Assessment of changes in secondary metabolites and growth of saffron under organic fertilizers and drought

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Abstract

Saffron (Crocus sativus L.) is an important source of natural Crocin, Picrocrocin, and Safranal, for the use of people, especially those with depression. However, there is no data on the growth of saffron under drought conditions affected by organic fertilizers under drought stress. However, water and nutrition management has a key role in saffron growth and quality. This study aimed to
evaluate the improvement of saffron growth and quality by fertilizer management under drought conditions. A two-year field experiment was performed in 2018 and 2019. Experiment treatments were irrigation regimes include control (field capacity) and drought (66% depletion of soil water) and fertilizer management comprise of control (without fertilizing), chicken manure, chemical fertilizer, 25% chicken manure + 75% chemical fertilizer (Ch25M75), 50% chicken manure + 50% chemical fertilizer (Ch50M50), and 75% chicken manure + 25% chemical fertilizer (Ch75M25). Drought reduced saffron growth characteristics, chlorophyll content, and yield. While the amount of proline and soluble carbohydrates increased and the relative water content decreased. However, the content of crocin, picrocrocin, and safranal increased by drought. Besides, mineral nutrition deficit reduced the growth characteristics, yield, and secondary metabolite content in saffron under both irrigation regimes. The integrated fertilizer management involves different proportions of chicken manure and chemical fertilizers were more effective than using only chemical fertilizers or chicken manure to improve the growth and biochemical traits of saffron in both irrigation regimes. Unlike the growth traits, the highest quality of saffron was obtained when the share of chemical fertilizer was higher than chicken manure (3:1). Therefore, the highest quality of saffron was obtained by replacing 25% of chemical fertilizer with chicken manure, and the greatest growth was acquired by replacing 75% of chemical fertilizer with chicken manure. Hence, the reduction of chemical fertilizer and use of chicken manure is recommended to improve saffron yield and quality under drought conditions.

Keywords: Saffron, Chicken manure, Fertilizer, Crocin, Proline, Drought
**Introduction**

Drought is the most momentous restricting agent in crop production worldwide. It has been suggested that the drought will reduce crop yields by between 55 to 70% in the late 21st century (Chai et al., 2016). Meanwhile, global food demand will increase by at least 70 percent by 2050. Hence, access to food security would be the main challenge over the world (Keating et al., 2014). Improving many agronomic approaches e.g., irrigation management, soil nutrient management, and developing drought-resistant species are appropriate solutions to overcome the problem (FAO, 2017). Drought often increased the accumulation of secondary metabolites in medicinal and spice herbs, on the contrary, it may be reduced their dry mass. Rather, the secondary metabolites percentage is increased per dry weight unit by reducing the dry matter accumulation.

Saffron (*Crocus sativus* L.) as the most expensive spice is a perennial plant that belongs to the Iridaceae family. Saffron has low water requirements and high economic capacity. Hence, it is widely cultivated in the arid and semi-arid regions of Iran such as the Khorasan and Fars provinces (Oftadeh et al., 2017). The saffron quality grade is determined based on the rate of yellow color, bitter taste, and aroma which is respectively dependent on the amount of Crocin, Picrocrocin, and Safranal. They are the main secondary metabolites in the stigma which are affected by genetic factors, fertilizer type, environmental stress, and drying procedure (Ahmad et al., 2011; Jami et al., 2020). The saffron water requirement is lower than other common crops during the first growing season (about 3600 m³ ha⁻¹) (Fallahi and Mahmoodi, 2018). However, it is reported that sufficient irrigation had an impressive effect on saffron yield increment (Fallahi et al., 2016). Aghhavani Shajaria et al. (2020) reported that irrigation management is an important factor in obtaining a higher stigma yield of saffron.

Providing mineral nutrition is another important factor to determine saffron yield (Amiri, 2008). Some studies have reported that there is a positive correlation between soil organic matter and
saffron yield. This increase is probably due to the release of nutrients, particularly phosphorus and nitrogen, and the improvement of soil physical properties (Negbi, 1999). Leaf nitrogen amount has a positive correlation with saffron yield, although safranal and crocin contents have a high correlation with the leaf phosphorus content (Naghdibadi et al., 2011; Parsa et al., 2018). Therefore, a sufficient nutrition supply can play an effective role in improving the quality and quantity of saffron yield. In agriculture, soil nutrient deficiencies are compensated by the use of chemical, organic, and biological fertilizers (Selim, 2020). In recent years, chemical fertilizer consumption has risen exponentially around the world, and it is responsible for a 50% increment of agricultural production over the twentieth century (Aziz et al., 2015). On the other, the excessive use of synthetic fertilizers causes serious problems e.g., deterioration of soil physio-chemical properties, soil nutrient deficiency, soil erosion, eutrophication, and food contamination (Wu and Ma, 2015; Selim, 2020). The consciousness of organic fertilizer ability has lately increased to replace a part of chemical fertilizers. Because Integrated Nutrition Management (INM) benefits are more known for improving plant growth and agroecosystem sustainability (Suge et al., 2011; Selim, 2020). The use of organic manure along with chemical fertilizers improved the physical, chemical, biological, and hydrological properties of the soil. It leads to the mitigation of drought detrimental effects and helps to access a more volume of water and nutrients by root expansion in the soil (Fallahi and Mahmoodi, 2018; Selim, 2020). Saffron yield has been reported that extremely affected by organic and chemical fertilizers (Jahan and Jahani, 2007; Koocheki and Seyyedi, 2015). However, chemical fertilizers especially nitrogen and phosphate have a significant impact on increasing the saffron stigma yield (Behzad et al., 1992). Koocheki and Seyyedi (2015) reported that the application of organic (cattle manure composts 25 Mg ha\(^{-1}\)) and chemical fertilizers (N 300 kg ha\(^{-1}\) + P 100 kg ha\(^{-1}\)) increased the number of flower and stigma yields.
Drought is caused to decrease uptake, redistribution, and transport of mineral nutrition in plants. Besides, diffusion and mass flow of mineral nutrition in the soil were reduced by a water deficiency (Gessler et al., 2017). A meta-analysis study revealed that drought reduced nitrogen and phosphorus contents in plant tissue (He and Dijkstra, 2014). Moreover, some studies have reported that drought can reduce nutrient absorption from the soil (Sardans and Peñuelas, 2012; Bista et al., 2018). Despite some studies performed to evaluate saffron response to irrigation or fertilizer management, limited studies have been conducted to improve the quality and quantity of saffron yield by fertilizer management under drought conditions. Thus, this study presumed that the utilization of appropriate chemical and organic fertilizers can alleviate the drought’s detrimental impact on saffron growth and secondary metabolites content.

Materials and methods

Farm location and treatments

A two-year field experiment was conducted as a split-plot arrangement based on a randomized complete block design with three replicates in Shahrekord, Iran (50° 39’ N and 52° 24’ E, 2300m elevation) during 2018 and 2019. Experimental treatments included irrigation regimes as the main plot and six fertilizer combinations as the subplot. Irrigation regimes included field capacity as control (soil water potential at -0.5 atm) and 66% soil water depletion from the field capacity as drought (soil water potential at -6.5 atm). The drought was applied after the end of flowering (beginning on November 10 and ending on May 10). Fertilizer combinations were included control (without fertilizing), chicken manure (3 Mg ha⁻¹), chemical fertilizer (100 kg ha⁻¹ urea + 150 kg ha⁻¹ K₂SO₄ + 45 kg ha⁻¹ FeSO₄ + 15 kg ha⁻¹ ZnSO₄ + 20 kg ha⁻¹ MnSO₄), 25% chicken manure + 75% chemical fertilizer, 50% chicken manure + 50% chemical fertilizer, and 75% chicken manure + 25% chemical fertilizer. Some chemical properties of the chicken manure are shown in Table 1.
The fertilizer treatments were applied after preparing the experimental plots. The soil water content was determined by the Eco tensiometer equipment. This apparatus was placed in the adjacent saffron corms before the irrigation was implemented. Then, the soil water potential was calculated daily from the recorded data. Therefore, irrigation intervals were determined for each irrigation regime. Two-year weather data included the change of minimum and maximum temperatures and precipitation value is presented in Fig. 1 as monthly.

**Table 1**

Chemical properties of used chicken manure.

<table>
<thead>
<tr>
<th>OM (%)</th>
<th>EC (ds m⁻¹)</th>
<th>pH</th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
<th>Na (%)</th>
<th>Fe (ppm)</th>
<th>Zn (ppm)</th>
<th>Cu (ppm)</th>
<th>Mn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65.88</td>
<td>5.87</td>
<td>7.4</td>
<td>2.11</td>
<td>1.65</td>
<td>1.82</td>
<td>6.1</td>
<td>0.92</td>
<td>0.36</td>
<td>714.2</td>
<td>296.4</td>
<td>68.4</td>
<td>411</td>
</tr>
</tbody>
</table>

**Farm operation and sampling**

The experimental farm was prepared by deep plowing, crushing great lumps, and smoothing the surface. The experimental plots were created with 4×2 m dimensions in September 2017. Each plot was contained 20 cultivating rows with a 20 cm distance from each other. Saffron corms were cultivated on the row with a 10 cm interval on October 7, 2017. The first irrigation was done for all plots on October 18. The sample of flowers was taken up randomly from each treatment on November 1 in 2018 and 2019. The leaf sampling was done between 10 to 20 March 2018 and 2019 before irrigation. The samples were transferred to the laboratory and after measuring fresh weight was dried in the shade. A part of the sample was immediately frozen in liquid nitrogen and was held for the biochemical analysis at −80°C. The physical and chemical properties of soil were investigated before field preparation (Table 2).
Flower traits

Flower number (FN) was measured in a square meter of each plot. Stigma and style after measuring flower fresh weight (FFW) separated from the flower and was measured their length. The flowers and stigmas were dried in shadow conditions at ambient temperature for 7 days.

Chlorophyll and carotenoid measurement

The chlorophyll and carotenoid amounts were measured by Arnon (1949) method. An appropriate amount of frozen leaf (1 g) was homogenized with 10 mL of 80% acetone by a mortar and pestle. The suspension solution was filtered and then the residual was washed to remove its color. Then, the absorbance of the extract was measured at 663, 645, 510, and 480 nm, respectively. The pigments content was calculated by the following equations and was expressed as mg g\(^{-1}\) fresh weight:

\[
\text{Chlorophyll a (Ch}_a) = 12.7 \times (A_{663}) - 2.69 \times (A_{645}) \times V/1000W
\]

(1)

\[
\text{Chlorophyll b (Ch}_b) = 22.9 \times (A_{645}) - 2.69 \times (A_{663}) \times V/1000W
\]

(2)

\[
\text{Chlorophyll Total (Ch}_T) = 20.2 \times (A_{645}) + 8.02 \times (A_{663}) \times V/1000W
\]

(3)

\[
\text{Carotenoid (Car)} = 7.6 \times (A_{480}) - 14.9 \times (A_{510}) \times V/1000W
\]

(4)

Relative water content (RWC)

RWC was determined with the leaf immersion method characterized by Kwasniewski et al., (2016). Fresh leaves were incision into 3 cm sections and were immediately weighted fresh weight (FW). Then, the leaf segments were immersed in deionized water (15 mL) in a petri dish and were maintained at a dark location for 24 hours. Then, the swollen leaf fragments take up from the water and were weighted as turgid weight (TW). In the following, the leaf parts were dried at 70°C for
24 hours till a constant weight was obtained, and then their weight was recorded as dry weight (DW). The RWC was measured as the following equation:

\[
RWC(\%) = \left(\frac{(TW - FW)}{(TW - DW)}\right) \times 100
\]  

(5)

**Proline content measurement**

The proline content was measured by frozen leaf crushing in sulfosalicylic acid with a mortar and pestle. The homogenate solution was centrifuged at 13,000g for 12 min. The extract (2 mL) was combined with an equal volume of Ninhydrin reagent and glacial acetic acid in a test tube. The reaction was finished after locating the test tube in boiling water for 1 h. Then, the reaction solution was cooled and toluene was added. The absorbance of the upper layer was read at 520 nm. The proline content was computed by proline standard curves and reported as µM g⁻¹ fresh weight (Bates et al., 1973).

**Sugar content measurement**

The sugar content was assayed according to the method of Dubois et al. (1956). The appropriate amount of frozen leaf was extracted with distilled water at 4°C. The extract was filtered and its volume reached 10 mm. Then, 1 mL of extract was reacted by aqueous phenol (5%) and sulfuric acid in a test tube, respectively. The reaction solution absorbance was read at 480 nm. Sugar amount was calculated by the glucose standard curve and was expressed as mg g⁻¹ fresh weight.

**Crocin, picrocrocin, and safranal assay**

Crocin, picrocrocin, and safranal were measured by ISO 3632-2 method (ISO 3632, 2014). Briefly, 50 mg of dried stigmas were added to distilled water (90 mL) in a 100 mL volumetric flask and
magnetically stirred in darkness for 1 h. Then, 10 mL distilled water was added to the volumetric flask, and the solution (0.5%, w/v) was incubated at ambient temperature in darkness for 24 h. The extract was diluted with distilled water (1:20) and filtered quickly to obtain a clear solution. Finally, the absorbance of picrocrocin, safranal, and crocin was recorded at 257, 330, and 440 nm, and their content was measured as the following equation:

\[ E^{1\%} = \frac{D \times 10000}{m(100 - h)} \]  

(6)

Where: D is the absorbance rate at 257, 330, and 440 nm; m is sample weight (g); h is moisture content of the sample.

**Statistical analysis**

Data analysis was performed by SAS 9.4 software. The Bartlett test was applied to evaluate the variance homogeneity. Duncan’s multiple range test was used for main factors comparison at a 5% confidence interval. The Least Square Means procedure was used for the comparison of the interaction of the main factors.

**Results**

**Saffron flower characteristics**

Drought and different fertilizers applications had a significant effect \((p \leq 0.01)\) on saffron flower characteristics included stigma length (SL), flower number, FFW, stigma fresh weight (SFW), flower dry weight (FDW), and yield. The flower traits and saffron yield in 2019 were higher than in 2018. Soil water potential reduction up to -6.5 atm reduced flower number in each square meter and stigma length about 12.3% and 7.1%, respectively. Moreover, flower fresh and dry weights
were decreased by drought to 11.7% and 13.7% compared with the control. Meanwhile, the saffron yield rate was reduced to 13.2% under drought conditions (Table 3).

The saffron yield or stigma dry weight had high positive correlation with SL ($r = 0.91, p \leq 0.01$), FN ($r = 0.99, p \leq 0.01$), FFW ($r = 0.99, p \leq 0.01$), SFW ($r = 0.98, p \leq 0.01$), and FDW ($r = 0.99, p \leq 0.01$). However, the stepwise regression showed that 99% of saffron yield changes were justified by flower dry weight in both irrigation regimes (Fig. 2). Therefore, it can be concluded that the stigma dry weight was dependent directly on the flower dry weight and indirectly on the stigma length, flower number, and flower fresh weight.

The flower traits include SL, FN, SWF, FFW, and FDW reduced without fertilizer application in both irrigation regimes. Although, this reduction was further in drought conditions and was 15%, 21.7%, 14.9%, 15.9%, and 28.6% for those traits, respectively. The saffron yield was also reduced down to 25% by water plus nutrition deficiency. The chicken manure and chemical fertilizer was the same influence on saffron stigma length. However, other flower traits and yield of fertilized saffron by chicken manure were higher from the chemical fertilizers. The combined fertilizer included organic and chemical fertilizers significantly increased the flower traits and yield in both irrigation regimes than their use alone. Albeit, the efficiency of combined fertilizer is reduced by decreasing the chicken manure share. Thus, the greatest flower traits and saffron yield were observed in Ch25M75 under both irrigation regimes. This treatment was more efficient in drought stress than optimal irrigation. Therefore, Ch25M75 increased the SL, FN, FFW, SFW, FDW, and yield by 63.6%, 78.9%, 79.7%, 78.9%, 110.3%, and 103.1% in deficit irrigation compared with the control, respectively. While these traits increased to 45.6%, 61%, 61.3%, 63.1%, 63.3%, and 64%, respectively, by Ch25M75 in the optimal irrigation. Reducing the share of chicken manure in drought conditions had more deleterious impacts than good irrigation regimes. So, the flower traits
and saffron yield were further reduced in drought stress (on average 50%) than the optimal irrigation by decrement chicken fertilizer share from 75% to 50% (Table 4 and Fig. 3).

Photosynthetic pigments content

The amount of leaf photosynthetic pigments was identical in 2018 and 2019 (Table 3). Drought and different fertilizers applications had a significant effect ($p \leq 0.01$) on the amount of photosynthetic pigment. Drought reduced the amount of Ch\(_a\), Ch\(_b\), and Ch\(_T\) by about 44% to 47% in both years, however, the Car amount had no significant change. In both irrigation regimes, the chemical fertilizer or chicken manure improved the content of total chlorophyll and Ch\(_a\) in 2018 and 2019. However, they had no significant effect on the Ch\(_b\) content compared with the control. In a good irrigation regime, the chemical fertilizer was more efficient to increase the average content of Ch\(_a\) than the chicken manure (31% and 20.5% were more than control, respectively). However, they were no significant differences in drought conditions. The combined fertilizer composed of chemical fertilizer and chicken manure further increased Ch\(_T\), Ch\(_a\), and Ch\(_b\) contents in both irrigation regimes. However, the highest amount of chlorophyll was obtained with various ratios of chemical fertilizer and chicken manure in each of the irrigation regimes. So that in drought stress, a higher amount of chicken manure was required. The greatest Ch\(_T\) amount under optimal irrigation conditions (10.16 mg g\(^{-1}\) FW) was obtained by replacing 25% of the used chemical fertilizer with chicken manure, while obtaining the greatest Ch\(_T\) content in drought conditions (5.96 mg g\(^{-1}\) FW) has required to replace 50% of the used chemical fertilizer with chicken manure. Besides, The Ch\(_a\) content in the combined fertilizers was higher than chemical fertilizers only in drought conditions in both years. Therefore, the highest amount of Ch\(_a\) in drought conditions was observed in Ch\(_{25}\)M\(_{75}\) (on average, 2.95 mg g\(^{-1}\) FW). the content of Ch\(_b\) in optimal irrigation conditions just improved in Ch\(_{25}\)M\(_{75}\) (on average, 5.2 mg g\(^{-1}\) FW) than the control in both years.
While in drought conditions, all combinations of chemical fertilizer and chicken manure significantly increased Ch₅ rate in both years (Table 5).

Fertilizing operations were reduced carotenoid content in drought conditions. However, only Ch₂₅M₇₅ (1.72 mg g⁻¹ FW) and Ch₅₀M₅₀ (1.75 mg g⁻¹ FW) significantly reduced carotenoid content in a good irrigation regime compared with the control (1.93 mg g⁻¹ FW) (Fig. 4).

**RWC, proline, and soluble carbohydrates (SC)**

The amount of RWC, proline, and soluble carbohydrates were similar in 2018 and 2019. Drought and different fertilizers applications had a significant impact (p ≤ 0.01) on RWC, proline, and soluble carbohydrates rates. Drought significantly increased proline content (2.3 times) and soluble carbohydrates amount (63%) compared with good irrigation regimes. In contrast, RWC reduced to 24.2% under drought conditions. In drought regimes, the lowest content of proline and soluble carbohydrates and the highest RWC value was observed in Ch₂₅M₇₅. So, in drought conditions, the average amount of proline and soluble carbohydrates in Ch₂₅M₇₅ was respectively 21.8% and 32.7% less than the control, on the contrary, RWC was 15.9% more than the control (Fig. 5).

**Crocin, picrocrocin, and safranal amounts**

Crocin amount was higher in 2018 (26.2%) than in 2019 (25.9%). However, the picrocrocin and safranal rates were identical in 2018 and 2019. Drought and different fertilizers utilization had a significant effect (p ≤ 0.01) on the content of crocin, picrocrocin, and safranal. Drought slightly improved the content of crocin (3.8%), picrocrocin (3.4%), and safranal (4.04%). Besides, the results showed that the highest amount of crocin, picrocrocin, and safranal was observed in
Ch75M25 in drought (on average, 29.02%, 74.8%, and 41.8%, respectively) and good irrigation regime. However, reducing the share of chemical fertilizers in the combined fertilizer reduced the amount of crocin, picrocrocin, and safranal compared to the use of chemical fertilizers alone (Table 5).

**Discussion**

Saffron growth was more in 2019 than in 2018, while the amount of photosynthesis pigments, RWC, proline, and soluble carbohydrates were similar in both years. The rainfall amount in February 2019 was lower than in 2018. In contrast, the temperature average in March 2019 was higher than in 2018. Since the soil moisture was adjusted in this study, the two-year difference in rainfall will not be the main factor in the growth of saffron. Besides, RWC amount and osmolytes accumulation in two years did not have significant differences which indicated water accessibility for treatments was similar in two years. Therefore, higher temperatures in 2019 caused an increase in the growth of saffron.

The features of saffron flower have a slight decrease by drought stress which indicates that saffron is a resistant plant to drought. The results were confirmed by other studies (Koocheki et al., 2015; Dastranja and Sepaskhah, 2019). Koocheki et al. (2014) reported that the number of saffron flowers, fresh dry weight, and stigma dry weight was significantly reduced by supplying 50% of saffron water requirement. Decreased access to water causes reduced cell turgor pressure, growth, and division (Kim et al., 2018). Therefore, drought can impose an adverse impact on saffron stigma length by decreasing cellular elongation and division. In contrast, water availability promotes more development of primordia in the corm, thus the appearance of leaves and flowers per stem and the area unit was increased.
Fertilizer application improved the growth and yield of saffron in both irrigation regimes. However, the combined fertilizer comprised of chicken manure and chemical fertilizers was more effective than their use alone. Organic fertilizer share determined the efficiency of fertilizer management because the saffron yield is reduced by decreasing the chicken manure in both irrigation regimes. However, this schedule was more efficient in drought stress. Saffron plant needs mineral elements for optimal growth, which must be provided from chemical, organic, and biological sources. Jahan and Jahani (2007) reported that saffron yield augmented with increasing manure consumption. However, the favorable manure impact elevated in the following years. Yarami and Sepaskhah (2015) showed that cow manure usage increased the saffron yield and harvest index. Therefore, the improvement of flower traits and saffron yield may be due to meeting the saffron nutritional needs through chemical or chicken fertilizers. Chicken manure used in this study had lower amounts of nitrogen, potassium, iron, zinc, and manganese than chemical fertilizers but had more phosphorus content. Therefore, it can be concluded that phosphorus can be a rate-limiting agent for saffron growth. Besides, chicken manure may have been effective in improving the morphological traits and saffron yield due to having a higher amount of organic matter, water retention ability, and cation exchange capacity. In contrast, Rezvani Moghaddam et al. (2007) reported that chemical and cow manure had a greater impact on the saffron flower and stigma yield than chicken manure. While Ghanbari et al. (2019) reported that the use of compost had a greater effect on improving flower number and flower dry weight than chemical fertilizers. Drought reduced photosynthesis pigment amount in saffron leaves, however, the content of Ch_b less decreased than the rate of Ch_a. Other studies have also reported that drought stress reduced chlorophyll content in various plant species (Chen et al., 2016; Abd Elbar et al., 2019; Afsahi et al., 2020). Ola et al. (2019) reported that water deficit in Thymus vulgaris L. reduced Ch_a and Ch_b.
to 25.5% and 19.5%, respectively. They expressed that drought stress reduced the chlorophyll content by preventing chlorophyll biosynthesis or chloroplast degradation. However, the latter has reported the result of increment reactive oxygen species (ROS) and lipid peroxidation. Moreover, they explained that less reduction of Ch$_b$ content is related to its protective role for the photosynthetic apparatus under drought regime. This may be the reason for the lower percentage reduction of Ch$_b$ and carotenoids in saffron leaves compared to Ch$_a$ under drought stress.

Using fertilizer improved the content of total chlorophyll and Ch$_a$ while having no significant effect on the Ch$_b$ content. Reducing chemical fertilizer share in the combined fertilizer further increased the content of Ch$_T$, Ch$_a$, and Ch$_b$ in both irrigation regimes. However, a higher amount of chicken manure was required in drought stress. So that, replacing 25% of the used chemical fertilizer with chicken manure in the good irrigation regimes and replacing 50% of it with chicken manure in drought conditions resulted in a higher number of photosynthetic pigments. These findings were confirmed by other studies (Jami et al., 2020; Rahimi et al., 2019; Afsahi et al., 2020). It is reported that the application of vermicompost fertilizer as 24 tons ha$^{-1}$ increased the total chlorophyll content of saffron (Jami et al., 2020). Heydari et al. (2014) confirmed that when the amount of chemical fertilizer was reduced by half and biological fertilizer was used, the amount of chlorophyll in saffron leaves had no significant reduction. Nasarudin et al. (2018) reported that plants have more N uptake and chlorophyll synthesis if fed by integrated fertilizing. Hence, light absorption capacity, photosynthate synthesis, growth, and yield are increased. Moreover, adding livestock manure to the soil increases the water maintenance ability, thus reducing the duration of the plant exposure to drought. Besides, drought stress reduced nutrient availability due to reducing root spread, the minerals mass flow in the soil, soil microbial activity, etc. (Selim, 2020).

Therefore, regardless of the chemical or organic source, fertilizing facilitated the access of mineral
elements and increased plant growth. On the other hand, the use of appropriate ratios of chemical
and organic fertilizers in addition to providing nutrients in the time required by the plant, due to
more water retention reduces the negative effects of drought stress. Fertilizing operations were
reduced carotenoid content in drought conditions. The carotenoid content in drought stress
increased to reduce photo-oxidative stress. However, carotenoid biosynthesis decreased by plant
fertilization and the reduction of the negative effects of drought stress. In contrast, chlorophyll
synthesis increased, which could be due to reduced free radical production and chlorophyllase
enzyme (Rahimi et al., 2019).

Drought increased the accumulation of proline and soluble carbohydrates, while RWC was
reduced. Increasing the content of soluble carbohydrates and proline in response to water deficit
has strongly been associated with an osmotic adjustment in plants. It is caused to protect
macromolecules and membrane integrity under drought stress (Arteaga et al., 2020). The main
superiority of a plant that accumulates the osmolytes is more preservation of leaf water potential.
Some authors reported that proline accumulation is a general indicator of leaf water loss and is
related to drought susceptibility (Dien et al., 2019). Xu et al. (2015) revealed that the amount of
soluble sugar significantly increased in roots and leaves of rice varieties by drought conditions,
however, the RWC has less reduction. This study revealed that proline is a key osmolyte in saffron
reaction to drought conditions. However, proline act as an antioxidant and is involved in free
radical scavenging. Soluble carbohydrates (such as glucose, fructose, etc.) help absorb and retain
more water in plant tissues by reducing the water potential, besides acting as an accessible source
of energy, a precursor to the synthesis of other compounds, etc. Hence, their lower accumulation
under drought conditions may be due to their greater catabolism (Dien et al., 2019; Arteaga et al.,
2020). Accumulation of proline and soluble sugars in saffron increased RWC. This subject was
confirmed by a significant negative correlation between RWC with proline \((r = -0.98, p \leq 0.01)\) and soluble sugars \((r = -0.95, p \leq 0.01)\).

Generally, the rates of proline and soluble carbohydrates were reduced by the fertilizer application in both irrigation regimes, and on the contrary, the RWC increased. Chicken manure reduced the amount of proline more than chemical fertilizer and increased the amount of RWC further. Similar results are obtained by other authors (Heidari et al., 2011; Mondal et al., 2017). Salehi et al. (2016) reported that the enhancement of leaf water content in chamomile plants treated with vermicompost might be due to increasing soluble sugar concentration in the leaves. Mohammadi et al. (2019) reported that bio-fertilizer application under drought conditions more reduced the content of proline compared to chemical fertilizers utilization. One effect of soil water shortages on plants is nutrient availability reduction. Hence, plants will face nutrient deficiency when a long period is exposed to drought (Bista et al., 2018). Therefore, meeting plant nutrition requirements by using appropriate amounts of fertilizer can improve plant resistance to drought conditions. On the other hand, livestock manure can mitigate the negative effects of drought due to more water retention. Besides, Potassium ion plays a vital role in regulating plant water relations, and in this study, the amount of potassium ion in chicken manure was 62.2% higher than the used chemical fertilizer.

Water shortage slightly increased the content of crocin, picrocrocin, and safranal. Studies conducted on other plants have shown that drought increases secondary metabolites (Caser et al., 2018; Mandoulakani et al., 2017). Abd Elbar et al. (2019) reported that the total phenolic content of Thymus vulgaris L. increased under drought stress. Selmar and Kleinwachter, (2013) explained that the stomata are closed in drought conditions and prevent CO\(_2\) from entering into the leaves. Then, a fewer amount of CO\(_2\) has been fixed in the Calvin cycle and less NADPH is oxidized and
reduced. It is responsible for the oxidation and reduction imbalance in the plant tissues toward a greater reduced status. Hence, the plant employs all routes to NADPH consumption such as secondary metabolites biosynthesis. Crocin, picrocrocin, and safranal in saffron are derived from lycopene and it is also synthesized from geranyl-geranyl diphosphate. Therefore, in saffron under drought stress may more pyruvate be shifted to the Mevalonic acid and MEP pathways, and also the genes may be overexpressed in the carotenoid biosynthesis pathway. Radwan et al. (2017) reported similar results in *Salvia officinalis*. They revealed that under drought conditions, over-reduced status, the biosynthesis of plant growth regulators, biochemical pathways shift, and up-expression of the main genes involved in terpenoid synthesis is responsible for increasing monoterpene biosynthesis.

Every fertilizer utilization increased the amount of crocin, picrocrocin, and safranal in both irrigation regimes in 2018 and 2019. Rezaie et al. (2019) oppositely reported that the use of fertilizer reduced the content of saffron secondary metabolites. Soil nutrients have an important effect on the content of plants’ secondary metabolites. However, other factors such as soil microbial community, soil water content, environmental stresses, etc. affect the amount of them (Jami et al., 2020). Although there are no mineral nutrients in the chemical structure of crocin, picrocrocin, and safranal, their amount may be increased by using fertilizers due to improved photosynthesis conditions and increasing carbon fixation, glucose production, protein synthesis, etc. The impact of chemical and organic fertilizers has not been well studied on the saffron secondary metabolites except in a few studies (Ghanbari et al., 2019). This study showed that the use of chemical fertilizer was more effective than chicken manure in incrementing the amount of crocin, picrocrocin, and safranal in each irrigation regime. Feli et al. (2018) similarly reported that chemical fertilizer (100 kg ha⁻¹ urea) more improved the content of safranal than vermicompost and biofertilizer while was
no significant impact on crocin and picrocrocin amounts. However, a study showed that the amount of crocin and picrocrocin improved by utilization of 25 Mg ha\(^{-1}\) cow manure (Rezaian and Paseban, 2006). On the other hand, it is expressed that biofertilizers may have positive impress on the saffron quality due to their effect on the preparation of nutrients, hormones, and vitamins, synergistic impact on other microorganisms, influence on increasing glycoside biosynthesis, and their decomposition into secondary metabolite (Heydari et al., 2014). Therefore, it is concluded that the nutritional status of saffron besides increasing plant growth, plays the main role in its quality improvement.

The combined fertilizer was more effective to improve saffron quality than using only chemical fertilizer or chicken manure. so that, replacing 25% of chemical fertilizer with chicken manure was the best option for improving saffron quality. Similar results were obtained by other authors. Rasooli et al. (2013) explained that the simultaneous use of biofertilizers and chemical fertilizers has the most effect on the number of saffron apo-carotenoids. Gholizadeh et al. (2016) revealed that the utilization of compost, biofertilizers, nitrogen fertilizer, and the combination of the fertilizers had a significant effect on the content of saffron picrocrocin. Its reason may be related to improving soil physical and biological properties, more water retention, cation exchange capacity development, improving the carbon to nitrogen ratio, etc. by chicken manure and rapid supply of elements required for saffron by chemical fertilizers.

Conclusions

Drought stress reduced the growth traits and chlorophyll content of saffron. While the amount of proline and soluble carbohydrates increased and the relative water content decreased. On the other hand, supplying the minerals required by saffron increased growth traits, chlorophyll content, and
yield under drought or good irrigation regimes. The fertilizer management involves different proportions of chicken manure and chemical fertilizers were more effective than using only chemical fertilizers or chicken manure to improve the growth and biochemical traits of saffron. Thus, the highest amount of these traits was acquired when 75% of chemical fertilizer was replaced with chicken manure. Besides, drought increased the content of crocin, picrocrocin, and safranal, on the contrary, mineral nutrition deficit reduced their content. So that, supplying the saffron-required nutrients improved the secondary metabolites content. Furthermore, the use of combined fertilizer has increased the secondary metabolites in stigma. However, the use of higher proportions of chemical fertilizers in the combined fertilizer had a greater effect to increase secondary metabolites rates. Unlike the growth traits, the highest quality of saffron was obtained when the share of chemical fertilizer was higher than chicken manure (3:1). Thus, it can be concluded that under drought stress, the use of combined fertilizer further increased saffron growth, yield, and quality.
References


