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Yield and qualitative and biochemical characteristics of saffron (*Crocus sativus* L.) cultivated in different soil, water, and climate conditions

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Abstract. Saffron is highly valued for its unique aroma, taste, color, and medicinal properties. Iran is one of the most important saffron-producing countries. The present study aimed to investigate the effect of climatic and environmental characteristics of six sites (Shirvan, Faruj, Zavareh, Torbat-e Heydarieh, Ghayen, and Birjand) on the yield and qualitative, and biochemical characteristics of saffron. The studied sites were considered as treatments. The obtained data were analyzed based on a nested design, where the village within the site was considered an experimental error, and the farm within the village within each site was considered a sampling error. The Torbat-e Heydarieh treatment with altitudes of ~1323.3 m produced the maximum saffron flower yield (0.83 g m²), stigma yield (0.098 g m²), safranal content (15.8%), picrocrocin content (30.6%), and crocins content (69.3%). Evidently that the low maximum summer temperature in the area is one of the reasons for its superiority. The correlation analysis between traits shows that the maximum summer temperature had a significant negative correlation with saffron flower yield, stigma yield, and picrocrocin and crocin content. Results showed the highest total flavonoid and phenol content and DPPH activity related to Shirvan and Faruj. Although the results showed that selenium could increase the quantitative and qualitative yield of saffron, this requires further studies to confirm it. Based on the findings, it is concluded that I) qualitative and quantitative characteristics of saffron are strongly controlled by the environmental and climatic conditions and II) Razavi Khorasan province had a significant advantage in terms of flower and stigma yield and safranal, picrocrocin and crocin content of saffron and North Khorasan province in terms of biochemical characteristics.

Keywords: DPPH activity, maximum summer temperature, saffron flower yield, safranal content, selenium, stigma yield.

INTRODUCTION

Saffron (*Crocus sativus* L.) is one of the most expensive cash crops among medicinal plants in the world and thus it has been called “the red gold” (Cardone *et al.*, 2020). From ancient times, saffron has been used as a dye for fabrics, a condiment to enrich food, and a drug to treat various human diseases (Cardone *et al.*, 2019). Saffron is the most expensive spice globally, and it has a special place in Iran’s industrial and export products (Kafi *et al.*, 2018). In addition to the stigma and corm yield, the spice quality represents an important parameter that contributes to increase the saffron economic value. Quality is determined chemically by three main secondary metabolites: safranal ($C_{10}H_{14}O$, a major component of essential oil), picrocrocin ($C_{16}H_{26}O_7$, monoterpene glycoside, a precursor of safranal), and crocin ($C_{44}H_{64}O_{24}$, water-soluble crocetin esters), which are responsible for the odor, bitter taste, and color, respectively (Cardone *et al.*, 2020).

Saffron is mainly cultivated in Iran (the source of more than 90% of world production), followed by India, Spain, Morocco, Greece, and Italy (Babaei *et al.*, 2014; Shokrpour, 2019). Saffron global production is estimated at 418 t y^{-1} on 121,338 ha. It is known as beneficial for human health due to three main bioactive compounds: crocin, picrocrocin, and safranal (Cardone *et al.*, 2020). Saffron cultivation dates back to more than 750 years in the southern and central regions of Khorasan, Iran (Kafi *et al.*, 2018). Although Iran is the leading producer of this product in the world with an annual production of 200 tons of dry saffron from more than 60,000 ha of arable land, the maximum yield of saffron in Iran is about 7.5 kg ha^{-1} , and the average is 3.96 kg ha^{-1} , which shows a significant difference compared to countries such as Spain with 15 kg ha^{-1} and Pakistan with 9 kg ha^{-1} (Feli *et al.*, 2018).

Recently, several researchers studied how different parameters such as temperature, humidity, light, substrate, mother corm dimension, planting density and application of nutritional elements, affect stigma and corm yield (Ghanbari *et al.*, 2019; Cardone *et al.*, 2020). Moreover, many factors are conducting to the growth, development, and yield of saffron, the most important of which are climatic and environmental conditions (Rahimi *et al.*, 2017). Ecological and climatic conditions, e.g., temperature, soil, and water content, noticeably affect both the quantitative and qualitative traits of saffron (Amirnia *et al.*, 2014). Maleki *et al.* (2017) reported that the flowering and yield of this plant are directly related to ecological conditions and field management. Temperature is the most critical environmental factor in con-

trolling the growth and flowering in *Crocus* species. The optimum temperature for flower initiation and development of the corms is 23–27 °C (Rahimi *et al.*, 2017). Besides, saffron cultivation in the world shows that the adaptability to soil types, temperatures, and day length encourages its production from the Mediterranean basin to the Middle East (Baghalian *et al.*, 2010). This is why some scientists believe that although saffron reproduces only by vegetative propagation, it displays considerable morphological and biochemical variations between and within populations (Baghalian *et al.*, 2010; Moradi *et al.*, 2020). Saffron grows well in temperate and dry environments, while cold weather has a vital role in its vegetative growth. However, some reports suggest rainy autumns, mild winters, and warm summers as the optimal climatic conditions for this species (Ghorbani and Koocheki 2017; Hussain *et al.*, 2019). In this regard, Lage and Cantrell (2009) reported that the analysis of the environment effect on saffron quality showed that just the altitude affects crocins. Using principal component analysis (PCA), Cardone *et al.* (2019) demonstrated that the cultivation site with higher temperature and without excessive rain during the flowering period generated the best stigma yield with high-quality traits. In this regard, Perpina *et al.* (2013) showed that the most suitable areas for biomass production in Valencia (Spain) were located in the vicinity of residential zones. Maleki *et al.* (2017) reported that the soil and climatic variables have a major role in the development of saffron cultivation.

Secondary metabolic profiles have been studied in different plants such as saffron (Parizad *et al.*, 2019). Environmental conditions associated with the geographical origin, including altitude, temperature, rainfall, humidity, and soil properties, influence the plant development and growth and may have strong effects on the plant’s secondary metabolite production (Jelínek *et al.*, 2012; Parizad *et al.*, 2019). Parizad *et al.* (2019) reported that more than 92% of Iran’s saffron is cultivated in Razavi Khorasan and South Khorasan provinces. Hence, this work aimed to study the yield, quality, and secondary metabolites of saffron in six cities of North, Razavi, and South Khorasan provinces.

MATERIALS AND METHODS

Experimental conditions and plant materials

The experiments were conducted on saffron cultivated (three-year-old) under diverse environments, including six cities in North, Razavi, and South Khorasan provinces (Shirvan, Faruj, Zavareh, Torbat-e Heydarieh, Ghayen, and Birjand) during the growing seasons 2018-

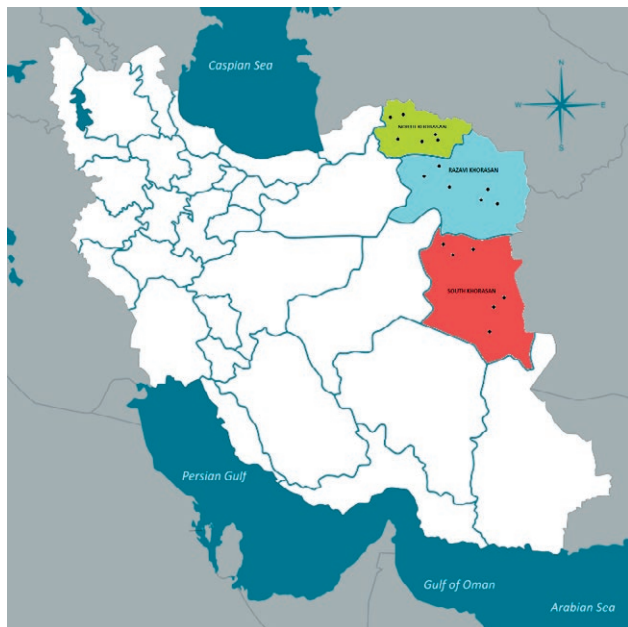


Fig. 1. Different sites location of North, Razavi, and South Khorasan provinces in the map of Iran.

2019. Six sites (with three villages in each site and three farms in each village) with different climates and altitudes were chosen for saffron cultivation (Fig. 1). In each site, geographical and climatic factors including relative humidity, maximum and minimum temperatures, annual frost days, average annual temperature, and rainfall were obtained from the nearest meteorology stations (Table 1).

Corms of 3.0–3.5 cm horizontal diameter and 14.0–19.0 g weight were sown in August at each site from Torbat-e Heydarieh origin. Corms were placed in raised beds, 80 cm wide and 30 cm high from soil level, with three rows of corms in each “bed” at 10 cm depth, 20 cm apart rows, and 5 or 6.7 cm within rows to the adopted density. The studied sites were considered treatments. The obtained data were analyzed based on a nested design. The village within the site was considered an experimental error, and the farm within the village within each site was considered a sampling error. In all areas, the soil was fallow in the previous year, and no fertilizer and no irrigation were applied. Weeds management was carried out by hand throughout the experiment.

Measurement of yield and qualitative characteristics

Whole flowers (in 1 m² for each farm) were manually picked daily early in the morning in the first hours after sunrise and before the flower had wholly opened.

Immediately after flower picking, stigmas were separated by hand and dried in a forced-air oven (BM-55E, Fanazma-Gostar Company, Iran) at 30 °C for 24 h. The flower and stigma yield was calculated per unit area of 1 m².

To measure the content of crocins (color agent), picrocrocin (bitter taste agent), and safranal (odor agent), stigmas related to each farm were dried at 55 °C for 45 min, then weighed and powdered for chemical analysis. The chemical analysis was performed according to ISO-3632 method, from which exactly the Iranian National Standard No. 259-2 has been adapted (Maleki Farahani and Aghighi Shahverdi, 2015). For this purpose, UV/Visible spectrophotometer (Jenway-6305, France) measuring the number of crocins, safranal, and picrocrocin, were used at wavelengths of 440, 330, and 257 nm, respectively. To compare the mean of the treatments, equation [1] was used (Esfanjani *et al.*, 2017).

$$E^{1\%}_{1\text{cm}} = 10000 \times \text{OD}/m (100-H) \quad [1]$$

Whereas: $E^{1\%}_{1\text{cm}}$ is aqueous saffron extract; OD: specific absorption, m: the weight of sample load in grams per 100 ml; and H: moisture content of the dry stigmas, which is usually between 8 and 10%.

Biochemical analyzes

To measure the biochemical analyzes such as anthocyanin, total flavonoid, and total phenol content as well as DPPH radical scavenging activity, sampling of total flowers in three replications was performed by the following methods.

Measurement of anthocyanin content

The anthocyanin content was measured spectrophotometrically as described previously (Sakamoto and Suzuki, 2019), with modifications. Fresh samples (total flower) were promptly dried in an oven at 55 °C for six h. Dried samples were weighed and soaked in 1 ml methanol containing 1% HCl and were incubated at 95 °C for 15 min. The sample was then cooled to room temperature. After removing the solids, the absorbance of the supernatant was measured at 533 nm, and a standard calibration curve was prepared using cyanidin-3-glucoside (CY).

Measurement of total flavonoid content

The total flavonoid content of samples' extracts (total flower) in different sites was determined by Esmaelian

Table 1. Details and environmental characteristics of the trials different sites of North, Razavi, and South Khorasan provinces of Iran.

Site number	Site name*	Province	Longitude (E)	Latitude (N)	Altitude (m.a.s.l)	Relative humidity (%)	Minimum winter temperature (°C)	Maximum summer temperature (°C)	Number of annual frost days	Average annual temperature (mm)	Average annual rainfall (mm)
S1	Shirvan	NK	57°99'111"	37°17'111"	1168.33	60	25.2	41.6	107	13.1	226.7
S2	Faruj	NK	58°17'222"	37°14'033"	1207.33	57	23.0	41.5	89	12.6	243.6
S3	Zavareh	RK	59°32'333"	35°16'689"	1370.33	45	24.0	41.0	89	14.5	245.0
S4	Torbat-e Heydarieh	RK	59°19'178"	35°39'089"	1323.33	45	24.6	40.6	88	14.3	246.2
S5	Ghayen	SK	58°95'856"	33°41'389"	1768.33	40	27.2	42.0	88	14.7	161.0
S6	Birjand	SK	59°34'467"	32°46'078"	1812.66	35	21.5	43.0	71	16.5	147.4

NK: North Khorasan; RK: Razavi Khorasan; SK: South Khorasan; m.a.s.l: meter above mean sea level.

* Shirvan villages: Vorg, Razmoghhan, Feizabad; Faruj villages: Siahdasht, Seghonbad, Mafraangha; Zavareh villages: Karizbala, Dolatabad, Safiabad; Torbat-e Heydarieh villages: Abrod, Damesk, Molkabad; Ghayen villages: Tajan, Andrik, Penhaei; Birjand villages: Nofrest, Galian, Chaj

et al. (2020) method. A 0.5 ml aliquot of 20 g L⁻¹ AlCl₃ ethanolic solution was added to 0.5 ml of extract solution. After one hour at room temperature, the absorbance at 240 nm was measured. The yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg ml⁻¹. Total flavonoids content was expressed as Quercetin (QE) (mg QE/g DW).

Measurement of total phenol content

The total phenol content was measured spectrophotometrically using the Folin–Ciocalteu method described previously (Baba *et al.*, 2015), with slight modifications. The samples (50 mg fresh weight of total flower) were homogenized with 500 µL of 90% methanol. The sample was then centrifuged at 10,000× *g* for 5 min. The supernatant (20 µL) was diluted with 680 µL of distilled water, and 50 µL of phenol reagent was mixed. After adding 300 µL of 5% sodium carbonate, the mixture was incubated at 25 °C for 30 min in the dark. The supernatant's absorbance was measured at 765 nm, and a standard curve was prepared using gallic acid (GA).

Measurement of DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined by using the method described by Sánchez-Vioque *et al.* (2012). The reaction mixture (total volume 3 ml), consisting of 0.5 ml of 0.5 M acetic acid buffer solution at pH 5.5, 1 ml of 0.2 mM DPPH in ethanol, and 150, 200, and 250 µmol ml⁻¹ solution, was shaken vigorously with various sam-

ples (total flower). After incubation at room temperature for 30 min, the remaining DPPH was determined by absorbance at 517 nm, and the radical scavenging activity of each sample was expressed using the ratio of the absorption decrease of DPPH (%) to that of the control DPPH solution (100%) in the absence of the sample. The radical scavenging activity was calculated (%) = 100(A-B)/A, where A and B are 517 nm absorption of the control and the corrected absorption of the sample reaction mixture.

Measurement of selenium, cadmium, arsenic, lead, and nickel content in saffron, water, and soil

The samples (soil and plant) were dried in the oven (60 to 65 °C). One-gram sample was added to 15 ml of a combination of three acids (nitric, perchloride, and sulfuric acids with 5:1:1 ratio, respectively). The mixtures were then digested at 80 °C until a clear solution was obtained, which was reduced to 50 mL using distilled water. The solutions were measured to determine the concentration of the heavy metals (Williams and Lambert, 1959). Also, the soils collected from the farms were transferred to the laboratory after drying and passing through 2 mm sieve. One-gram dry soil was mixed in a ratio of 1:3 with 6 M hydrochloric acid and concentrated nitric acid and heated for 24 h at 90 °C with nitric acid 2 M to a volume of 50 ml (Chen and Ma, 2001). Finally, the concentrations of heavy metals such as selenium, cadmium, arsenic, lead, and nickel in the saffron, water, and soils under study were determined using an atomic absorption spectrometer (Avanta-P model, GBC-Australia).

Statistical analysis

After checking the data distribution normality assumption through employing Kolmogorov-Smirnov and Shapiro-Wilk tests, data were analyzed using a Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version 9.2) and Minitab version 19. Statistical analysis was performed using nested design and means were compared with a Least Significant Difference (LSD) test at $p \leq 0.05$. The p -values of less than 0.05 were considered statistically significant. Data collected from different sites have been compared by correlation coefficients, PCA, and cluster analysis by Statistical Analysis System software and Minitab software.

RESULTS

Quantitative (flower and stigma yield) and qualitative (safranal, picrocrocin, and crocins content) traits

According to the variance analysis of the studied traits (Table 2), there were significant differences among cultivation sites concerning the saffron quantitative (flower and stigma yield) and qualitative (safranal and crocins content) characteristics ($p \leq 0.05$). The S4 treatment (Torbat-e Heydarieh) with altitudes of ~1323.33 m.a.s.l produced the maximum saffron flower yield (0.83 g m^{-2}), stigma yield (0.098 g m^{-2}), safranal content (15.8%), picrocrocin content (30.6%), and crocins content (69.3%). Results showed the lowest saffron flower yield (0.63 g m^{-2}), safranal content (11.7%), and picrocrocin content (22.2%) were achieved in S5 (Ghayen). Also, Zavareh (S3) and Birjand (S6) showed the lowest stigma yield (0.081 g m^{-2}) and crocins content (39.9%), respectively (Table 3).

Biochemical traits

As shown in Table 2, the effect of treatment (sites) was significant on total flavonoid content ($p \leq 0.01$), total phenol content ($p \leq 0.01$), and DPPH activity in 150, 200, and 250 $\mu\text{mol ml}^{-1}$ concentrations ($p \leq 0.05$). Means comparison showed the highest total flavonoid content (14.01 and 14.14 $\text{mg QE g}^{-1} \text{ DW}$) and total phenol content (125.5 and 126.5 $\text{mg GA g}^{-1} \text{ DW}$) and DPPH activity related to S1 (Shirvan) and S2 (Faruj). Moreover, Zavareh (S3) site produced the maximum means of total flavonoid and phenol content (14.16 $\text{mg QE g}^{-1} \text{ DW}$ and 126.6 $\text{mg GA g}^{-1} \text{ DW}$). While S5 (Ghayen site) had the lowest total flavonoid (13.4 $\text{mg QE g}^{-1} \text{ DW}$) and phenol (117.3 $\text{mg GA g}^{-1} \text{ DW}$) content and DPPH activity in 150, 200, and 250 $\mu\text{mol ml}^{-1}$ concentrations (29.9, 39.8, 59.6%, respectively) (Table 3). The results indicated that saffron cultivated in S1 and S2 sites (North Khorasan province) has the highest phenol and flavonoids content as well as total antioxidant activity (DPPH) and S5 and S6 sites (South Khorasan province) has the lowest range of mean traits.

Heavy metals content in water, soil, and saffron

The analysis of saffron, soil, and water in saffron cultivation in the studied areas is different in terms of the amount of heavy elements namely, selenium, lead, nickel, cadmium, and arsenic. Data analysis results indicated that there was selenium in saffron, cadmium in soil, arsenic in saffron and soil, and nickel and lead in saffron, water, and soil. The analysis of variance of the data showed a significant difference in the saffron selenium in different sites (Table 4). As shown in Fig. 2, the highest saffron selenium was related to S4 (2.021 mg

Table 2. Variance analysis of yield, qualitative, and biochemical traits of saffron plant in the different sites of North, Razavi, and South Khorasan provinces of Iran.

S.O.V	df	Mean square (MS)										
		Saffron flower yield	Stigma yield	Safranal content	Picrocrocin content	Crocins content	Anthocyanin content	Total flavonoid content	Total phenol content	DPPH ($\mu\text{mol ml}^{-1}$)		
										150	200	250
Treatment	5	0.0534*	0.00039*	22.07*	85.70ns	994.9*	0.052ns	0.916**	145.6**	268.2*	271.9*	472.3*
Experimental error*	12	0.0152	0.00011	8.19	36.49	357.6	0.060	0.168	19.83	68.77	76.72	133.4
Sampling error**	36	0.0022	0.00004	0.098	0.27	0.74	0.018	0.0033	0.34	0.21	0.28	0.33
CV (%)	-	6.21	7.91	2.28	2.11	1.52	4.38	0.41	0.47	1.24	1.15	0.86

ns: non-significant; * and **: significant at 5 and 1% probably level, respectively.

* Village within site was considered as an experimental error.

** Farm within the village within each site was considered as a sampling error.

Table 3. Mean comparison of yield, qualitative, and biochemical traits of saffron plant in the different sites of North, Razavi and South Khorasan provinces of Iran.

Sites	Saffron flower yield (g m ⁻²)	Stigma yield (g m ⁻²)	Safranal content (%)	Picrocrocin content (%)	Crocins content (%)	Anthocyanin content (mg CY g ⁻¹ DW)	Total flavonoid content (mg QE g ⁻¹ DW)	Total phenol content (mg GA g ⁻¹ DW)	DPPH activity (%) (μmol ml ⁻¹)		
									150	200	250
S1	0.80±	0.091±	14.5±	25.4±	59.8±	3.21±	14.01±	125.5±	41.3±	51.0±	72.3±
	0.02 ab	0.003 abc	0.22 ab	1.38 a	5.7 ab	0.05 a	0.053 a	0.42 a	2.13 ab	2.63 a	2.65 a
S2	0.78±	0.092±	14.0±	24.8±	54.9±	3.11±	14.14±	126.5±	44.5±	53.2±	74.8±
	0.01 ab	0.002 ab	0.16 ab	0.50 a	3.1 ab	0.04 a	0.066 a	0.95 a	1.94 a	2.11 a	2.80 a
S3	0.69±	0.081±	12.02±	24.2±	63.9±	3.19±	14.16±	126.6±	38.8±	47.1±	72.4±
	0.02 bc	0.002 c	0.28 b	0.30 a	0.33 a	0.05 a	0.014 a	0.17 a	0.26 abc	0.074 ab	0.192 a
S4	0.83±	0.098±	15.8±	30.6±	69.3±	3.04±	13.5±	119.7±	32.7±	40.1±	58.3±
	0.03 a	0.002 a	1.11 a	1.93 a	3.06 a	0.06 a	0.133 bc	1.48 b	1.22 cd	1.06 b	2.56 b
S5	0.63±	0.081±	11.7±	22.2±	52.3±	3.10±	13.4±	117.3±	29.9±	39.8±	59.6±
	0.01 c	0.002 bc	0.21 b	0.38 a	0.43 ab	0.07 a	0.067 c	0.36 b	1.2 d	0.62 b	0.92 b
S6	0.79±	0.090±	14.2±	22.2±	39.9±	3.03±	13.90±	120.3±	35.4±	45.1±	63.3±
	0.02 ab	0.004 abc	0.38 ab	0.30 a	3.34 b	0.05 a	0.01 ab	0.21 b	0.53 bcd	0.22 ab	0.30 ab
LSD	0.126	0.011	3.11	3.40	19.90	0.25	0.42	4.57	8.51	8.99	11.86

Data are expressed as the mean ± standard error (n = 3) based on three replicates at each site. Means with different letters in a column show differences at a significance level of 5% according to Duncan's multiple range test (DMRT). Shirvan (S1); Faruj (S2); Zavareh (S3); Torbat-e Heydarieh (S4); Ghayen (S5); Birjand (S6).

Table 4. Mean comparison of the heavy metals content in water, soil, and saffron cultivated in the different sites of North, Razavi, and South Khorasan provinces of Iran.

S.O.V.	df	Mean square (MS)														
		Selenium (Se)		Lead (Pb)		Nickel (Ni)		Cadmium (Cd)		Arsenic (As)						
		Saffron	Water	Soil	Saffron	Water	Soil	Saffron	Water	Soil	Saffron	Water	Soil			
Treatment	5	0.55**	-	-	139.1*	54.9ns	26.2**	3.78ns	2.85*	88229.4*	-	-	0.086*	91.9**	-	15.8**
Experimental error*	12	0.080	-	-	35.43	63.14	2.58	7.90	0.87	17847.3	-	-	0.021	16.38	-	1.98
Sampling error**	36	0.0151	-	-	0.014	0.41	0.115	0.013	0.010	3.72	-	-	0.00005	0.0008	-	0.042
CV (%)	-	7.33	-	-	3.10	20.80	3.51	3.67	45.29	1.85	-	-	18.21	1.63	-	3.68

ns: non-significant; * and **: significant at 5 and 1% probably level, respectively.

* Village within site was considered as an experimental error.

** Farm within the village within each site was considered as a sampling error.

kg⁻¹), and the lowest was S5 (1.435 mg kg⁻¹). The water and soil of sites lacked selenium.

The highest lead content in saffron (11.92 mg kg⁻¹), water (6.75 mg kg⁻¹), and soil (11.62 and 11.79 mg kg⁻¹) were related to S2, S1, and S1 and S5 sites, respectively. The lowest saffron lead in the S1 and S5 (1.903 and 1.824 mg kg⁻¹) was observed. S4 and S2 showed the lowest means of soil and water lead (7.983 and 0 mg kg⁻¹), respectively. Overall, the results showed that the highest lead content was in North Khorasan province, and the other two areas did not differ much in terms of the average of this trait (Fig. 3).

Nickel content in soil was much higher than water and plant samples in all studied sites. Also, soil nickel

content in Khorasan Razavi province increased by 88.4% and 68.8% compared to North and South Khorasan, respectively. The highest soil and saffron nickel was achieved at S3 (235.2 mg kg⁻¹) and S6 (3.761 mg kg⁻¹), respectively. In contrast, S5 and S1 showed the lowest means of soil and saffron nickel content (19.15 and 2.088 mg kg⁻¹), respectively. Only the nickel was observed in the water sample of the S4 site (1.38 mg kg⁻¹) (Fig. 4).

The results showed that the cadmium was observed only in the soil of the S1 site by 0.241 mg kg⁻¹, and other sites and water and saffron lacked this element (Fig. 5).

As shown in Fig. 6, the highest soil arsenic content (6.985 mg kg⁻¹) was related to the S3 site, and the lowest

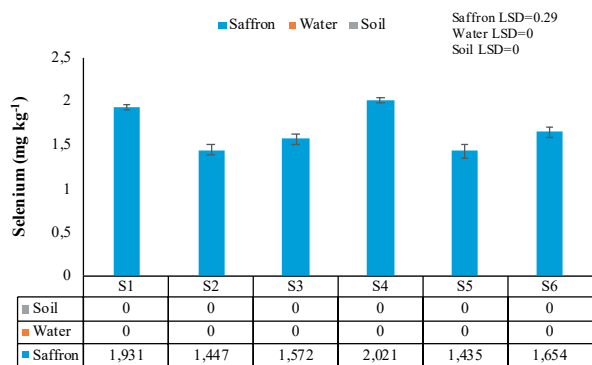


Fig. 2. Selenium content in water, soil, and saffron cultivated in the different sites of North, Razavi, and South Khorasan provinces of Iran (Shirvan (S1); Faruj (S2); Zavareh (S3); Torbat-e Heydarieh (S4); Ghayen (S5); Birjand (S6)).

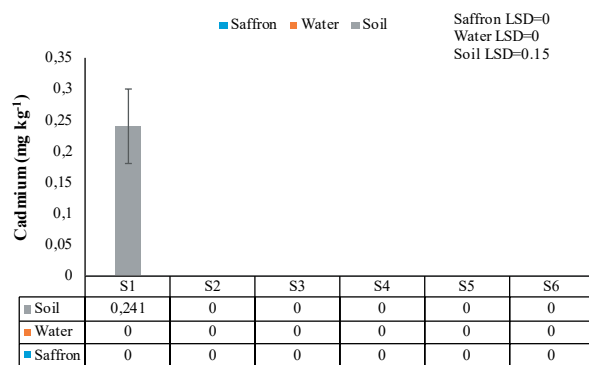


Fig. 5. Cadmium content in water, soil, and saffron cultivated in the different sites of North, Razavi and, South Khorasan provinces of Iran (Shirvan (S1); Faruj (S2); Zavareh (S3); Torbat-e Heydarieh (S4); Ghayen (S5); Birjand (S6)).

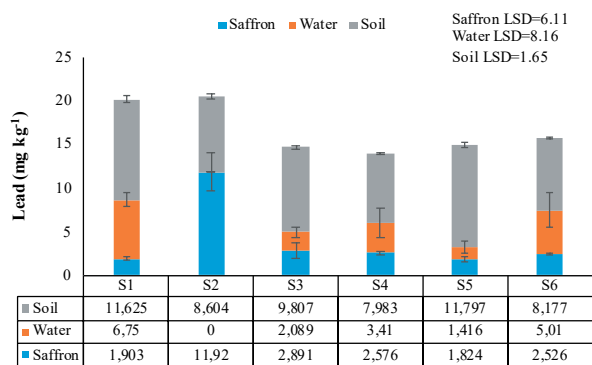


Fig. 3. Lead content in water, soil, and saffron cultivated in the different sites of North, Razavi, and South Khorasan provinces of Iran (Shirvan (S1); Faruj (S2); Zavareh (S3); Torbat-e Heydarieh (S4); Ghayen (S5); Birjand (S6)).

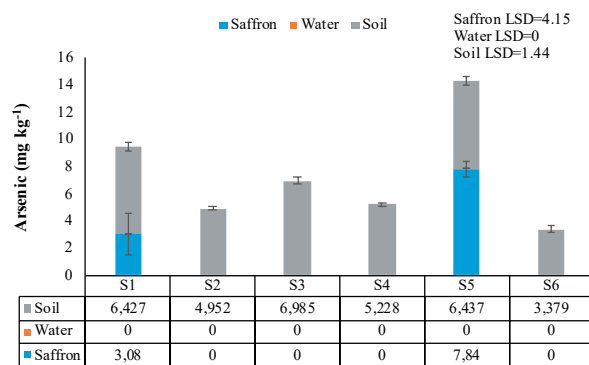


Fig. 6. Arsenic content in water, soil, and saffron cultivated in the different sites of North, Razavi and South Khorasan provinces of Iran (Shirvan (S1); Faruj (S2); Zavareh (S3); Torbat-e Heydarieh (S4); Ghayen (S5); Birjand (S6)).

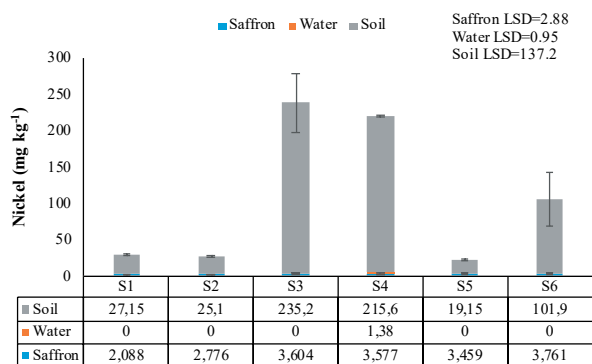


Fig. 4. Nickel content in water, soil, and saffron cultivated in the different sites of North, Razavi, and South Khorasan provinces of Iran (Shirvan (S1); Faruj (S2); Zavareh (S3); Torbat-e Heydarieh (S4); Ghayen (S5); Birjand (S6)).

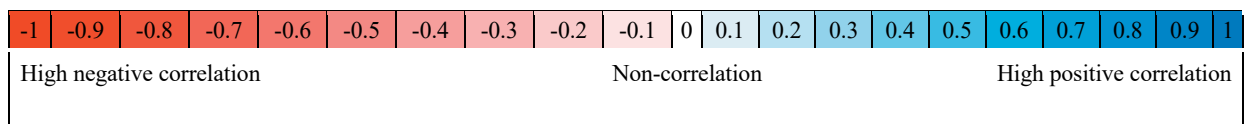
(3.379 mg kg⁻¹) to S6. Water samples from the tested sites did not contain arsenic. Also, saffron samples grown at S1 and S5 sites had arsenic elements (3.08 and 7.84 mg kg⁻¹, respectively).

Simple correlation coefficient, PCA, and cluster analysis

Pearson’s correlation coefficient matrix of all the measured variables with quantitative (flower and stigma yield) and qualitative (safranal, picrocrocin, and crocins content) traits is reported in Table 5. There were significantly negative and positive correlations among quantitative and qualitative properties as well as environmental and climatic characteristics. For example, the stigma yield was significantly and positively correlated with saffron flower yield, safranal and picrocrocin content, and

Table 5. Pearson correlation coefficient between environmental and climatic traits and biochemical attribute with flower and stigma yield and quality of saffron in different sites of North, Razavi, and South Khorasan provinces of Iran.

	Saffron flower yield	Stigma yield	Safranal content	Picrocrocin content	Crocins content
Saffron flower yield	1				
Stigma yield	0.60	1			
Safranal content	0.85	0.68	1		
Picrocrocin content	0.59	0.47	0.72	1	
Crocins content	0.24	0.16	0.27	0.78	1
Anthocyanin content	0.13	-0.01	0.09	0.04	0.04
Total flavonoid content	0.24	-0.01	0.21	0.07	-0.04
Total phenol content	0.24	0.03	0.22	0.19	0.14
DPPH-150	0.16	-0.02	0.16	0.01	-0.14
DPPH-200	0.09	-0.12	0.09	-0.09	-0.23
DPPH-250	0.05	-0.16	0.08	-0.01	-0.08
Se-saffron	0.40	0.28	0.44	0.50	0.32
Pb-saffron	0.20	0.20	0.08	-0.03	0.09
Ni-saffron	-0.13	-0.25	-0.19	0.04	0.24
As-saffron	-0.31	-0.18	-0.27	-0.04	0.16
Pb-water	0.15	0.18	0.09	-0.02	-0.07
Ni-water	0.00	0.21	-0.03	0.16	0.19
Pb-Soil	-0.36	-0.26	-0.36	-0.21	0.06
Ni-Soil	0.09	-0.05	0.06	0.29	0.42
Cd-Soil	0.28	0.23	0.18	0.14	0.21
As-Soil	-0.54	-0.49	-0.51	-0.09	0.28
Relative humidity	0.25	0.28	0.18	0.22	0.31
Minimum winter temperature	-0.38	-0.21	-0.27	0.05	0.29
Maximum summer temperature	-0.37	-0.55	-0.12	-0.55	-0.70
Number of annual frost days	0.04	0.02	0.04	0.22	0.43
Average annual temperature	-0.12	-0.15	-0.09	-0.25	-0.38
Average annual rainfall	0.25	0.20	0.19	0.48	0.60
Altitude	-0.30	-0.23	-0.19	-0.34	-0.44
Latitude	0.27	0.22	0.20	0.31	0.41
Longitude	-0.10	-0.03	-0.16	-0.09	-0.09



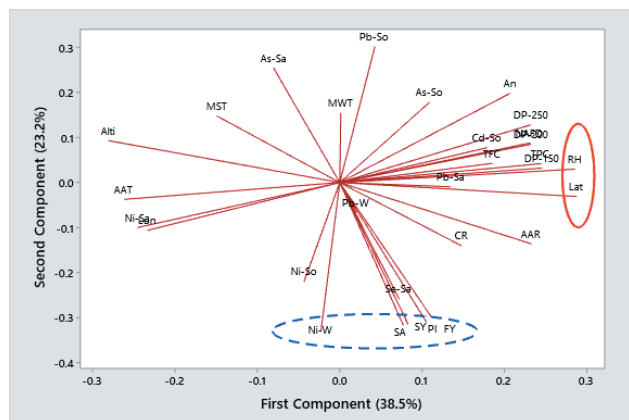


Fig. 7. Principal components analyses for yield, quality, and biochemical characteristics as well as environmental and climatic traits.

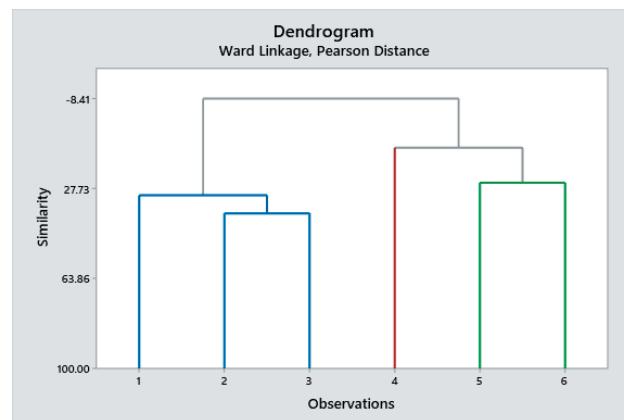


Fig. 8. Cluster analysis for yield, quality, and biochemical characteristics as well as environmental and climatic traits.

Se-saffron content. However, the saffron flower yield and stigma yield were negatively and significantly correlated with the Pb-soil, As-soil, and the maximum summer temperature. Interestingly, Se-saffron and latitude had a positive effect on increasing the flowers and stigmas yield as well as quality. While increasing the altitude and maximum summer temperature leads to a decrease in yield and quality in saffron. Moreover, saffron's biochemical traits had no significant negative or positive correlation with quantitative and qualitative yield. In

general, the results of this section showed that increasing climatic parameters, average annual rainfall, average humidity, and latitude leads to increased quantitative and qualitative performance.

Table 6. Cluster analysis and grouping for yield, quality, and biochemical characteristics saffron.

Variable	Cluster 1	Cluster 2	Cluster 3	Grand centroid
Saffron flower yield	0.76	0.84	0.71	0.76
Stigma yield	0.09	0.10	0.09	0.09
Safranal content	13.55	15.84	13.04	13.76
Picrocrocin content	24.84	30.63	22.26	24.95
Crocins content	59.58	69.38	46.18	56.75
Anthocyanin content	3.18	3.05	3.07	3.12
Total flavonoid content	14.11	13.55	13.65	13.86
Total phenol content	126.21	119.77	118.83	122.68
DPPH-150	41.55	32.77	32.67	37.13
DPPH-200	50.48	40.18	42.52	46.11
DPPH-250	73.20	58.34	61.46	66.81
Se-saffron	1.65	2.02	1.54	1.68
Pb-saffron	5.57	2.58	2.17	3.94
Ni-saffron	2.82	3.58	3.61	3.21
Pb-water	1.03	0.00	3.92	1.82
Pb-Soil	2.95	3.41	3.21	3.11
Ni-Soil	0.00	1.38	0.00	0.23
Number of sites in each cluster	3	1	2	-

Regarding the relationship among quantity, quality, biochemical, environmental, and climatic characteristics, all studied characteristics were analyzed by using PCA. Combined, PC1 (38.5%) and PC2 (23.2%) accounted for 61.7% of the total variance of the data (Fig. 7). The first PC (PC1) is characterized by relative humidity and latitude. This is appropriate because the Pearson correlation showed a significant correlation between yield and quality traits with relative humidity and latitude (Table 5). The second PC (PC2) is characterized by flower and stigma yield and quality attributes such as picrocrocin and safranal content. According to the findings, the PC1 can be called climatic and environmental factor, and the PC2 can be called quantitative and qualitative yield.

The goal of cluster analysis is to build a tree diagram where the sites that were viewed as most similar by the attributes in the study are placed on branches that are close together. As shown in Table 6 and Fig. 8, six sites were classified into three clusters, the first cluster included 3 sites (S1, S2, and S3), the second had 1 site (S4), and the S5 and S6 as the third cluster. The first cluster in terms of biochemical characteristics such as anthocyanin, total flavonoid, and total phenol content, as well as DPPH activity and the second cluster in terms of quantitative and qualitative characteristics including flower and stigma yield and safranal, picrocrocin, and crocins content had the highest coefficients.

DISCUSSION

The purpose of this study was to compare the quantitative and qualitative performance of saffron in six sites of major saffron producing provinces in Iran. The results showed significant differences in saffron flower yield, stigma yield, crocin, and safranal content in the studied areas. Rahimi *et al.* (2017) reported that the significant difference among the cultivation areas in terms of quality and quantity traits confirms the findings of the present study. Saffron quantitative and qualitative characteristics depend on the concentration of its primary metabolites and environmental conditions. According to the mean comparison and cluster analysis, saffron grown in S4 (Torbat-e Heydarieh: Khorasan Razavi province) resulted in significantly higher yield and quality than those produced in the other sites. It seems that the low maximum summer temperature in the area is one of the reasons for its superiority. In this regard, the correlation analysis shows that the maximum summer temperature had a significant negative correlation with saffron flower yield, stigma yield, picrocrocin and crocin content. On the other hand, a specific climatic parameter in the S4 site is the low maximum summer temperature and the high average annual rainfall. Accordingly, the simple correlation analysis shows that the average annual rainfall had a significant positive correlation with saffron quantitative and qualitative characteristics. Kamyabi *et al.* (2014) and Maleki *et al.* (2017) reported that annual rainfall and temperature had the highest impact on saffron cultivation among environmental factors, which confirms the findings of the present study. Temperature is the most important environmental factor controlling *Crocus* species' growth and flow (Haghighi *et al.*, 2020). Moreover, Gresta *et al.* (2009) reported that temperature certainly plays a role in flowering induction and flower appearance. Saffron has been successfully grown under different geographic locations in the world (Husaini, 2014). This crop can be cultivated in temperate, semi-arid, and arid areas with 1500–2800 m.a.s.l (Rahimi *et al.*, 2017). Ghorbani and Koocheki (2017) reported that the favorable climatic conditions for saffron's high yields are warm summers, autumn rains, and mild winters.

Environmental conditions such as altitude may also affect saffron yield and quality (Cardone *et al.*, 2019; Parizad *et al.*, 2019). In the current study, a negative relationship was obtained between the altitude and the quantitative and qualitative characteristics of saffron (Table 5). It seems that increasing the altitude too much leads to a decrease in the quantity and quality of saffron. This study's findings showed that an altitude of more than 1350 m.a.s.l decrease quantity and quality of

the yield. In general, the altitude of 1250 to 1350 m.a.s.l seems desirable in achieving maximum quantitative and qualitative yield. However, in contrast with Al Madini *et al.* (2019), who point out that saffron cultivation is possible at altitudes of 1500 to 2800 m.a.s.l, our results show that the optimal altitude for maximum quantitative and qualitative yield was about 1300 m.a.s.l. However, saffron can also be cultivated with good yields in very different environmental conditions (Caser *et al.*, 2019); a combination of certain environmental factors can be important to reach the optimum qualitative and quantitative yield (Rahimi *et al.*, 2017). Finally, correlation analysis showed transparent relationships within quantitative parameters, within qualitative parameters, and between quantitative and qualitative traits (Gresta *et al.*, 2009).

Biochemical properties of saffron such as flavonoid and total phenol content as well as DPPH free radical scavenging at different concentrations of the extract (150, 200, and 250 $\mu\text{mol ml}^{-1}$) showed significant differences at these sites. However, a decreasing trend was observed in the mean of total phenol and flavonoid metabolites as well as antioxidant activity of DPPH from north to south of Khorasan. Along with this trend, the climatic parameters of relative humidity and the number of annual frost days from North to South Khorasan decreased. In the climatic data, the low average annual temperature and altitude in North Khorasan province and the highest number of annual frost days are the index parameters of these sites. These three climatic factors seem to be environmental stressors of the plant. In response to these conditions, the plant changes its physiological interactions to increase defense mechanisms such as total phenol, flavonoids, and activity of DPPH free radical scavenging (Xu *et al.*, 2015; Hashim *et al.*, 2020). Zargoosh *et al.* (2019) reported that the effect of site on the antioxidant potential (DPPH) and total phenol amount of *Scrophularia striata* L. was significant. Therefore, it can be stated that the complexity of the effect of ecological factors on the one hand, and the emergence of different chemical processes in the plant under such effects, on the other hand, has led to the synthesis of various compounds with antioxidant potential in a plant in different regions (Zargoosh *et al.*, 2019). Although genetic processes guide the production of active ingredients in medicinal plants, environmental factors are strongly influenced (Figueiredo *et al.*, 2008). Therefore, environmental factors such as temperature cause changes in the growth of medicinal plants, as well as the quantity and quality of their active components, such as essential oils, glycosides, steroids, and alkaloids. The determinants of plant production are climate, soil, and geographic location. These factors can have a sig-

nificant impact on increasing or decreasing the quantity and quality of plant performance (Liu *et al.*, 2015; Zargoosh *et al.*, 2019). Researchers believe that many factors, such as water, air, soil, altitude (m.a.s.l), and differences between species, extraction methods, and antioxidant measurements, affect the number of secondary metabolites in plants, including phenol and flavonoids (Hashim *et al.*, 2020; Mykhailenko *et al.*, 2020). Overall, the antioxidant properties and habitat effects the number of secondary metabolites (Zargoosh *et al.*, 2019).

The heavy metals content (selenium, lead, nickel, cadmium, and arsenic) in water, soil, and saffron cultivated in the different sites of North, Razavi, and South Khorasan provinces indicated that the results present a wide variation. Selenium, lead, nickel, and arsenic were observed in saffron samples in the studied sites. On the other hand, in these sites' soil, there was lead, nickel, cadmium, and arsenic. Overall, the S4, S6, and S5 sites had the lowest averages in terms of lead, arsenic, and nickel, respectively. In other words, the high amount of heavy metals in South Khorasan province compared to the other two provinces leads to the conclusion that saffron grown in this province due to the high heavy metals content is less marketable and harms the consumer. The correlation between traits showed that the most negative effect on flower and stigma yield was related to soil arsenic followed by soil lead.

Esmaeili *et al.*, (2013) reported that the low contents of heavy metals (lead, nickel, cadmium, and arsenic) are also important for the plant's quality. The results showed that the concentration of heavy metals in plant samples other than lead for the S2 site was lower than the standard level in other cases. Uptake of various elements by plants through the root system from the soil depends on the particular plant, botanical structure of specific tissue, soil type, and element (Shahid *et al.*, 2018). Besides, microelements can enter the plant from the external environmental compartments (Esmaeili *et al.*, 2013; Liu *et al.*, 2018).

A significant point was the presence of selenium in saffron samples. This element had a positive and significant correlation with the quantitative and qualitative yield of saffron. In this regard, various researchers have expressed the positive effects of selenium. For example, Shahverdi *et al.*, (2018) reported that selenium is a useful element for plant growth and production, which plays an important role in growth and biochemical traits such as antioxidant activity. Some studies have shown that selenium is an essential element for humans and animals, which plays some beneficial roles in higher plants (Iqbal *et al.*, 2015). Selenium application caused an increasing growth in mung bean and wheat under both stressed and non-stressed conditions (salinity, drought, and heat

stresses) (Shahzadi *et al.*, 2017; Shahverdi *et al.*, 2018; Shahverdi *et al.*, 2020).

CONCLUSION

The current study focused on the effect of different climatic parameters on quantitative, qualitative, and biochemical characteristics of saffron in the six strategic saffron production regions (North, Razavi, and South Khorasan provinces) in Iran. Based on the present study results, qualitative and quantitative characteristics such as flower and stigma yield, crocins, safranal, and picrocrocin content are strongly controlled by the soil properties and climatic conditions in Shirvan, Faruj, Zavareh, Torbat-e Heydariyeh, Ghayen, and Birjand regions. The saffron flower and stigma yield were more in Torbat-e Heydariyeh (S4) than in other sites, while biochemical attributes were higher in S1 and S2 (Shirvan and Faruj sites) than that of other sites. Low maximum summer temperature and high relative humidity were two features of the climate index in the S4 region. This study's attractive result was the positive and significant relationship between selenium and quantitative and qualitative yield of saffron. It seems that the presence of selenium in the growth medium of the saffron increases the yield. Because there was a positive and significant correlation between selenium and quantitative and qualitative yield of saffron, in the saffron samples related to S4, a higher average of the element was observed compared to other sites. Based on the findings, it is concluded that Razavi Khorasan province (Especially Torbat-e Heydariyeh site) had a significant advantage in terms of quantitative and qualitative yield of saffron and North Khorasan province (Shirvan and Faruj sites) in terms of biochemical characteristics.

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