Journal of Medicinal Plants and By-products (2022) 2: 171-179
https://doi.org/10.22092/jmpb.2021.355393.1389

Original Article

Evaluating the Application of Organic and Chemical Fertilizers for Safranal, Crocin, and Picrocrocin of Saffron (*Crocus sativus* L.) under Dryland Farming System

Keivan Kianimanesh[1], Mohammad Hossein Lebaschi[2*], Kamkar Jaimand[2], Vahid Abdosi[1] and Seyed Reza Tabaei Aghdaee[2]

[1]Department of Horticulture Sciences and Agronomy, Agriculture and Food Science college, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Research Institute of Forests and Rangeland, Agricultural Research, Education and Extension Organization (AREEO) Tehran, Iran

Article History

Received: 30 July 2021
Accepted: 15 August 2021

Keywords
Medicinal plant
Dryland farming Delfard
Saffron active ingredients
Stomatal density

ABSTRACT

Crocus sativus L. is one of the most valuable medicinal plants and spices. In recent decades, interest in using organic fertilizers as sustainable agricultural has been increased. Cultivation of saffron under dryland farming, in addition to the production of this valuable spice, could prevent erosion, especially in slops. This experiment evaluated the effect of organic and inorganic fertilizers on active ingredients (safranal, crocin, picrocrocin), macronutrients (NPK), and stomatal properties of saffron (*C. sativus* L.) in a research station (Hamand) near Tehran. The plants were treated with cattle manure (20 t/ha^{-1}) and foliar application of Delfard (7 kg/ha$^{/}$) and Floral P (2.5 kg/ha) in a completely randomized block design (CRBD) with three replications in 2015-16. The results of liquid chromatography (HPLC) showed decreasing in safranal content (0.04-0.11 µg/g) organic and inorganic fertilizers, whereas the fertilizers enhanced crocin (85-146µg/g) and picrocrocin (3.5-10.5 µg/g) concentrations. Although there were no significant differences between the N concentration of floral and control. Organic and delfard-treated plants showed higher N compared to control. A significant increase of leaf P concentration was obtained in plants treated with inorganic realizers, where floral and delfard resulted in 3.5 and 2.2-fold improvement of P content compared to control, respectively. Delfard had a significant effect in the enhancement of K. The stomatal size remained unchanged, Although their density decreased over fertilizer application. According to the results, it could be concluded that manure, delfard and floral fertilizers can improve the nutritional value of saffron such as crocin and picrocrocin concentrations under dry farming conditions.

INTRODUCTION

Saffron is one of the most important medicinal and spice plants of Iran. The cultivated species of saffron is *Crocus sativus* L. It is mainly cultivated in Khorasan and in some other regions of Iran. Saffron, the dried red stigmata of *C. sativus* L. flower, is the most expensive of spices. Its price in the retail market is five euros per gram. Saffron adds its faint, delicate aroma, pleasing flavor, and magnificent yellow color to foods**.** Cultivation of saffron under dryland farming in addition to the production of this valuable spice could prevent erosion, especially in slops. Saffron (*C. sativus* Linnaeus) is a perennial plant of the Iridaceae family [1]. There is an increasing interest in saffron cultivation in different parts of the world due to its high application [2,3]. Iran with the best climate for saffron is well-known to produce high-quality saffron [3]. The annual production of saffron is about 300 tons per year all over the world, where Iran covers about 80 % of this production [1]. The high-quality saffron in the world belongs to Khorasan province, east of Iran [4]. Stigma is a well-

*Corresponding author: Research Institute of Forests and Rangeland, Agricultural Research, Education and Extension Organization (AREEO) Tehran, Iran
Email Address: lebaschy@rifr-ac.ir

known tissue of saffron, which is a costly spice and used as a food flavoring, a coloring agent, and as a traditional herbal medicine [5]. The high-quality color and taste of saffron correspond to crocins, the glycoside derivatives of crocetin, picrocrocin (mainly responsible for the bitter taste), and safranal (monoterpene aldehyde). Safranal is formed by hydrolysis from picrocrocin during drying and storage [1,6]. These active ingredients are antioxidant, anti-tumor, analgesic, anti-inflammatory, anti-cough, insulin resistance, etc.

The noticeable increase in world food production needs to use the different elicitors and soil amendments. The adverse effects of extensive application of inorganic fertilizers on herbal products have been well addressed [7]. Chemical fertilizers by contaminating the soil and water are a great risk for human being's health [8]. In agro-ecosystems, the application of synthetic toxic chemical pesticides restricts the soil fertility and growth of cultivated crops [9]. In Iran, in order to compensate for the deficiency of nutrients in soil, chemical fertilizers are being used in the large amount [10]. To minimize the accumulation of pollutants in agro-ecosystems, the reduction of toxic chemicals should be managed in agricultural practices. In this regard, organic products are considered as an alternative to sustainable agriculture development. Recently in Iran, the use of eco-friendly compounds has been raised. The effect of organic inputs can guarantee both agricultural production and nature conservation. The current approach is to find an appropriate substitute of chemical fertilizers by organic materials that are cost-effective and eco-friendly. Bio and organic due to its capability in the enhancement of plant growth by converting nutritionally important elements such as N and P from unavailable to available form via biological process [7,11].

The changes of different plant ingredients due to the use of synthetic and organic fertilizers have been addressed in medicinal plants. The functional effect of organic fertilizer on *Aloe vera* L. [12], *Ocimum basilicum* L. [13], *Satureja mutica* [7], and *Satureja macrantha* L. [10]. Seyyedi *et al*. [14] showed the increased yield of saffron with mineral fertilizer. Moreover, Kianimanesh *et al*. [1] showed the changes in essential oil composition and phenolic content of saffron under organic fertilizers. However, there is little information on the effective ingredients and nutritional value of saffron under the management of fertilizers. Therefore, the aim of the study was to assess the effects of manure, delfard and floral P fertilizer on safranal, crocin, and picrocrocin contents and also leaf macronutrients of saffron under dry farming conditions. The findings of this study would be highly effective in the management of soil amendment to meet high-quality saffron.

MATERIAL AND METHODS

Experimental site and design

A two-year field experiment was conducted at Hamand Absard research station (52º 21′ E and 35º 43′ N), belonging to the research institute of Forests and Rangelands, Tehran province. It was carried out in a completely randomized block design (CRBD) with three replications in 2015 and 2016. The minimum and maximum temperatures were respectively -15 °C in January–February and 35 °C in July–August with the mean annual amount to be 12 °C. The mean annual rainfall was 340 mm. Fig. 1 represents the ambrothermic diagram during 2015 and 2016. Table 1 describes soil features of the case study.

Agronomic practices

The experimental field was prepared to apply plow, disk, and leveler according to the local practice for saffron production. Each plot was 5 × 10 m. Planting depth was 12 cm, corm spacing on the row was 8 cm and row spacing was 35 cm. The average density was 35 corms per square meter. Weed management was carried out by hand-removal over growing seasons.

Fertilizer application

Table 2 shows the fertilizer compounds. Before corm planting, cattle manure (20 t/ha) was scattered onto the soil surface and then mixed into the soil. Delfard (7 kg/ha) as a special fertilizer for saffron and floral P (2.5 kg/ha) as the main fertilizer in Iranian soils was used with a foliar application at January 15 and March 15. At the end of phonological stage, the plants were harvested at the November 1 to November 20.

Leaf minerals

The dry ash method was applied to assay the minerals in leaf black cumin. The samples were dried in an oven at 70°C. To obtain the white ash, 1g dried leaf was transferred into ceramic vessels and slowly subjected to 500°C in the oven. The obtained ash was cooled by desicator , which each sample was subsequently changed with 20 mL 1N HCl, and put in the sand bath for 30 min. The samples were elutriated in a 100 mL volumetric balloon. Potassium (K) concentrations were measure by flame photometer (Model 410, Corning, Halstead, UK) in the volumetric balloons (Fig. 2a) [15]. Phosphorous (P) was measured by yellow method with vanadate-molybdate (Fig. 2b) [16]. P concentration was determined at 430 nm using the spectrophotometer (Shimadzu, UV3100). N was measured by Kjeldahl digestion (Model Jenway PFP7, UK) [17].

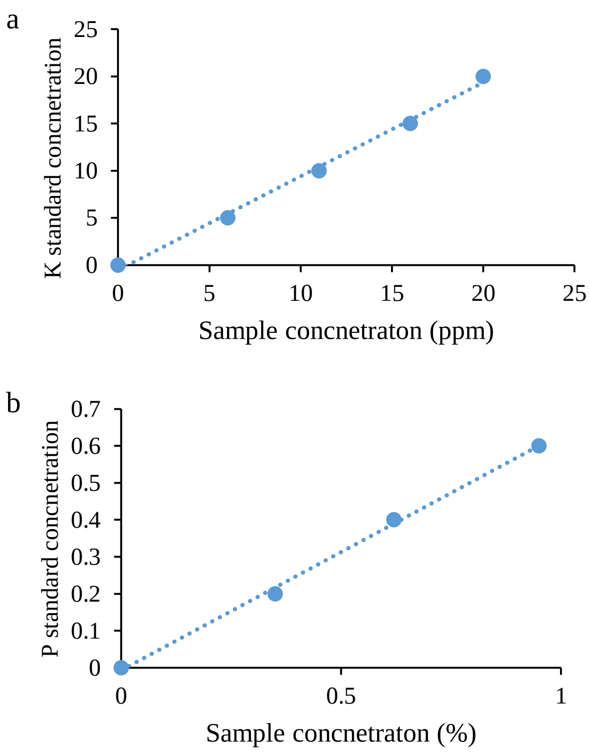

Fig. 2 Potassium (K, a) and prosperous (P, b) standards

Anatomical analyses

For investigation of the anatomical parameters viz. stomatal density and size as well as glandular trichomes, dry leaf samples were analyzed via scanning electron microscopy (SEM, SU 3500, Hitachi, Japan). In this regard, the gold was used for coating the dry samples in the vacuum coating unit (SG 110, Iran) according to the method reported by Khosropour *et al*. [18]. Image J software was applied to measure the anatomical properties.

Extraction procedure of safranal, crocin, and picrocrocin

Samples are collected in two steps. The first stage of 24 stigma samples, November 6, 2016 .The second stage, one year later, 24 stigma samples, November 6, 2017.

In order to achieve the separation of the saffron components from all samples, a methanolic extract was prepared using 0.1 mg of saffron stigmas and shake with 5 ml of methanol in a sonicate device (sound vibration) to transfer its material to the solvent. Samples were collected at room temperature and in the dark while constantly being stirred. They were filtered using Nylon Acrodisc Waters 13 (0.45 um) membranes and stored at –20ºC for further analysis (Tarantilis *et al*., 1995).

HPLC conditions

HPLC analyses were performed using a KNUAR liquid chromatograph with WellChrom 2000, consisting of a quaternary pump Maxi-Star K-1000, Column, an Erospher 100 C_{18}, 4mm and 250mm, 5mm column was used for this analysis with a column flow of 1 mL/min. Using a solvent system of Mobile phase: Acetonitrile/Methanol/Water (45:45:10), (%v/v). In a one-liter Erlenmeyer flask (19.4.2) add 450 ml of Acetonitrile, 450 ml of methanol and 100 ml of bisdistilled water. Homogenize and filter the mixture with 0,45 µm membrane filters (19.4.3) in a glass solvent degasser (19.4.1), detector Spectrophotometer K-2500, Safranal was detected at 310nm, all crocins were detected at 440 nm and picrocrocin at 250nm. Flow intensity: 0.6ml/min., Injected sample: 20 µmolµl., Duration: 40 minutes

Standard preparation and drawing of the calibration curve

The standards used in this experiment of Safranal compounds, with molecular formula $C_{10}H_{14}O$ and molecular mass 150.21 g/mol, in the amount of 5 ml were purchased from Sigma Company. The combination of crocins with molecular formula $C_{44}H_{64}O_{24}$ and molecular mass 976.972 g/mol/ in the amount of 100 mg was purchased from Sigma. The combination of picrocrocin with molecular formula $C_{16}H_{26}O_7$ and molecular mass 330.37 g/mol in the amount of 100 mg was received from Shahid Beheshti University, Research Institute of Plants and Medicinal Raw Materials from Dr. Doost, where they had specialized. The amount of safranal, crocin and picrocrocin compounds with different concentrations was prepared from three samples. Safranal composition was prepared with concentrations of 2.5, 10, and 25ppm (Fig. 3a). Crocin was prepared with concentrations of 250, 500, 1000, 1500, and 2000ppm (Fig. 3b). The combination of picrocrocin was prepared with concentrations of 125, 250, 500, 750, and 100 ppm (Fig. 3c). Then, according to the drawing of the calibration curves of the three combinations, their mass was measured.

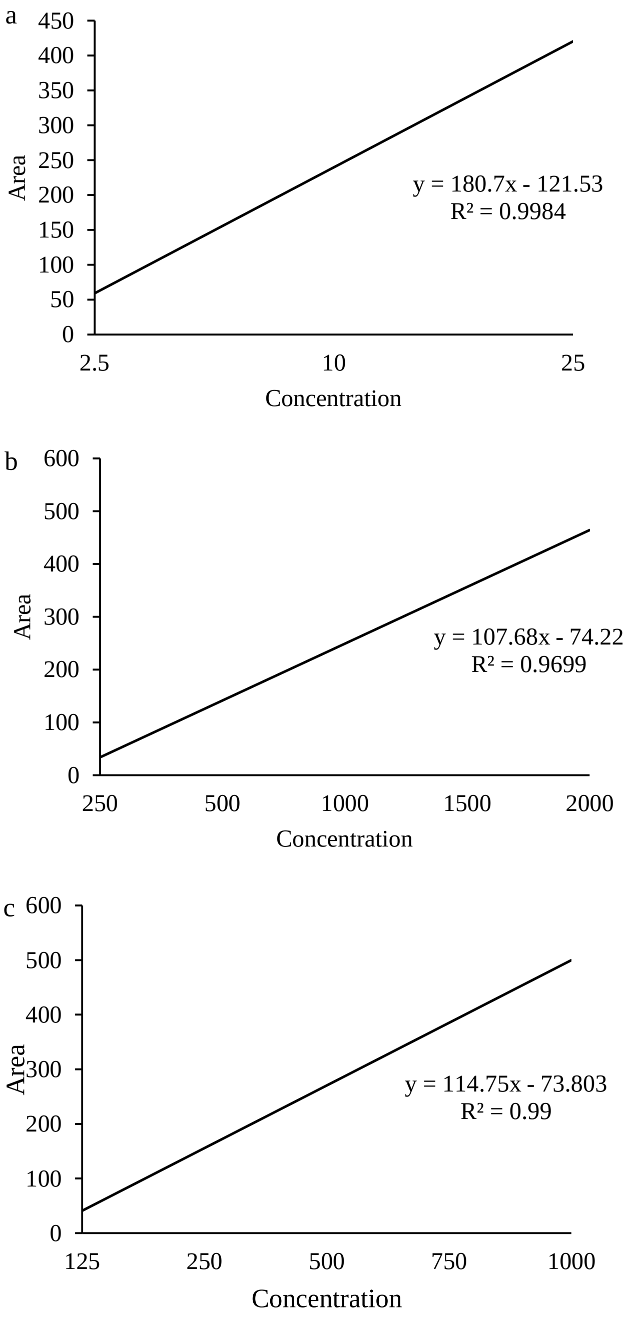

Fig. 3 Standard curves of safranal (a), crocin (b), and picrocrocin (c) of saffron

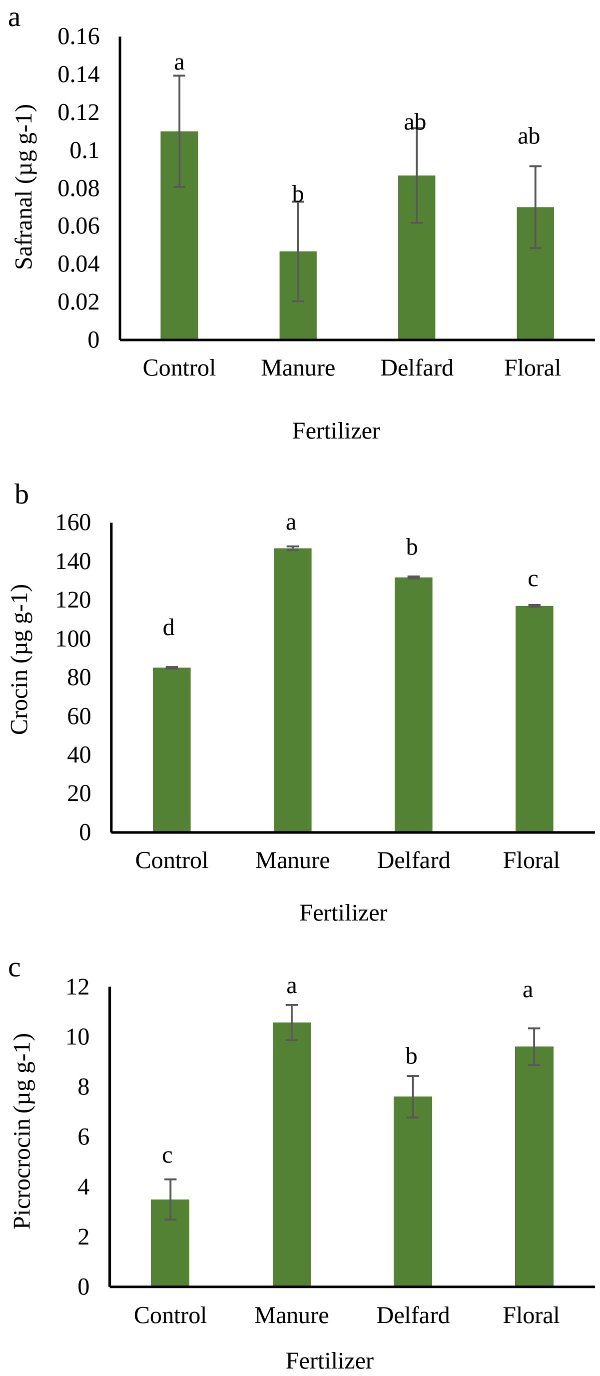

Fig. 4 Safranal, crocin, and picrocrocin concentrations of saffron under organic and inorganic fertilizers. Values are means ± standard deviation (SD) of three replications (n= 3). Different letters show statistically significant differences among treatments at $P \leq 0.05$.

Statistical Analysis

The data (n=3) were subjected to one-way analysis of variance (ANOVA) and using the SAS software package for Windows (SAS, version 9.3, SAS Institute, Cary, NC). Duncan's multiple range tests showed the comparison of mean values. The data were statistically investigated at 5% probability level.

RESULTS

Safranal, crocin, and picrocrocin concentration

The fertilizers significantly changed the safranal content ($P \leq 0.05$). Safranal content was decreased when organic and inorganic fertilizers had been used. The significant reduction of safranal was recorded in plants treated with organic fertilizers. Organic, delfard, and floral fertilizer reduced safranal by 57, 21, and 36%, respectively, as compared to control (Fig. 4a). Unlike safranal, crocin in all plants treated with organic and inorganic fertilizer was higher than in control plants. Compared to control, crocin increased by 72%, 54%, and 37% with organic, delfard and floral fertilizers, respectively (Fig. 4b). In addition, picrocrocin was increased by applying the fertilizers. The 3, 2.1, and 2.7-fold increases of picrocrocin were obtained in plants treated with organic, delfard, and floral fertilizers, respectively, compared to control (Fig. 4c). In addition, the amount of main compounds of saffron in different areas of Iran showed significant changes due to the geographical and ecological variations (Table 3).

Leaf macronutrients

The concentrations of macronutrients (NPK) in the leaves of saffron were significantly influenced by fertilizers ($P \leq 0.05$). The concentrations of macronutrients (NPK) in the leaves of saffron were significantly influenced by fertilizers ($P \leq 0.05$). Although there was no significant change of N concentration between floral and control, organic and delfard-treated plants showed higher N compared **to** control. It ranged from 14.3 mg/g DW in control to 21.6 mg/g DW in organic fertilizer (Fig**.** 5a). The significant increase of leaf P concentration was observed in plants treated with inorganic realizers, where floral and delfard resulted in 3.5 and 2.2-fold enhancement of P content, respectively, compared to control (Fig. 5b). For K, delfard had a remarkable role in its increment compared to control. Delfard, organic, and floral fertilizers increased K content by 31, 26, and 22%, respectively, when compared to control (Fig. 5c).

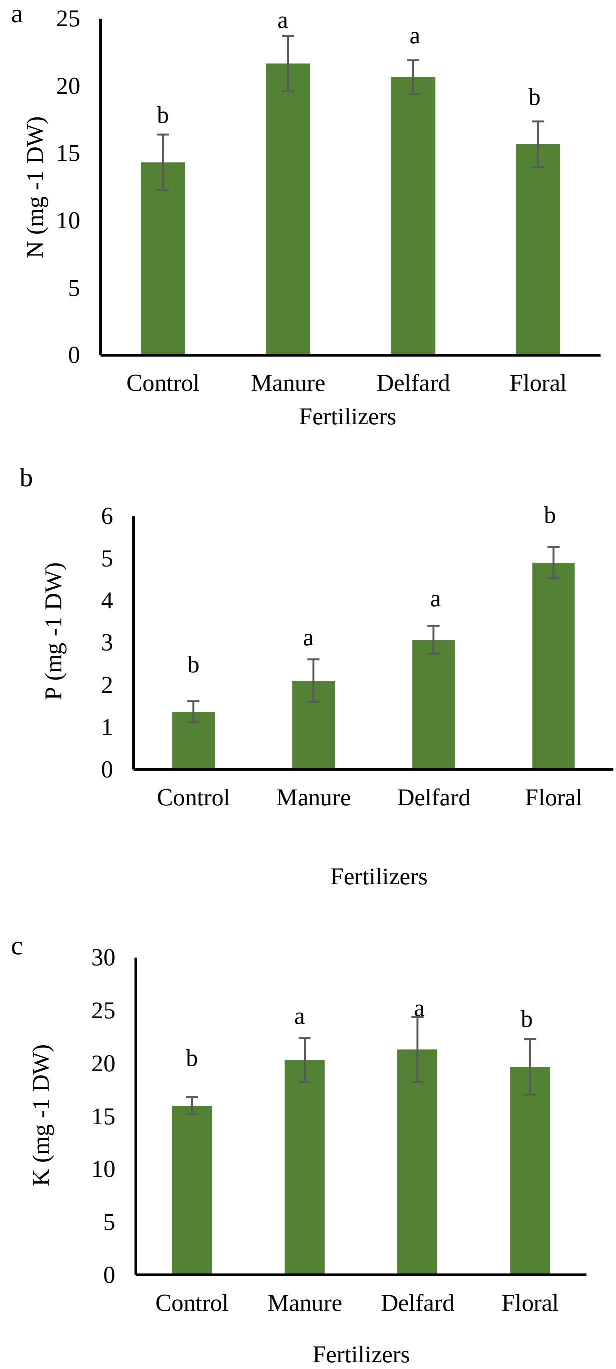

Fig. 5 Leaf NPK concentration of saffron under organic and inorganic fertilizers. Values are means±standard deviation (SD) of three replications (n=3). Different letters show statistically significant differences among treatments at $P \leq 0.05$.

Stomatal Properties

Table 4 shows the stomatal changes under organic and inorganic fertilizer application. Although there wass no significant change of plant stomatal size, its density was significantly affected by fertilizers ($P \leq 0.05$). The stomatal density decreased over fertilizer application. It ranged from 5.6 stomata m^{-2} in floral fertilizer to 9 stomata m^{-2} in the control treatment.

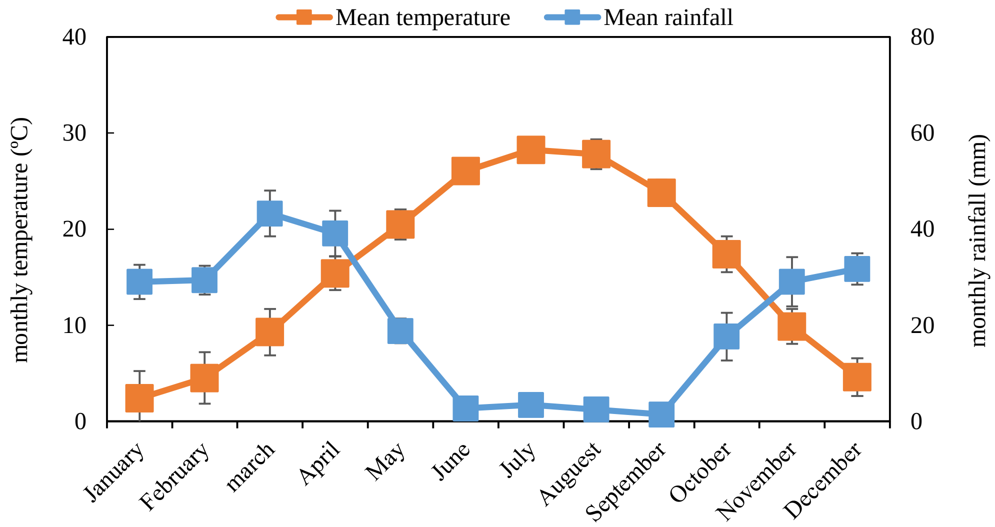

Fig. 1 Mean temperature and rainfall in the case study

Table 1 The soil characteristics of the experimental field

Year	pH	EC	OC (%)	N (%)	P (mg/kg)	K (mg/kg)	Sand (%)	Silt (%)	Clay (%)
2015	8.3	0.84	1.2	0.96	18	272	21	45	32
2016	8.1	0.85	1.3	0.99	21	279	20	47	33

Table 2 The characteristics of fertilizers used in the study

Fertilizer	N (%)	P (%)	K (%)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Manure	3.2	1.6	3	55.4	3456	378.5	203.6
delfard	12	8	4	500	2000	1000	1000
Floral	11	20	11	500	1500	1000	1000

Table 3 The comparison of saffron compounds after six months of storage

Treatments	Middle spring			End autumn		
	Safranal (ppm)	Crocin (ppm)	Picrocrocin (ppm)	Safranal (ppm)	Crocin (ppm)	Picrocrocin (ppm)
SRT Sample of the first year of plot 1		2456.65	83.13	11.13	506.28	2.61
SRT Sample of the first year of plot 2		2362.67	3.04	0.09	34.83	2.62
SRT Sample of the first year of plot 3		2568.83	80.50	0.05	111.89	5.54
C plot witnessed the second year		2520.24	120.96	-	78.92	5.96
M manure plot second year		2428.02	105.82	-	144.40	10.71
D delfa manure plot second year		2755.57	136.43	-	132.81	8.38
P phosphorus fertilizer plot second year		2445.98	108.72	-	116.81	9.58
Aeroponic sample		2392.72	113.21	-	76.47	5.44
Ferdous sample		2492.52	88.94	-	61.06	13.36
Faroje sample		2395.98	156.17	-	50.03	16.91
Bashroieh sample		2380.21	286.77	-	82.47	13.76
Shabestar sample		2555.91	128.24	-	39.82	21.40
Ghaen sample		397.33	170.98	-	47.34	13.37
Manjeel Abad sample		491.5	154.69	-	87.09	21.78
Kashmar sample		2377.84	86.32	-	9.09	45.98
Vermicompost sample		521.01	107.79	-	45.98	3.10
Goolerang sample		8.54	36.61	-	-1	6.29

Table 4 Stomatal size and density of saffron under organic and inorganic fertilizers.

Fertilizer	Stomatal length	Stomatal width	Stomatal density
Control	2.30 ± 0.43 a	2.30 ± 0.65 a	9.0 ± 0.81 a
Organic	2.11 ± 0.67 a	2.11 ± 0.67 a	6.0 ± 0.82 b
Delfard	1.8 4 ± 0.25 a	1.8 4 ± 0.45 a	7.6 ± 0.94 ab
Floral	1.83 ± 0.16 a	1.83 ± 0.53 a	5.6 ± 1.24 b

Values are means±standard deviation (SD) of three replications (n=3). Different letters show statistically significant differences among treatments at $P \leq 0.05$.

DISCUSSION

The safranal, crocin, and picrocrocin concentrations were 0.04-0.11, 85-146, and 3.5-10.5 µg/g. Safranal is a monoterpene aldehyde, which is useful for treating a large range of diseases in human such as most concerns. The amount of safranal and picrocrocin was lower than that previously reported in Iran [4], which can be due to the change of climate for saffron. Saffron quality mainly depends on the variety, growing conditions, and nutritional status [20]. Ghanabri *et al*. [4] showed picrocrocin, safranal, and crocin concentrations were in the range of 68–93, 39-45, and 217–219, respectively. The values of picrocrocin, safranal, and crocin in saffron stigmata were reported between 52–78, 36–50, and 117–350, respectively, depending on corm provenances and environmental conditions [21]. The safranal, crocin, and picrocrocin concentrations of saffron were significantly changed under fertilizer treatments. The change of active ingredients can be due to the biochemical pathways of producing the corresponded compounds that are strongly corresponded to external factors like soil and foliar application of organic and inorganic fertilizers [22].

According to our results, fertilizers improved crocin and picrocrocin, whereas decreased safranal content. Ghanbari *et al*. [4] represented improvement of picrocrocin and safranal contents under organic fertilizers; however, crocin content remained unchanged. These observations are suggesting that nutritional status in parallel with increased plant growth, play an important role in the improvement of saffron quality. Similar to our results, Rezaian and Paseban [23] reported increased crocin and picrocrocin contents by 25 Mg/ha cow manure compared with the control while the safranal concentration was decreased. It seems that changes in apo-carotenoids content, in saffron in addition to the fertilizer type, depended on other factors in each experiment.

The inorganic and organic fertilizers increased N, K and P contents. Delfard consists of macro and micronutrients, which are necessary for plants. It is a special fertilizer for saffron and its impact on saffron quality and quantity were reported [1]. Delfar and floral fertilizers increase the availability in rhizosphere and plant can uptake these essential elements properly [1]. Soils often lack these nutrients, either naturally, or as a result of over cultivation or other environmental factors. In cases where soils are lacking, nutrients must be put back into the soil in order to create the ideal environment for optimal plant growth [7]. Organic and inorganic fertilizers contain the essential elements for plant growth and development. Manure increases the availability of N and P for plants by N fixation and it dissolves P while giving off no unpleasant odor or stink in the process. Similarly, the increase of leaf minerals has been reported under manure [7,24] and inorganic fertilizers [10,25].

Stomata are the main structures that control the exchange of gases between atmosphere and plant, which their anatomy maybe influenced by many environmental conditions [26]. In our study, the use of fertilizers alleviates the undesirable environment for saffron. Most studies indicated that the stomatal density was enhanced under stress conditions [18,27]. We found the size of stomata not affected by fertilizer. In line with our study, Mirzaie *et al*. [28] showed organic and bio fertilizers did not change the size of stomata in lemongrass plants.

CONCLUSIONS

Fertilizers have an important role in improving soil fertilization and plant productivity. Recently, the main purpose is based on eco-friendly fertilizers. In the present study, the main saffron fertilizers were evaluated concerning active ingredients and nutritional values. The response of main ingredients was different to fertilizers. All fertilizers can improve leaf minerals under dry farming conditions. Animal manure due to its environmental friendly attributes and low cost, compared to delfard and floral, can be recommended to improve saffron quality. In the cases that we need more safranal, the use of fertilizers has negative effects, but it is useful for obtaining the increased crocin and picrocrocin. Finally concluded that saffron production could be qualitative and practical under dry farming in semi-arid region of Iran with 330 mm annual rain fall.

REFERENCES

1. Kianimanesh K., Lebaschi M.H., Jaimand K., Abdossi V., Tabaei-Aghdaei S.R. The Changes in Yield, Biochemica l Properties and Essential Oil Compounds of Saffron (*Crocus Sativus* L.) Plants Treated with Organic and Inorganic Fertilizers Under Dryland Farming System. J Med Plant By-prod. 2020.
2. Dhiman N., Kharkwal H. Biosynthesis and Derivatization of the Major Phytoconstituents of Saffron. In *Saffron*. Academic Press. 2020;83-92.
3. Tabibian S.A., Labbafi M., Askari GH., Rezaeinezhad A.R., Ghomi H. Effect of gliding arc discharge plasma pretreatment on drying kinetic, energy consumption and physico-chemical properties of saffron (*Crocus sativus* L.). J Food Engin. 2020;207:109766.
4. Ghanbari-Niaki A, Saeidi A, Kolahdouzi S, Aliakbari-Baydokhty M, Ardeshiri S, Abderrahman, AB, Zouhal H. Effect of *Crocus Sativus* Linnaeus (saffron) supplementations combined with circuit resistance training on total creatine kinase, lactate dehydrogenase and creatine kinase MB levels in young untrained men. Sci Sport. 2019;34:53-58.
5. Pandita D. Saffron (*Crocus sativus* L.): phytochemistry, therapeutic significance and omics-based biology. In *Medicinal and Aromatic Plants*. Academic Press. 2020;325-396.
6. Ameri R., Azizi M., Mollafilabi A. Effects of gibberellic acid and naphthalene acetic acid on saffron plant (*Crocus sativus* L.) under field conditions. J Appl Hort. 2019;21:123-130.
7.Saki A., Mozafari H., Asl K.K., Sani B., Mirza M. Plant yield, antioxidant capacity and essential oil quality of Satureja mutica supplied with cattle manure and wheat straw in different plant densities. Commun Soil Sci Plant Anal. 2019;50:2683-2693.
8. Srivastav A.L. Chemical fertilizers and pesticides: role in groundwater contamination. In *Agrochemicals detection, treatment and remediation* (pp. 143-159). Butterworth-Heinemann. 2020.
9. Niu J., Liu C., Huang M., Liu K., Yan D. Effects of foliar fertilization: a Review of current status and future perspectives. J Soil Sci Plant Nutr. 2020;1-15.
10. Bakhtiari M., Mozafari H., Asl K.K., Sani B., Mirza M. Bio-organic and inorganic fertilizers modify leaf nutrients, essential oil properties, and antioxidant capacity in medic savory (*Satureja macrantha* L.). J Biol Res. 93(1). 2020;93:1-9.
11. Baniyaghoub M., Mozafari H., Karimzadeh K., Rajabzadeh F., Azimi R. The Changes in Yield and Chemical Profile of Essential Oil and Leaf Minerals of *Satureja macrantha* L. Under Combined Manure and NPK Fertilizer. J Med Plant By-prod. 2020.
12. Muneer N.Y., Rabee K. M. Effect of different types of organic fertilizer on some secondary metabolic compounds product of cactus (*Aloe vera* L.). The Iraq J Agri Sci. 2017;48:422.
13. Burducea M., Zheljazkov V.D., Dincheva I., Lobiuc A., Teliban G.C., Stoleru V., Zamfirache M.M. Fertilization modifies the essential oil and physiology of basil varieties. Ind Crop Prod. 2018;121;282-293.
14. Seyyedi S.M., Ebrahimian E., Rezaei-Chiyaneh E. Saffron daughter corms formation, nitrogen and phosphorous uptake in response to low planting density, sampling rounds, vermicompost and mineral fertilizers. Commun Soil Sci Plant Anal. 2018;49:585-603.
15. Williams V., Twine S. Flame photometric method for sodium, potassium and calcium. In: Peach K, Tracey MV, editors. Modern methods of plant analysis. Berlin: Springer. 1960;3-5.
16. Tandon H.L.S., Cescas M.P., Tyner E.H. An acid-free vanadate-molybdate reagent for the determination of total phosphorus in soils. Soil Sci Soc Am J. 1968;32:48-51.
17. Bradstreet, R.B. (1954). Kjeldahl method for organic nitrogen. *Analytical Chemistry*. 1954;26:185-187.
18. Khosropour E., Attarod P., Shirvany A., Pypker T.G., Bayramzadeh V., Hakimi L., Moeinaddini M. Response of Platanus orientalis leaves to urban pollution by heavy metals. J Foret Res. 2019;30:1437-1445.
19. Cossignani L., Urbani E., Simonetti M.S., Maurizi A., Chiesi C., Blasi F. Characterisation of secondary metabolites in saffron from central Italy (Cascia, Umbria). Food Chem. 2014;143:446-451.
20. Rabani-Foroutagheh M., Hamidoghli Y., Mohajeri S.A. Effect of split foliar fertilisation on the quality and quantity of active constituents in saffron (*Crocus sativus* L.). J Sci Food Agri. 2014;94:1872-1878.
21. Lage M., Cantrell C.L. Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. Sci Hort. 2009;121:366-373.
22. Farag M.A., Hegazi N., Dokhalahy E., Khattab A.R. Chemometrics based GC-MS aroma profiling for revealing freshness, origin and roasting indices in saffron spice and its adulteration. Food Chem. 2020;331:127358.
23. Rezaian S., Paseban M. The effect of micronutrients and manure fertilizers on the quantity and quality of Khorasan saffron. Acta Hort. 2007;739:155-168.
24. Adekiya A.O., Agbede T.M., Aboyeji C.M., Dunsin O., Ugbe J.O. Green manures and NPK fertilizer effects on

soil properties, growth, yield, mineral and vitamin C composition of okra (Abelmoschus esculentus (L.) Moench). J Saudi Soci Agri Sci. 2019;18:218-223.

25. Weldegebriel R., Araya T., Egziabher Y.G. Effect of NPK and blended fertilizer application on nutrient uptake and use efficiency of selected sorghum (*Sorghum bicolor* (L.) Moench) varieties under rain-fed condition in Sheraro District, Northern Ethiopia. Mom Ethiop J Sci. 2018;10:140-156.
26. Steinthorsdottir M., Vajda V., Pole M. Significant transient pCO2 perturbation at the New Zealand Oligocene-Miocene transition recorded by fossil plant stomata. Palaeogeogr Palaeoclimatol Palaeoecol. 2019;515:152-161.
27. Haworth M., Scutt C.P., Douthe C., Marino G., Gomes M.T.G., Loreto F., Centritto M. Allocation of the epidermis to stomata relates to stomatal physiological control: stomatal factors involved in the evolutionary diversification of the angiosperms and development of amphistomaty. Environ Exp Bot. 2018;151:55-63.
28. Mirzaie M., Ladanmoghadam A.R., Hakimi L., Danaee E. Water stress modifies essential oil yield and composition, glandular trichomes and stomatal features of lemongrass (*Cymbopogon citratus*) inoculated with arbuscular mycorrhizal fungi. J Agri Sci Technol. 2020;22:1575-1585.