicotyledonous plants [22]. Therefore K both directly and indirectly affected quality and quantity production. Also Iron is essential for the synthesis of chlorophyll. It is involved in nitrogen fixation, photosynthesis, and electron transfer [27]. As an electron carrier, it is involved in oxidation-reduction reactions [28]. It is required in protein synthesis and is a constituent of hemoprotein. It is also a component of many enzymes and involved in respiratory enzyme systems as a part of cytochrome and hemoglobin [27]. The catalytic function of iron depends on its electronic structure, which can undergo reversible changes through several oxidation states differing by one electron [23]. The results of studies on Zn have been shown that Zinc aids plant growth substances and enzyme systems. Zinc, along with Cu, has been shown to be a constituent of the enzyme superoxide dismutase [25]. Zinc is required for the synthesis of tryptophane. As tryptophane is also a precursor of indoleacetic acid, the formation of this growth substance is also indirectly influenced by zinc. With regard to the role of these elements, increase of these elements can increase picocrocin. Also the content of picrocrocin in saffron that was planted in Gonabad experiment was more than Bam one. Although the difference between years was not significant.

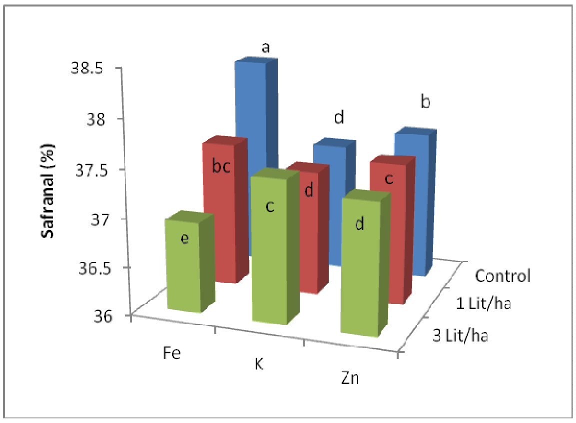

Figure 5. Effect of type of element in dose of application on safranal

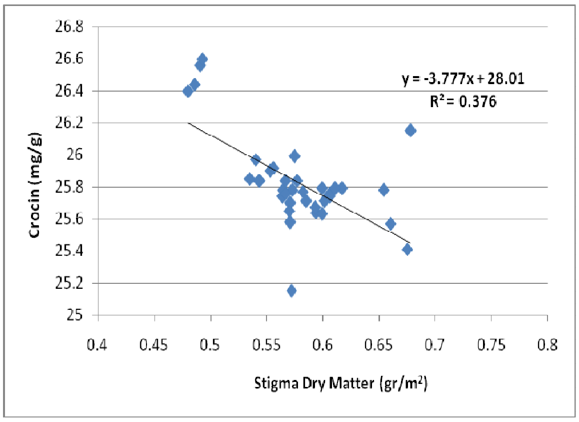

Figure 6. Correlation between stigma dry matter and crocin

Crocin

According to the analysis variance (table 2) the effects of elements, dose of application and interaction of element in dose of application were significant at 1% probability level. The most content of crocin was obtained from control treatment (table 3). Increasing concentration of elements was reduced crocin. As in the case of safranal it seems that there is no possibility of increasing factors affecting the saffron quality simultaneously. Crocin content in tow location was not significant too. Crocin content in saffron that obtained from 2011 experiment was more than 2010. The relationship between dry weight and crocin showed a negative correlation between them (fig 6).

CONCLUSION

1- It could be concluded that, use of nutrients especially Fe had positive effects on saffron quality and quantity characteristics.
2- The amounts of picrocrocin and safranal were reduced by increasing the dose of element application.
3- The highest of stigma dry matter was obtained when Fe was used (3 kg/ha).
4- There are relatively strong positive correlation between stigma dry matter and stigma length, also between stigma dry matter and leaf area index (LAI).

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