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Influence of some pre and post-harvest practices on quality of saffron stigmata

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ABSTRACT

Saffron (*Crocus sativus* L.) yield and quality are affected by many pre and post-harvest activities. Therefore, four separate experiments were carried out to investigate the effect of some agronomic (irrigation, fertilization, and organic/ conventional cultivation) and post-harvest practices on color parameters (*L*, *a*, *b*, *h*°, and *C*) and apocarotenoid content (crocin, picrocrocin, and safranal) in stigmata. Required plots for the first and the second experiments were constructed in September 2013 and again in 2014 for corm planting. Sampling of the first experiment was done one year after each planting. When two years passed since corm planting, sampling was done for the second experiment. In the first study, the combined effect of the production system (one-year-old organic or conventional fields) and drying temperature (25 °C at shade; 55 and 75 °C in the electric oven) were evaluated during two flowering seasons of 2014 and 2015. In both production systems, oven-dried samples at 55 °C had better quality. In the second experiment, the organic and non-organic stigmata (obtained from two-year-old fields in flowering seasons of 2015 and 2016) were stored for 1, 2, or 3 years under room temperature in a dark place. Color coordinates (*a*, *a/b,* and *C*) increased by longer storage duration. The maximum crocin and safranal contents were obtained from organic samples, which were being stored for less than one year. In the third experiment, the effect of irrigation level (3600, 4200, and 4600 $m^3 ha^{-1}$) and fertilization (humic acid, *Rhizophagus irregularis,* and unfertilized control) was evaluated during two flowering seasons (2016 and 2017) on saffron yield and quality. Deficit irrigation treatment (3600 $m^3 ha^{-1}$), associated with the use of humic acid, had the highest yields of flower and stigmata. However, the effect of mycorrhizal inoculation was negative on flowering. Lower water availability plus mycorrhizal inoculation had the highest picrocrocin and safranal content but the lowest *C*, *a/b,* and *a* color parameters. In the fourth experiment, 48 stigmata samples were collected, and then the relationships between color parameters and apocarotenoids content were evaluated using correlation and regression procedures. Safranal had a correlation with *a* (-0.411**), *b* (0.295*), *a/b* (-0.454**) and *h*° (0.410**). Similarly, crocin had a positive correlation with *b* and *h*°, but a negative one with *a* and *a/b* indexes. Overall, the results of four separate experiments revealed that saffron stigmata quality is highly affected by pre and post-harvest practices. It was also concluded that stigmata quality assessment is somewhat possible through measurement of color parameters as a quick and cheap method.

1. Introduction

Saffron, as a member of Iridaceae, is an annual herbaceous plant. However, due to the annual corm proliferation, its perennial cultivation is more common (Koocheki et al., 2019). Despite some doubts, Iran and Greece have been suggested as the possible regions for its origination (Behdani and Fallahi, 2015; Rashed-Mohassel, 2020). Saffron distribution areas are mainly in 30–50 °N and 10 °W to 80 °E. Nowadays, Iran is the leading saffron producer globally, with around 108,086 ha cultivation areas and 376 tons annual dry stigmata production, which includes about 90 % of the global production (Behdani and Fallahi, 2015; Ahmadi et al., 2018).

Stigmata are the main economic part of saffron flowers with many applications in cosmetics, perfumery, food (as flavoring and coloring agent), medical and pharmacological industries (Tong et al., 2015; Chaouqi et al., 2018). Stamen and petal also can be used mainly in the

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food industry (Behdani and Fallahi, 2015). The aromatic profile of saffron contains many different compounds (around 150), mostly terpenes, terpene alcohols, and their esters (Rocchi et al., 2019). The primary chemical secondary metabolites in stigmata are crocins, picrocrocin, and safranal, responsible for color, taste, and aroma, respectively (Li et al., 2018).

The quality of saffron stigmata has significant importance for most consumers. The concept of quality in medicinal plants in general and particularly in saffron includes items such as purity, authenticity, no adulteration, no microbial and radioactive contaminations, no toxins residual, no contamination with heavy metals, and the presence of a sufficient amount of secondary metabolites (Behdani and Fallahi, 2015; Koocheki and Milani, 2020). ISO 3632 standard is a basis for the qualitative classification of saffron based on data obtained by spectrophotometric analysis (Rocchi et al., 2019).

Saffron apocarotenoids (crocin, picrocrocin, and safranal) are the leading qualitative indicators and being formed because of carotenoids cleavage (mainly zeaxanthin and β-carotene) by carotenoid cleavage dioxygenases (CCDs). The cleavage of carotenoids leads to the production of crocetin and hydroxy-β-cyclocitral. After glucosylation by glycosyltransferases, crocetin forms crocin, and hydroxy-β- cyclocitral forms picrocrocin. Finally, hydrolysis of picrocrocin leads to safranal production (Baba and Ashraf, 2016).

Many pre-harvest activities such as climatic conditions and corm origin (Cardone et al., 2019), corm planting density (Koocheki and Seyyedi, 2016), soil properties (Behdani and Fallahi, 2015), water availability (Koocheki et al., 2016), fertilization (Fallahi and Mahmoodi, 2018a) and organic or conventional production system (Behdani and Hoshyar, 2016), can affect saffron stigmata quality. Koocheki and Seyyedi (2016) reported that relatively severe water stress could increase secondary metabolites concentrations in saffron stigmata, despite a decrease in replacement corms growth. It was also confirmed by Hosseini and Rahimi (2016) that an increase in safranal, picrocrocin, and crocin contents in stigmata would be obtained by intensifying moisture stress in saffron cultivation. Results of Koocheki et al. (2016) showed that although four irrigation rounds (in August, October, November, and April) are highly recommended for favorable flowering, the highest picrocrocin and crocin contents were gained under no irrigation conditions.

Ghanbari et al. (2019), in a study on saffron, reported that the effect of mycorrhizal inoculation and chemical fertilizer was negative on the content of total phenolic, while the impact of compost fertilizer was positive. Also, antioxidant activity in stigmata increased by organic amendments application. In another study, it was concluded that biological phosphorus fertilizer improved the stigmata yield and crocin content in comparison with the chemical one, while the maximum values of safranal and crocin obtained in the integrated application of those fertilizers (Naghdi Badi et al., 2011). Behdani and Hoshyar (2016) confirmed that stigmata obtained from organic fields had more secondary metabolites, antioxidant, and cytotoxic properties than non-organic samples. Caser et al. (2019a) revealed that saffron root inoculation with *Rhizophagus intraradices,* increased the antioxidant activity and the content of quercitrin, crocin II, and picrocrocin in stigmata. However, some reduction in other valuable compounds was observed. Aimo et al. (2010) reported that 4-trans-crocin concentration in the stigmata obtained from corms inoculated with mycorrhiza was more than non-inoculated corms. Another study in a soilless system showed that *R. intraradices* had more benefits to saffron than the mix of *R. intraradices* and *Funneliformis mosseae* in terms of stigmata apocarotenoids content (Caser et al., 2019b). Based on the results of Ahmadi et al. (2017) application of humic acid as an organic source of nutrients also led to an increase in crocin, picrocrocin and safranal concentrations in stigmata and anthocyanin content in petals.

Post-harvest activities such as drying methods and storage duration can also affect the quality of saffron. Stigmata are traditionally dried at shade, under ambient temperature, which, in addition to prolonging the drying period, also increases the risk of contamination (Fallahi et al., 2018). Fancello et al. (2018) showed that the frequency of isolated bacterial from the shade and sun-dried saffron were more than oven-dried samples. Chaouqi et al. (2018) concluded that the concentrations of kaempferol-3-sophoroside-7-glucoside, crocins, picrocrocin, and safranal in oven-dried stigmata (at 40 °C) were more than shade-dried ones. Also, shade-dried stigmata, during one year of storage, lost around half of their crocins, picrocrocin, and kaempferol-3-sophoroside-7-glucoside, while safranal showed an increasing trend during storage. Results of Maggi et al. (2010) revealed that stigmata samples after different storage duration (< 1 year, 3–4 and 8–9 years) had different compounds. The highest ranges of compounds with spicy and freshly cut grass aromatic notes was found in the sample with less than one-year storage, while the highest contents of vegetable notes compounds were detected in samples stored for a long time. Comparing three methods of stigmata drying, Bolandi and Ghoddusi (2006) found that microwave oven (300 W) had the best effect on stigmata quality. The same authors also found that, although crocin content decreased, safranal increased during six months of stigmata storage. Similarly, Sereshti et al. (2018) reported that after storage of stigmata for two years, the intensity of color reduced, while their aroma increased. Mollafilabi et al. (2020) found that the content of apocarotenoids in oven-dried stigmata decreased when drying temperature increased from 40 to 60 and 80 °C. Their results also revealed that microwave and freeze-drying are appropriate methods to maintain the quality of stigmata during drying. Koocheki (2020) reviewed different methods of stigmata drying and reported that traditional drying results in carotenoids degradation. Drying by infrared radiation needs lower drying time and energy consumption and increases the values of crocin and safranal. In the microwave dehydration method, stigma dries in a shorter time and at a lower temperature, which leads to higher color strength, aroma, and taste. Finally, freeze-dried stigmata have high safranal and crocin and lower moisture content, although higher initial investment and longer drying time (Koocheki, 2020).

The visual appearance of food products such as spices, which is determined mainly by surface color, is a determinant factor in its acceptance by the consumer. The other interesting point is that color-related parameters can be correlated with internal qualitative indices such as nutritional values or visual or non-visual deficiencies, enabling us to quickly assess product quality at a minimal cost (Pathare et al., 2013; Fallahi et al., 2017). Quality evaluation of saffron is mainly done by spectrophotometry or chromatography methods, which are less or highly expensive, time-consuming, and labor-intensive (Chaouqi et al., 2018; Rocchi et al., 2019). In some previous studies, faster determination of safranal (Maggi et al., 2011) and crocins (Li et al., 2018) in saffron has been investigated. However, the proposed methods still require some time and capital. Therefore, it is valuable to develop a fast and non-expensive procedure for stigmata quality evaluation. Accordingly, in this experiment, besides the assessment of the effect of some pre and post-harvest activities on stigmata quality, we aimed to investigate the relation between color parameters (colorimetry) and the quality of the stigmata through correlation and regression procedures to offer a possible indirect, rapid and cheap way for evaluation of saffron quality.

2. Materials and methods

2.1. Effect of the production system and drying temperature on stigmata quality

This experiment was carried out in factorial layout based on a completely randomized design with three replications during the 2013–14 and 2014–15 growing seasons in the University of Birjand, Iran. Experimental factors were: 1- production system (PS) including organic (OPS) and conventional (CPS) and 2- drying temperature of obtained stigmata from each PS, with three levels (25, 55, and 75 °C).

Corms were planted on 11th September of 2013 and 2014 (Fig. 1), with a density of 100 corms per m^{-2} (20 and 5 cm distances between and along the single rows, respectively) in three plots (each 10 m^2) for each PS. Planting was done at the start of each cultivation season, namely, a new plantation was considered for the second year of the study (Fig. 1). The weight of mother corms used for the experiment was about 8 g in both years. Flowers obtained from the first flowering seasons (autumn, 2013, and 2014) were not harvested, because the experimental treatments had not been applied yet. During the first growing seasons (from mid-autumn 2013, up to mid-spring 2014 and again from mid-autumn 2014, up to mid-spring 2015, for the plots of the first and the second year, respectively), the agronomic activities in each PS were exerted according to Table 1.

Soil in experimental site had a loam texture (sand = 41 %, silt = 43 % and clay = 16 %) with bulk density of 1.41 g cm^3. Before the beginning of the experiment, soil water content at field capacity (FC) was determined to be about 27 % (w/w). Irrigation was applied when the soil water content reached about 55 % of FC (45 % depletion of available water). The amount of water required to bring soil moisture to the FC (up to a depth of 35 cm as the depth of root expansion in this experiment) was 600 $m^3 ha^{-1}$. The amount of water consumed during the growing season was about 3600 m^3 ha^{-1}, used as basin irrigation in both PS. The amount of precipitation during the saffron growing season was about 108 mm.

At the start of the second flowering seasons (autumn, 2014, or 2015), when the fields had one-year-old, flowers were harvested daily from all plots separately (Fig. 1). Then, stigmata related to the flowers of each plot were separated by hand and dried under three different temperatures, including 25, 55, and 75 °C. Flowers obtained from each plot were divided into three parts, and each part was assigned to a specific temperature. In the treatment of 25 °C, stigmata were dried under laboratory conditions at shade for eight days (as traditional drying method). For 55 and 75 °C treatments, an electric oven was used for about 2.5 and 2 h, respectively. In both years, at the end of the flowering stage, the total sum of stigmata in each drying treatment was gathered to be used in qualitative evaluations.

In both years, stigmata color parameters were measured using a colorimeter (TES 135, Shenzhen Youfu Tools Co., Ltd., Taiwan). Then results were expressed as Hunter color values of *L*, *a,* and *b*. *L* is used to denote lightness, *a* redness and greenness, and *b* yellowness and blueness. *L*, ranges from black = 0 to white = 100. For parameter *a*, a positive number indicates red and a negative number shows green [ranging from +60 (red) to -60 (green)], while for parameter *b* positive and negative numbers indicate yellow and blue, respectively [ranging from +60 (yellow) to –60 (blue)] (Kortei et al., 2015; Fallahi et al., 2017; Khayyat et al., 2018). Moreover, color intensity (saturation) or Chroma (*C*) and Hue angle (*h*°) were determined by Eqs. 2 and 3, respectively. Chroma parameter is used to determine the degree of difference of a hue compared to grey color with the same lightness. Higher *C* values mean higher color intensity. For hue angle, 0° or 360° = red-purple (a lower hue value shows a redder product), 90° = yellow, 180° = bluish-green, and 270° = blue, or intermediate colors between adjacent pairs of these basic colors (McGuire, 1992; Pathare et al., 2013).

$$\text{Chroma} = \left(a^2 + b^2\right)^{0.5} \quad (2)$$

$$\text{Hue angle} = tg^{-1}\left(\frac{b}{a}\right) \quad (3)$$

Saffron apocarotenoids content was measured according to ISO 3632. This method has two stages, including aqueous extract preparation and UV–vis analysis. For the preparation of saffron extract, powdered stigmata initially passed through a sieve (0.5 mm pore diameter). Then, 500 mg of sieved sample was transferred to a 1000 mL volumetric flask, and 900 mL of distilled water was added. The solution was shaken away from the light for 1 h at 1000 rpm using a magnetic stir bar. Afterward, the flask reached a volume of 1000 mL using distilled

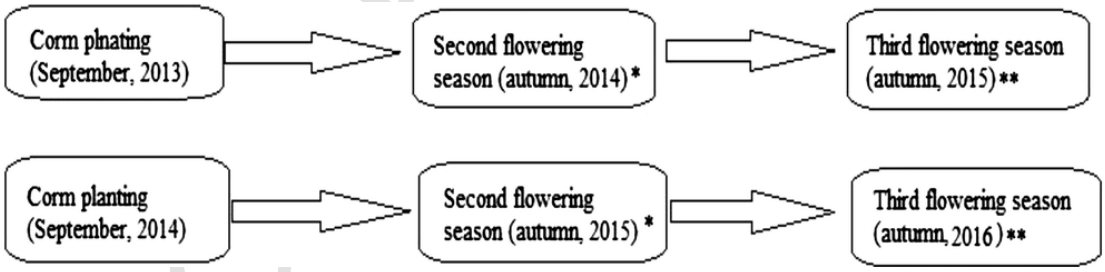

Fig. 1. A simple presentation of the sampling method for experiments 2-1 and 2-2.

Table 1
Agronomic practices during the first growing season (2013-14) for organic and conventional production systems.

Production system	Pre-planting corm disinfection by benomyl (2 per 1000)	Cow manure application (ton ha^{-1})	Pre-planting application of super-phosphate (kg ha^{-1})	Urea application after first and second irrigations and at the end of Feb. (kg ha^{-1})	Hand weeding	Super-Galant herbicide application at the rate of 1.5 L ha^{-1}	Nutrient spraying in March 8, using 20:20:20 NPK
Organic	No	30	0	0	2 times (Jan 18-March 8)	0	0
Conventional	Yes	0	150	150	0	2 times (Jan 18-March 8)	4 in 1000

water and then was stirred to obtain a homogenized solution. After that, 20 mL of solution was transferred to a 200 mL volumetric flask and was filled to the mark, using distilled water, and then the diluted solution was homogenized by agitation. Finally, the solution was rapidly filtered through a filter while being kept away from direct light. In the second stage, spectral characteristics of the obtained extract were monitored by scanning from 200 to 700 nm using a spectrophotometer. Picrocrocin, safranal, and crocin content were expressed as direct readings of the absorbance of 1 % aqueous solution of dried stigmata at 257, 330, and 440 nm, respectively (ISO 3632, 2010). The absorbance at the mentioned wavelengths was evaluated using a 1 cm pathway quartz cell. Double distilled water was used as the reference solution. For calculating the amount of each component, Eq. 3 was used (Cossignani et al., 2014).

$$A_{1cm}^{1\%}(\lambda max) = \frac{D \times 10000}{M \times (100 - H)} \quad (3)$$

In which, D = specific absorbance at 257, 330, and 440 nm, M = sample weight in g and H = sample moisture and volatile content. For measurement of H, a stigmata sample, after weighing, was placed uncovered in an oven set at 103 ± 2 °C for 16 h. Then, moisture and volatile matter percentage was calculated using Eq. 4 (Cossignani et al., 2014).

[(initial mass of sample-final mass)/initial mass] × 100. (4)

Experimental data were subjected to analysis of variance (ANOVA). Data analysis was done using SAS 9.1. Means were compared using the Tukey test (Honestly Significant Difference = HSD) at 5% level of probability. To show the significant difference in the simple and interaction effects of experimental factors, the *P*-values were reported in the tables.

2.2. Effect of the production system and storage duration on stigmata quality

In this experiment, the hypothesis was that the stability of stigmata quality during the post-harvest stage could be different between samples obtained from organic (OPS) or conventional (CPS) production systems. For this purpose, a factorial experiment was carried out based on a completely randomized design with three replications. Experimental factors were PS (OPS *vs.* CPS) and storage duration of dried stigmata (1, 2, and 3 years).

The PSs (OPS and CPS) were similar to the first research (see part 2.1). However, stigmata samples were obtained from the third flowering season (autumn of 2015 and 2016), when the saffron field was two years old (Fig. 1). Chemical analysis showed no significant difference between OPS and CPS in terms of stigmata quality when the field was one year old (see part 3.1). Accordingly, in this experiment, operations related to each PS continued for another year (Fig. 1). According to Fig. 1, experimental plots were created in September 2013 and again in 2014 to be used for corm planting. One year after corm planting (in autumn 2014 and 2015 for 2013 and 2014 plantations, respectively, when the field was one year old), flower sampling was done for the first experiment (see part 3.1), while, when two years were passed since the corm planting, flower sampling was done for the present experiment (in autumn 2015 and 2016, when the field was two years old). It should be noted that about one month after each corm planting, flowering occurred, but these flowers were not used in the experiment because the experimental treatments had not yet been applied. Therefore, flowers of the second and the third flowering seasons (when filed was one and two years old, respectively) were used for the first and the second (present) experiments, respectively (Fig. 1).

In this experiment, the agronomic operations during the first growing seasons (from autumn 2013 or 2014, up to mid-spring 2014 or 2015), were similar to the first experiment (see part 2.1 and Table 1). However, during the second growing seasons (from autumn 2014 or 2015 up to mid-spring 2015 or 2016) there was no superphosphate fertilizer application, while cow manure and urea chemical fertilizer were used at the rate of 10 ton ha^{-1} and 50 kg ha^{-1}, respectively. The other practices were similar to the first growing seasons.

At the start of the third flowering seasons (autumn 2015 and 2016, when the fields had two-years-old), flowers in each plot were harvested daily (Fig. 1). Then, stigmata were separated and dried under room temperature (~ 25 °C) at shade for eight days (traditional drying). It should be noted that the traditional method is less suitable for stigmata drying than new drying methods such as microwave dehydration and freeze-drying (Koocheki, 2020). At the end of the flowering period, total stigmata obtained from each plot were placed into three dark glasses and transferred to the storage room to be stored for 1, 2, or 3 years. Storage condition includes 17–22 °C of temperature, 25–30 % of relative air humidity in absolute darkness. The stored stigmata were evaluated qualitatively when 1, 2, and 3 years had passed from the beginning of storage. Therefore, in each studied year, the total number of stigmata samples was 18 (2 PS × 3 storage duration × three replicates). Measured parameters were the content of crocin, picrocrocin, and safranal and Hunter's color parameters, which the methods of their measurement were described in the first trail. Data analysis was also similar to the first experiment (see part 2.1).

2.3. Effect of irrigation and fertilization on stigmata quality

To evaluate the effect of irrigation and organic fertilization on saffron stigmata quality, a factorial experiment was conducted based on a randomized complete block design with three replications in Sarayan (33 °N, 58°E, and 1450 masl, with a semi-arid climate, annual precipitation of 110 mm and mean annual temperature of 17ºC), Iran. Experimental factors were three levels of fertilization (humic acid, mycorrhizal inoculation along with an unfertilized control) and three levels of water availability (3600, 4200, and 4600 $m^3 ha^{-1}$), which were applied during two successive growing seasons (2015–16 and 2016–17).

In each replicate, there were nine plots (2 × 3 m) in which corms (~8 g) were planted with a density of 100 corms per m^2 (10 cm distances between and along the single rows) at a depth of 15 cm, in early September 2015. The main characteristics of field soil, as well as humic and mycorrhiza properties, are presented in Table 2. Mycorrhizal fungi were applied below each corm at planting time, while humic acid was used in two equal parts (with pre and post-flowering irrigations) during both growing seasons. Mycorrhizal species was obtained from TuranBiotech Company, which was prepared by trap culture method on berseem clover (*Trifolium alexandrinum* L.). The amount of consumed water was similar in both years, and its volume was measured by the contour. Flood irrigation was done separately for each plot using pipes.

Flower harvesting was done daily for two weeks in the autumn of 2016 and 2017. Then, stigmata were separated and dried under laboratory conditions at shade for eight days. At the end of flowering seasons, flower and stigmata yields were determined in both studied years. Also, stigmata obtained from the second flowering season (autumn 2017) were used for qualitative evaluation. Stigmata chemical analysis and color parameters measurements, as well as data analysis, were similar to what was described in the first trail (part 2.1).

2.4. Correlation between color parameters and apocarotenoids content in stigmata

This study aimed to investigate the relationship between the color characteristics of saffron stigmata with the content of its apocarotenoids. For this purpose, 48 different stigmata samples produced in the flowering season of 2018 were prepared from the market in Birjand and Mashhad cities. Then, their color parameters and apocarotenoids

Table 2
Some chemical and physical properties of soil, humic acid and mycorrhizal fungi used in the experiment.

Soil								
Sand (%)	Clay (%)	Silt (%)	Soil texture	pH	P_{ava} (ppm)	K_{ava} (ppm)	N_{total} (%)	Organic matter (%)
25	45	30	Silt-loam	8.3	36	245	0.065	0.77
Humic acid						**Mycorrhiza**		
Humic acid extract	Humic acid	Fulvic acid	K_2O	Brand (produced in Spain)	Rate of application (kg ha^{-1})	Spices	Number of live spores per g soil	Rate of application (g per plant)
85	68	17	13	©Humixtract	12	*Rhizophagus irregularis*	140	10

(crocin, picrocrocin, and safranal) content were measured according to those methods presented in the first experiment (see part 2.1). Finally, correlations between all indices were assessed using SAS 9.2, and wherever correlation was significant, linear regression using Excel was used to predict the relationship between the desired indicators.

3. Results and discussion

3.1. Impact of the production system and drying temperature on stigmata quality

The production system (PS) had no significant effect on stigmata qualitative parameters during both years of the study (Table 3). No impact of PS on saffron quality is discordance with the results of Behdani and Hoshyar (2016) and Fallahi and Mahmoodi (2018a). They found that organic production systems (OPS) had more impact on improving stigmata quality. In this experiment, fields related to both organic and conventional (CPS) production systems were one-year-old. Therefore, it seems that the quality of stigmata is briefly affected when the field is under a PS for a short time. This opinion supported by the results of Jami et al. (2020) on saffron, who reported that the effect of OPS was more in a two-year-old than a one-year-old field. They stated that this issue is associated with the perennial growth habit of the plant, which most often its quality rises from the second year onwards. Shahandeh (2020) also said that the beneficial effect of OPS on saffron quality is less noticeable in the first year of plant growth because the most nutrients in organic manure will not be released in the first growing season. Similarly, Rezaie et al. (2019) reported that OPS and CPS had a no-significant effect on stigmata quality during the first growing season.

Drying temperature (DT) significantly affected on most qualitative traits of stigmata during both studied years (Table 3). Oven-dried samples had more *L* (lightness), *b* (yellowness), *C* (saturation or chroma which describes the color intensity), and *h°*, but lower *a/b* ratio (in the first year) in comparison with the shade-dried stigmata (Table 3). Accordingly, it seems that the oven-dehydrated samples had a better appearance because they were brighter. In the study of Carmona et al. (2005), stigmata dried at higher temperatures, had better appearance due to more intracellular pores but lower tissue cohesion than those dried at room temperature. The *h°* for dried stigmata under different temperatures measured between 14–15° (at 25 °C) and 19–20° (at 75 °C) (Table 3), showing a dark red color of the product (Tham et al., 2018), with more darkness at lower drying temperatures.

In confirmation of our results, findings of Tham et al. (2018) in the roselle showed that the *b* colorimetric index (yellowness) in calyx samples increased with an increase in drying temperature due to non-enzymatic browning reaction. Decrease in *L* and h^o, which was observed in shade-dried stigmata (Table 3), indicates sample browning (Muliterno et al., 2017). Therefore, these samples had lower quality. Raina et al. (1996) also found that stigmata obtained by drying at the range of 35–50 °C had pigments concentration near to that observed in fresh samples with excellent texture, color, and a bright appearance. They concluded that low drying temperature increases the drying period, which leads to enzymatic degradation of crocin (responsible for stigmata color). In contrast, very high temperature leads to thermal degradation of pigments. Therefore, more desirable color parameters of oven-dried stigmata in our study are probably due to a considerable decrease in the drying period (2–2.5 h), which perhaps has reduced the breakdown of pigments.

In both years, the highest amount of crocin obtained from stigmata that were dried in the oven at 55 °C, followed by 75 °C and shade-dried (25 °C) samples (Table 3). Mortezapour et al. (2014) also found that increasing the drying temperature from 40 to 60 °C improved the color strength of saffron stigmata. They reported that the retention of crocin is highly affected by the drying period, which decreased by 62 %, with raising the temperature. In the study of Atyane et al. (2017), crocin content in drying temperature of 60 °C was much higher than lower (30 and 45 °C) and higher (75 and 90 °C) temperatures. In the study of Tong et al. (2015), crocin content increased when drying temperature increased from 50 to 70 °C. However, they emphasized that crocin is a thermos-labile substance, and when the temperature is very high, it would be released due to the destruction of chromoplast. Delshad and Hakimzadeh (2017) reported that crocin as a carotenoid, is exposed to thermal and oxidative degradation. They found that besides the air temperature, other parameters are also important during the drying process of stigmata, where the best conditions for the oven drying process were 51 °C for 112 min and 0.5 cm thickness of the sample layer. In this regard, Carmona et al. (2005) also confirmed that higher temperatures and shorter drying times produced higher crocin than the low-temperature long-term drying treatment. Therefore, for obtaining the highest coloring strength, it can be concluded that when drying temperature is high, the drying period should be reduced and *vice versa*.

During both studied years, stigmata that were dried in an oven at 55 °C had more safranal content compared to the shade-dried sample (Table 3). Considerable safranal content at higher drying temperatures is most probably due to the thermal conversion of picrocrocin (Maghsoodi et al., 2012). Although, Mortezapour et al. (2014) obtained higher safranal in stigmata dried at 40 than 60 °C. In the study of Tong et al. (2015) also the content of safranal at 50 °C was higher than 60 and 70 °C. However, in the study of Atyane et al. (2017), safranal content was more when it dried in the oven at 75 and 90 °C, compared with the lower temperatures. It seems that these differences are mainly due to differences in drying duration, and it is necessary to reduce the drying period with a great deal of obsession by increasing the drying temperature (Delshad and Hakimzadeh, 2017). Yao et al. (2019) concluded that the optimal drying temperature for saffron stigmata was 100 °C for just 20 min. In high temperatures, safranal content increases due to direct thermal conversion of picrocrocin, although enzymatic conversion inactivates at temperatures above 60 °C (Gregory et al., 2005). Some studies had used a combination of high and low temperatures to obtain higher safranal content. For example, Gregory et al. (2005) obtained stigmata with around 25 times more safranal content

Table 3
Means comparison for the simple effect of production system and drying temperature on saffron stigmata quality.

Treatments	*L*		*a*		*b*		*a/b*		*C*		h^o		Crocin ($\lambda_{1cm}^{1\%}$)		Safranal ($\lambda_{1cm}^{1\%}$)		Picrocrocin($\lambda_{1cm}^{1\%}$)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
	Production system with one year old																	
Organic	22.97^{a}	24.58^{a}	37.90^{a}	32.12^{a}	11.02^{a}	9.56^{a}	3.52^{a}	3.38^{a}	89.64^{a}	76.69^{a}	16.51^{a}	17.92^{a}	158.31^{a}	156.01^{a}	24.00^{a}	23.24^{a}	76.15^{a}	70.43^{b}
Conventional	24.04^{a}	24.58^{a}	37.19^{a}	32.73^{a}	11.58^{a}	8.78^{a}	3.29^{a}	3.75^{a}	90.11^{a}	75.38^{a}	17.53^{a}	16.66^{a}	164.96^{a}	166.76^{a}	25.13^{a}	22.82^{a}	78.69^{a}	79.48^{a}
P-value	0.284	0.999	0.595	0.668	0.399	0.064	0.292	0.216	0.849	0.641	0.320	0.367	0.315	0.061	0.489	0.695	0.321	0.002
	Drying temperature (°C)																	
25	21.28^{b}	25.98^{a}	37.43^{a}	30.37^{a}	9.74^{b}	8.65^{b}	3.98^{a}	3.55^{a}	84.53^{b}	71.39^{b}	14.19^{b}	15.88^{a}	140.14^{b}	156.28^{b}	21.24^{b}	20.97^{b}	79.85^{a}	77.25^{a}
55	24.16^{ab}	23.09^{a}	39.31^{a}	33.54^{a}	11.63^{a}	10.08^{a}	3.38^{ab}	3.34^{a}	93.77^{a}	80.33^{a}	16.69^{b}	16.90^{a}	190.74^{a}	170.76^{a}	26.48^{a}	28.04^{a}	78.58^{a}	74.98^{a}
75	25.08^{a}	24.67^{a}	35.90^{a}	33.38^{a}	12.74^{a}	8.77^{b}	2.85^{b}	3.80^{a}	91.32^{ab}	76.38^{a}	20.18^{a}	19.10^{a}	154.03^{b}	157.12^{b}	25.98^{ab}	20.07^{b}	74.84^{a}	73.10^{a}
P-value	0.017	0.154	0.148	0.153	0.003	0.017	0.004	0.430	0.024	0.051	0.001	0.176	<0.0001	0.055	0.036	<0.0001	0.286	0.358

In each column and for each experimental factor, means with at least one similar letter had no significant different based on HSD test.

when they used a short (20 min) initial drying at high temperatures (80–92 °C) followed by drying at a lower temperature (43 °C), compared with stigmata that were dried only at lower temperatures.

The better quality of oven-dried stigmata compared with the shade dried samples (Table 3) is similar to those reported by Atyane et al. (2017) and Chaouqi et al. (2018). Drying under room temperature needs a longer drying duration, which leads to biodegradation of active components, probably due to enzymatic activity. However, high temperatures in the oven will also cause thermal degradation (Maghsoodi et al., 2012). The interaction effect of PS and DT was also significant on *a* (redness), *C,* picrocrocin and crocin contents of stigmata (Table 4). The highest values of *a*, *C*, and crocin obtained from organic stigmata, which were dried in the oven at 55 °C. Stigmata produced under CPS also showed the highest content of crocin when they dried at 55 °C (Table 4). The better quality of stigmata under OPS is associated with the multifunctional role of inputs that are used in such systems. Comprehensive providing of nutritional needs of the plant by theses inputs can increase the production of organic acids, vitamins, and phytohormones as indirect factors in improving the quality of plant products (Naghdi Badi et al., 2011; Fallahi and Mahmoodi, 2018a). In both PS_s, the content of picrocrocin decreased when drying temperature in the oven was more than 55 °C (Table 4), which is probably due to the direct thermal conversion of picrocrocin to safranal (Gregory et al., 2005).

3.2. Impact of the production system and storage duration on stigmata quality

In both studied years, PS exerted a significant effect on the crocin and picrocrocin contents in stigmata (Table 5). The content of crocin and picrocrocin in stigmata that were produced under OPS was around 4 and 5% more than CPS samples, respectively (Table 5). These results are discordance with the results of the experiment reported in 3.1 (Table 3), in which the saffron field was one year old. In section 3.1, it was discussed that why saffron does not show a significant reaction to PS during the first growing season (one-year-old field). Therefore, unlike the first experiment (section 3.1), in the present study, the field was under each PS for two years, which made the effect of the PS on the quality more obvious. Jami et al. (2020) also found that the impact of PS on saffron quality increased with field age increase. Higher stigmata quality in OPS is in a good agreement with the results of Behdani and Hoshyar (2016), who reported that the content of crocin, picrocrocin, and safranal in stigmata obtained from OPS were 18, 17, and 29 % more than CPS, respectively. Similar results were gained by Fallahi and Mahmoodi (2018a), and it was considered relevant to balanced nutrients availability, proper plant photosynthesis, and production of more carbohydrates in OPS.

The effect of storage duration (SD) was significant on the *a*, *a/b*, *C* color parameters in stigmata (Table 5). In both years, the lowest values of *a, a/b,* and *C* were belonged to stigmata, which were stored for one year, while there was no significant difference between two and three years storage durations (Table 5). To the best of our knowledge, there is no report on the effect of storage duration on saffron color parameters. In a study on paprika (*Capsicum annuum* L.), the *L*, *a*, *b* and *C* values significantly decreased after one-month storage. However, with longer storage, the color changes were not significant, probably due to depletion of oxygen from the headspace of the storage bottles, thereby a decrease in ongoing carotenoids oxidation (Topuz et al., 2009). In another study on 36 plants traditionally consumed in Spain as infusions, in 32 % of the samples redness (*a*) increased after three months of storage at room temperature, while this index in remaining plants was stable or decreased. However, under severe conditions (50 °C), the value of *a* increased in 80 % of the plant samples, which probably is related to the development of the Maillard reaction (Jiménez-Zamora et al., 2016). Tham et al. (2019) also reported that enhancement in *C* value is probably the result of enzymatic browning reaction. According to the above-mentioned information, the changes in color parameters during the storage is heavily depending on plant type and storage conditions. Overall, in the present study, more than one-year storage duration led to more darkening and lower appearance quality of stigmata.

SD also significantly affected crocin and safranal content in stigmata in both years of the study (Table 5). The highest values of safranal and crocin obtained from stigmata that were stored for one year. However, there was no significant difference between the storage duration of two and three years in terms of the content of these two main compounds (Table 5). In a good agreement with our results, Raina et al. (1996) concluded that over two years storage period, the content of crocin reduced, but its loss was varied depending on the moisture content. One reason for crocin reduction is that probably the conversion of crocetin to crocins is reversible. Moreover, unknown enzymatic activates, heat, or light can degrade crocins during storage (Sereshti et al., 2018).

The interaction effect of PS and SD was significant on the *a/b* ratio, crocin, and safranal content of stigmata, at least during one of the studied years (Table 6). In both PS, the highest *a/b* ratio was gained when stigmata samples were stored for two or three years, while its lowest values were recorded for one-year storage duration (Table 6). The highest amounts of crocin and safranal were obtained from OPS and storage duration of one year. In both PS, the highest crocin content was obtained when storage duration was one year (Table 6). In both PS and during both years of the study, there was no significant difference between two and three years of storage duration in terms of safranal content, while the storage time of one year had the best status (Table 6). Chaouqi et al. (2018) reported that during one-year storage of stigmata, the concentration of safranal increased, while picrocrocin content decreased. They stated that safranal could not be found in the fresh stigmata, and it is formed during the dehydration, processing, and storage by picrocrocin hydrolysis, so that samples with higher levels of picrocrocin were the same ones with high safranal content. However, in the present study that the shortest storage duration was one year, there was no contrary relationship between safranal and picrocrocin contents. Also, the content of safranal decreased when the stor-

Table 4
Means comparison for the interaction effect of production system and drying temperature on saffron stigmata quality.

Production system	Drying temperature (°C)	*a*	*C*	Crocin ($\lambda_{1cm}^{1\%}$)		Picrocrocin($\lambda_{1cm}^{1\%}$)
		2014	2014	2014	2015	2015
Organic	25	37.68^{ab}	84.01^{b}	125.45^{c}	175.15^{ab}	80.40^{ab}
	55	41.96^{a}	98.21^{a}	196.63^{a}	137.54^{c}	63.03^{c}
	75	34.08^{b}	86.69^{b}	152.87^{bc}	155.33^{bc}	68.79^{bc}
Conventional	25	37.19^{ab}	85.06^{b}	154.84^{bc}	137.41^{c}	74.11^{abc}
	55	36.65^{ab}	89.32^{ab}	184.85^{ab}	203.98^{a}	86.93^{a}
	75	37.73^{ab}	95.95^{a}	155.19^{bc}	158.91^{bc}	77.41^{ab}
P-value	–	0.049	0.032	0.050	< 0.0001	0.0006

In each column, means with at least one similar letter had no significant different based on HSD test.

Table 5
Means comparison for the simple effect of production system and storage duration on saffron stigmata quality.

Treatments	*L*		*a*		*b*		*a/b*		*C*		h^{o}		Crocin ($\lambda_{\frac{1\%}{1cm}}$)		Picrocrocin		Safranal ($\lambda_{\frac{1\%}{1cm}}$)	
	Production system with two-years old																	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Organic	19.07^{a}	20.97^{a}	33.16^{a}	32.24^{a}	14.20^{a}	14.23^{a}	2.34^{a}	2.30^{a}	90.71^{a}	89.24^{a}	23.35^{a}	23.65^{a}	216.44^{a}	220.42^{a}	79.88^{a}	80.78^{a}	35.22^{a}	36.00^{a}
Conventional	20.54^{a}	22.78^{a}	33.21^{a}	32.33^{a}	14.71^{a}	14.72^{a}	2.28^{a}	2.26^{a}	92.02^{a}	90.58^{a}	24.02^{a}	24.63^{a}	208.00^{b}	210.60^{b}	76.00^{b}	76.85^{b}	34.88^{a}	36.15^{a}
P-value	0.120	0.066	0.973	0.945	0.503	0.532	0.316	0.494	0.638	0.625	0.681	0.575	0.006	0.001	0.018	0.027	0.337	0.827
	Storage duration (year)																	
3	20.00^{a}	22.31^{a}	35.91^{a}	34.95^{a}	15.02^{a}	15.01^{a}	2.40^{a}	2.36^{a}	97.33^{a}	95.71^{a}	22.77^{a}	23.11^{a}	203.33^{b}	203.45^{b}	78.66^{a}	78.27^{a}	33.50^{b}	34.50^{b}
2	20.23^{a}	22.23^{a}	33.90^{ab}	33.09^{ab}	14.70^{a}	14.75^{a}	2.32^{ab}	2.28^{a}	93.21^{a}	91.98^{a}	23.47^{a}	23.84^{a}	200.65^{b}	204.96^{b}	76.00^{a}	78.20^{a}	33.00^{b}	32.70^{b}
1	19.18^{a}	21.10^{a}	29.75^{b}	28.82^{b}	13.64^{a}	13.67^{a}	2.21^{b}	2.19^{a}	83.54^{b}	82.04^{b}	24.82^{a}	25.49^{a}	232.66^{a}	238.11^{a}	79.16^{a}	80.98^{a}	38.66^{a}	41.02^{a}
P-value	0.600	0.492	0.010	0.007	0.308	0.349	0.043	0.110	0.004	0.003	0.578	0.518	<0.0001	<0.0001	0.192	0.168	<0.0001	<0.0001

In each column and for each experimental factor, means with at least one similar letter had no significant different based on HSD test.
In Exp 1 and Exp 2, stigmata samples were belonged to the flowering season of 2015 and 2016, respectively, and then stored for 1, 2 or 3 years.

Table 6
Means comparison for the interaction effect of production system and storage duration on saffron stigmata quality.

Production system	Storage duration	*a/b*	Crocin ($\lambda_{\frac{1\%}{1cm}}$)	Safranal ($\lambda_{\frac{1\%}{1cm}}$)	
		Exp. 1	Exp. 1	Exp. 1	Exp. 2
	3	2.33^{ab}	206.6^{b}	32.66^{c}	33.50b
Organic	2	2.43^{a}	204.6^{b}	33.33^{c}	32.00b
	1	2.25^{ab}	238.0^{a}	39.66^{a}	42.50a
	3	2.47^{a}	200.0^{b}	34.33^{c}	35.50b
Conventional	2	2.21^{ab}	196.7^{b}	32.66^{c}	33.40b
	1	2.16^{b}	227.3^{a}	37.66^{b}	39.55a
P-value	–	0.056	0.014	0.002	0.021

In each column, means with at least one similar letter had no significant different based on HSD test.
In Exp 1 and Exp 2, stigmata samples were belonged to the flowering season of 2015 and 2016, respectively, and then stored for 1, 2 or 3 years.

age period increased to more than one year (Table 5). Maggi et al. (2010) also reported that the safranal content of stigmata increased by storage duration increase up to three years, but longer storage reduced that, while HTCC as a possible reservoir for safranal production disappeared during storage. Sereshti et al. (2018) also found a negative correlation between safranal with crocin and picrocrocin content in stigmata during two years of storage, where the intensity of stigmata color and taste reduced, while its aroma increased.

Overall, based on our measurements (Table 5) and previous studies (Bolandi and Ghoddusi, 2006; Sereshti et al., 2018), crocin has a negative correlation with the storage time. However, we obtained contradictory results in terms of picrocrocin and safranal. It is believed that during storage of stigmata, picrocrocin progressively decomposes and produces safranal; thereby the aroma of stigmata increases while their bitter taste reduces during the time (D'Auria et al., 2006; Chaouqi et al., 2018; Sereshti et al., 2018). In our study, picrocrocin did not break down significantly over time, and thereby, there was no enhancement in safranal content (Table 5). The reason for the lack of hydrolysis of picrocrocin during storage was not apparent to us. However, it may be related to the low water content of samples (~6%) during the storage period. In a previous study, it was concluded that picrocrocin hydrolysis reduced considerably when the stigmata water content was lower (Bolandi et al., 2008).

3.3. Interaction effect of irrigation and fertilization on stigmata yield and quality

Simple and interaction effects of irrigation and fertilization were significant on the saffron flower and stigmata yields during both growing seasons (Table 7). Lower water availability and application of humic acid improved the yields of saffron, while mycorrhizal inoculation had a negative effect (Table 7). The best and the worst treatments in terms of flower and stigmata yields were humic acid combined with water application of 3600 $m^3 ha^{-1}$ and mycorrhizal inoculation in all levels of water availability, respectively (Table 8). Better performance of saffron under lower water availability, when the age of the field is below two years, is in accordance with those reported by Fallahi and Mahmoodi (2018a, b) and Koocheki et al. (2020). The negative effect of mycorrhizal fungi on saffron yield is probably due to the use of inappropriate symbiont species (Behdani and Fallahi, 2015). In support of this opinion, Caser et al. (2019b) reported that inoculum type and environmental and growing conditions are critical in evaluating the usefulness of mycorrhizal fungi for saffron flowering. They found that single application of *Rhizophagus intraradices* was more effective to saffron flowering than the mixture of *R. intraradices* and *Funneliformis mosseae*. In addition, inoculation with *R. intraradices* under open field increased

Table 7
Means comparison for the simple effect of irrigation and fertilization on saffron yield and quality.

Treatments	Flower yield (kg ha^{-1})		Stigmata yield (kg ha^{-1})		*L*	*a*	*b*	*a/b*	*C*	Safranal ($\lambda_{\frac{1\%}{1cm}}$)	Crocin ($\lambda_{\frac{1\%}{1cm}}$)	Picrocrocin ($\lambda_{\frac{1\%}{1cm}}$)
	2016	2017	2016	2017	2017	2017	2017	2017	2017	2017	2017	2017
Irrigation level ($m^3 ha^{-1}$ during growing season)												
4600	162.3^{b}	422.6^{a}	2.44^{b}	6.42^{a}	26.06^{a}	25.02^{a}	12.01^{b}	2.23^{a}	89.66^{a}	23.89^{b}	163.88^{b}	72.76^{b}
4200	175.3^{ab}	430.2^{a}	2.74^{a}	6.44^{a}	22.55^{a}	26.61^{a}	13.14^{b}	2.30^{a}	87.04^{a}	25.86^{b}	211.33^{a}	74.55^{b}
3600	194.1^{a}	448.1^{a}	2.76^{a}	6.83^{a}	25.54^{a}	22.10^{a}	15.65^{a}	1.38^{b}	85.90^{a}	38.14^{a}	152.76^{b}	81.33^{a}
P-value	0.013	0.160	0.045	0.071	0.130	0.061	0.003	0.0004	0.818	<0.0001	0.002	<0.0001
Fertilization												
Control	179.3^{ab}	428.7^{b}	2.53^{b}	6.51^{b}	25.24^{a}	22.22^{b}	16.97^{a}	1.34^{b}	91.23^{a}	26.61^{b}	187.30^{a}	74.81^{b}
Humic	193.7^{a}	471.7^{a}	2.98^{a}	7.10^{a}	22.94^{a}	27.24^{a}	12.79^{b}	2.22^{a}	97.09^{a}	25.67^{b}	207.30^{a}	75.38^{ab}
Mycorrhiza	158.7^{b}	400.4^{b}	2.44^{b}	6.07^{b}	25.97^{a}	24.27^{ab}	11.05^{b}	2.34^{a}	74.28^{b}	35.60^{a}	133.36^{b}	78.44^{a}
P-value	0.006	0.0002	0.001	0.0002	0.230	0.037	<0.0001	0.0002	0.004	0.0005	0.0004	0.0295

In each column and for each experimental factor, means with at least one similar letter had no significant different based on HSD test.

Table 8
Means comparison for the interaction effect of irrigation and fertilization on saffron yield and quality.

Water availability ($m^3 ha^{-1}$)	Fertilization	Flower yield (kg ha^{-1})		Stigmata yield (kg ha^{-1})		*A*	*b*	*a/b*	*C*	Safranal ($\lambda_{\frac{1\%}{1cm}}$)	Crocin ($\lambda_{\frac{1\%}{1cm}}$)	Picrocrocin ($\lambda_{\frac{1\%}{1cm}}$)
		2016	2017	2016	2017	2017	2017	2017	2017	2017	2017	2017
	Control	146.3^{b}	427.7^{b}	2.19^{c}	6.50^{b}	22.11^{abc}	15.56^{abc}	1.47^{cd}	87.07^{ab}	23.26^{cd}	258.28^{ab}	68.04^{c}
4800	Humic	173.3^{ab}	439.7^{b}	2.61^{abc}	6.66^{b}	24.26^{ab}	10.09^{cd}	2.47^{abc}	99.96^{a}	15.06^{d}	170.66^{cd}	70.13^{bc}
	Mycorhiza	167.3^{ab}	400.6^{b}	2.52^{bc}	6.11^{b}	28.68^{ab}	10.39^{cd}	2.75^{ab}	81.93^{ab}	33.34^{bc}	162.68^{cd}	80.09^{a}
	Control	193.6^{ab}	433.3^{b}	3.02^{ab}	6.49^{b}	23.92^{abc}	18.72^{a}	1.32^{cd}	99.47^{a}	15.34^{d}	186.4^{bcd}	76.14^{ab}
4200	Humic	186.0^{ab}	454.0^{ab}	2.91^{abc}	6.78^{ab}	25.04^{ab}	11.17^{bcd}	2.31^{abc}	79.33^{ab}	35.67^{abc}	313.91^{a}	76.13^{ab}
	Mycorhiza	146.3^{b}	403.3^{b}	2.29^{bc}	6.05^{b}	30.89^{ab}	9.54^{d}	3.28^{a}	82.32^{ab}	26.57^{cd}	133.66^{de}	71.39^{bc}
	Control	198.0^{ab}	425.3^{b}	2.37^{bc}	6.55^{b}	20.63^{bc}	16.62^{ab}	1.25^{cd}	87.14^{ab}	41.23^{ab}	117.20^{de}	80.26^{a}
3600	Humic	222.0^{a}	521.6^{a}	3.41^{a}	7.87^{a}	32.43^{a}	17.11^{a}	1.90^{bcd}	111.98^{a}	26.28^{cd}	237.33^{abc}	79.88^{a}
	Mycorhiza	162.3^{b}	397.3^{b}	2.51^{bc}	6.07^{b}	13.25^{c}	13.21^{a-d}	0.99^{d}	58.58^{b}	46.90^{a}	103.73^{de}	83.84^{a}
P-value		0.047	0.057	0.006	0.058	0.0002	0.018	0.003	0.012	<0.0001	<0.0001	0.0009

In each column, means with at least one similar letter had no significant different based on HSD test.

stigmata yield, while in the soilless system improved their quality. In a study on saffron, Jami et al. (2020) found that, during the first flowering season, there was a minimum effect of mycorrhiza on flowering. Their results during the second flowering season also revealed that consuming more than 7.5–10 g per plant of soil containing fungus spore had no beneficial effect on flowering.

Irrigation management had a significant effect on the *a*, *b*, and *a/b* color parameters, and the content of all three main apocarotenoids in stigmata (Table 7). Lower water availability (3600 $m^3 ha^{-1}$) led to a lower *a* and *a/b* ratio but higher *b*, safranal, and picrocrocin content. The highest value of crocin obtained at the middle level of water availability (4200 $m^3 ha^{-1}$), while there was no significant difference between the two other irrigation treatments (Table 7). In the study of Koocheki et al. (2016) also, the highest content of crocin and picrocrocin, as two main secondary metabolites, obtained under lower water availability conditions, which was considered as a possible adaptability mechanism against drought stress. Similarly, the results of another study revealed that crocin and picrocrocin contents in stigmata increased by reducing the amount of consumed water (Koocheki and Seyyedi, 2016). Overall, the application of 3600 $m^3 ha^{-1}$ water during a growing season produced stigmata with lower darkness, higher yellowness, and more secondary metabolites. Besides producing stigmata with better appearance and quality, this treatment (3600 $m^3 ha^{-1}$) can be considered in terms of water productivity as a crucial factor for crop production in semi-arid regions. In a similar study on saffron, the application of 3600 $m^3 ha^{-1}$ water plus cow manure caused a good flowering and replacement corm growth and improved water productivity during the first and the second growing seasons compared with the application of 7200 $m^3 ha^{-1}$ (Fallahi and Mahmoodi, 2018a). Accordingly, it can be concluded that saffron has good compatibility to deficit irrigation under arid and semi-arid climates in terms of both qualitative and quantitative parameters (Koocheki et al., 2020).

The effects of nutritional treatments were significant on the *a*, *b*, *a/b*, *C,* and the content of apocarotenoids in saffron stigmata (Table 7). Both humic acid and mycorrhizal treatments produced stigmata with higher *a* and *a/b*, but lower *b*, than the unfertilized control treatment (Table 7). Mycorrhizal symbiosis decreased *C* colorimetric index and crocin content by 22 and 40 %, respectively, but increased the contents of safranal and picrocrocin by 34 and 5 %, compared with the control (no-symbiosis treatment), respectively (Table 7). In a similar study, corm inoculation with a combination of *Rhizophagus intraradices* and *Funneliformis mosseae* reduced the content of crocin and picrocrocin. In contrast, alone application of *R. intraradices* increased both of them (Caser et al., 2019a). Therefore, possibly the qualitative response of saffron to different species of mycorrhizal fungi can be different. Production of terpenoids like safranal is heavily dependent on phosphorous and nitrogen availability (Aalizadeh et al., 2018). Accordingly, higher content of safranal under mycorrhizal inoculation is probably due to more availability of those nutrients. In support of this theory, Naghdi Badi et al. (2011) and Aalizadeh et al. (2018) concluded that saffron corm inoculation with nitrogen-fixing and phosphorus solubilizingbacteria such as *Bacillus subtilis, Azotobacter chrococum, Pseudomonas putida*, *Pantoea agglomerans,* and *Pseudomonas aeruginosa* had a positive effect on saffron apocarotenoids content.

Humic acid application caused an 11 % enhancement in crocin content in comparison with its content in stigmata that were obtained from unfertilized plants (Table 7). Golzari Jahan Abadi et al (2017) also reported that humic acid increased the content of crocin and picrocrocin in stigmata. In a study on saffron, cow manure as a source of humic substances increased the content of safranal by three times and the content of crocin by 33 % compared to unfertilized plants. Additionally, although chemical fertilizer did not affect crocin content, cow manure increased that by 48 % (Fallahi and Mahmoodi, 2018a). It has been reported that organic and biological fertilizers can be effective on the quality of saffron through the providing of hormonal substances and water-soluble vitamins as well as by the production of primary compounds useful in the biosynthesis of glucosides and their decomposition into secondary compounds (Heidari et al., 2014).

The combined effect of water availability and fertilization was significant on almost all color parameters and apocarotenoids concentrations in stigmata (Table 8). Nutrition by humic acid and mycorrhizal fungi caused an increase in *a* and *a/b* values compared with the control in all levels of water availability, except for mycorrhizal symbiosis at a lower level of water consumption (3600 $m^3 ha^{-1}$), which produced the lower values of those indices among all combined treatments. Against *a* value, *b* index decreased in all three irrigation treatments, when nutritional treatments exerted on the plant (Table 8). Irrespective of the irrigation level, mycorrhizal symbiosis decreased *C* index compared with the unfertilized treatment, while humic acid application increased that by 15 and 28 %, respectively, in high (4800 $m^3 ha^{-1}$) and low water availability levels in comparison with the unfertilized control treatment (Table 8).

Mycorrhizal symbiosis, as the best fertilization treatment, increased the content of safranal by 44, 73, and 14 % in high, medium, and low water availability levels, respectively. However, mycorrhizal inoculation reduced the crocin content in all three irrigation levels compared with the control treatment, although humic acid application improved that by 68 and 128 % in medium and low levels of water availability, respectively (Table 8). Caser et al. (2019a,b), by studying mycorrhizal inoculation in saffron, found that the inoculum type is critical in terms of obtained stigmata quality. Their results showed that the crocin content of stigmata decreased when corms were inoculated with a mixture of *Rhizophagus intraradices* and *Funneliformis mosseae*. In contrast, single application of *R. intraradices* enhanced its concentration.

Overall, mycorrhiza had a positive effect on the content of safranal in stigmata (Table 8). The positive role of mycorrhizal inoculation (*Rhizophagus intraradices*) on stigmata picrocrocin and safranal concentrations has previously been reported by Caser et al. (2019b) in a soilless system. In inoculated plants, some cytological changes such as an increase in the number of plastids and mitochondria occur, which activates the tricarboxylic acid cycle and the plastid biosynthetic pathways, thereby enhancing the production of primary and secondary metabolites. Moreover, higher photosynthetic activity in the host plant results in more production of primary metabolites, which are predecessors of secondary metabolites (Pedone-Bonfim et al., 2015). In a similar study on saffron, the interaction effect of water availability and fertilization was significant on stigmata quality, where crocin content decreased under low water availability combined with no-fertilization or chemical fertilizer application, while cow manure application had a positive effect on crocin content (Fallahi and Mahmoodi, 2018a). Lower water consumption increased the content of safranal, regardless of nutrition treatment (Table 8). The highest safranal content was obtained by mycorrhizal inoculation combined with the application of 3600 $m^3 ha^{-1}$ water, which was 23 % more than no-fertilization combined with the application of 4800 $m^3 ha^{-1}$ water (Table 8).

3.4. Correlation between color parameters and apocarotenoids content in stigmata

The correlation between safranal with *a* and *a/b* ratio was negative, while there was a positive correlation between safranal with *b* and h^o (Table 9). R^2 values for correlations of safranal with *a*, *b*, *a/b,* and h^o were 0.17, 0.20, 0.21, and 0.26, respectively. Crocin correlations with *b* and h^o were positive, but its correlation with *a* and *a/b* was negative (Table 9). The values of R^2 for correlation of crocin with *a* (0.26), *b* (0.1), *a/b* (0.18), and h^o (0.19) were relatively low. Additionally, the correlation of *a* with *b* and h^o was negative, but its correlation with *C* was positive. Index of *b* showed a positive and negative significant cor-

Table 9
Correlation between different apocarotenoids and color parameters in saffron stigmata.

	L	*a*	*b*	*a/b*	*h*°	*C*	Safranal	Crocin	Picrocrocin
L	1								
a	−0.023^{ns}	1							
b	−0.028^{ns}	−0.365**	1						
a/b	−0.012^{ns}	0.669**	−0.86**	1					
h°	0.001^{ns}	−0.750**	0.882**	−0.927**	1				
C	−0.046^{ns}	0.602**	**0.521****	−0.138^{ns}	0.065^{ns}	1			
Safranal	0.027^{ns}	−0.411**	0.295*	−0.454**	0.410**	−0.112^{ns}	1		
Crocin	−0.228^{ns}	−0.442**	0.288*	−0.429**	0.435**	−0.157^{ns}	0.753**	1	
Picrocrocin	0.074^{ns}	−0.203^{ns}	0.002^{ns}	−0.190^{ns}	0.100^{ns}	−0.168^{ns}	0.252^{ns}	0.173^{ns}	1

ns: no-significant, * and ** significant at 5 and 1% probability level, respectively.

relation with *h*° and *C*, respectively. Also, the correlation between crocin and safranal was positive (Table 9), so that, with an increase in safranal content in stigmata, the content of crocin increased (Fig. 2). Among all correlations between qualitative traits and color indices, the highest R^2 value (0.55) was found for the crocin x safranal correlation (Fig. 2). Overall, it was concluded that stigmata that were redder and darker in appearance had lower levels of apocarotenoids.

Moratalla-López et al. (2019) said that colorimetry is a possible analytical technique for saffron quality analysis. Ordoudi et al. (2018) found that colorimetry is a rapid, promising tool to detect the presence of unrealistic colors (adulteration) in saffron. In a study on saffron, it was reported that the correlations found between color parameters and crocin content were not very high. In that study, among all correlations, parameter *a* was more appropriate for examining the coloring potential (crocin content) of stigmata (Cuko et al., 2004). Ordoudi and Tsimidou (2004) stated that *a* and *h*° color parameters could offer some preliminary evidence as to whereas red colorants are present in an aqueous saffron extract. Yadollahi et al. (2007) observed a good correlation ($R^2 = 0.84$) between the chroma and coloring strength values in saffron stigmata.

Color measurement is an indirect measure of quality factors like flavor and pigment contents such as carotenoids. This method is simple, fast, and correlates well with other physicochemical characteristics (Pathare et al., 2013). Currently, saffron phytochemical analysis is done by different methods such as high-performance liquid chromatography, thin-layer chromatography, gas chromatography-mass spectrometry, and extraction techniques (Anuar et al., 2017; Li et al., 2018). Colorimetry is one of the low cost and easy methods that can probably estimate the quality of saffron stigmata. A preliminary study on saffron *L*, *a* and *b* coordinates showed the relationship between the color and the quality of stigmata. In that study, a linear correlation was obtained between the chromatic parameters and coloring power (Alonso et al., 2003). Anuar et al. (2017) found that *C* and *b* had a significant positive correlation with luteolin but not with kaempferol and quercetin.

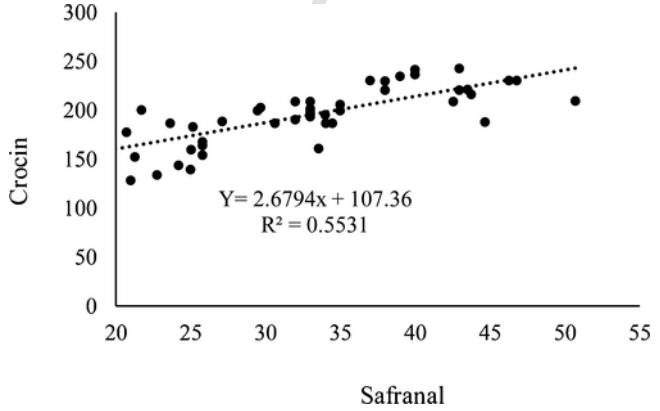

Fig. 2. Regression between crocin and safranal content in saffron stigmata.

Fallahi et al. (2019) also reported that crocin had a positive correlation with *L* and *b* but negatively related to *a* color parameter. In the current study, we found that colorimetry might represent, to some extent, the apocarotenoids content of saffron stigmata. However, further researches are needed to fully understand the extent of this relationship.

4. Conclusion

Results of current experiments revealed that stigmata color and quality are severely affected by pre and post-harvest practices. Saffron grown under organic production system had better quality than inorganic one, especially in the fields that were under organic management for more than a year. Also, stigmata had proper amounts of apocarotenoids under low water availability, especially when combined with humic acid application and mycorrhizal inoculation. In terms of post-harvest practices, stigmata drying in the oven at 55 °C and storage duration below one year improved the apocarotenoids content. Finally, we found that a quick evaluation of the safranal and crocin content of stigmata from the color parameters is somewhat possible. For a more accurate evaluation of the relationship between colorimetric data and stigma quality, it is suggested that in future studies, the correlation between data obtained from the HPLC method and color indices be also evaluated.

Declaration of Competing Interest

None.

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