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Replacing chemical fertilizers with organic and biological ones in transition to organic farming systems in saffron (*Crocus sativus*) cultivation

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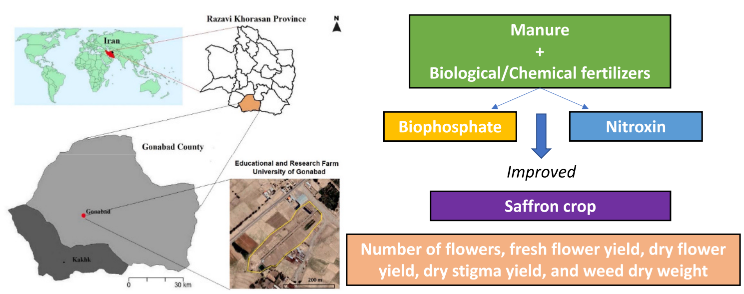
HIGHLIGHTS

- The effect of manure on the saffron traits was significantly positive.
- The combination of manure and biological or chemical fertilizers improved saffron crop.
- The combination of manure and chemical fertilizer or biophosphate improved saffron stigma quality.
- Biophosphate and nitroxin biofertilizer improved saffron yield.

GRAPHICAL ABSTRACT



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ABSTRACT

To evaluate the response of saffron to animal manure, and biological and chemical fertilizer in an arid climate, an experiment was performed as split plots based on a randomized complete blocks design with three replications during three consecutive crop growth seasons (2015–2018) at the Research Farm of University of Gonabad, Iran. The experimental treatments included application (60 t ha^{-1}) and non-application (control) of manure as the main plot and the use of biosulfur (5 kg ha^{-1}), biophosphate (3 L ha^{-1}), nitroxin (3 L ha^{-1}), chemical fertilizer (150, 100, and 100 kg ha^{-1} of urea, triple superphosphate, and potassium sulfate, respectively), and no fertilizer application (control) as the sub-plot. The results showed a highly significant response of the quantitative traits of saffron to the application of manure, which increased the leaf, flower, and corm indices of saffron by a mean of 15.1–35.7% than control. The interaction effect of manure with biological and chemical fertilizers for leaf, flower, and weeds indices of saffron was significant. There was no significant difference between the interaction treatments of manure and chemical fertilizer with nitroxin and biophosphorus fertilizers in most of the mentioned traits in the three experiment years. The simultaneous application of these fertilizers increased the average by about 60, 105, 135, 110, 165, and 55% of the leaf dry weight, the number of flowers, fresh flower yield, dry flower yield, dry stigma yield, and weed dry weight of saffron, respectively as compared to control. There was no significant difference between the chemical fertilizer with nitroxin or biophosphate in terms of the

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effect on the traits related to saffron corm so the use of these fertilizers, as compared to control, increased replacement corm weight, replacement corm size, and bud number per corm by, respectively, about 35, 60, and 40% on average. The chemical and biological fertilizers improved the content of crocin, picrocrocin, and safranal of saffron stigma. The best results were obtained from the use of chemical fertilizers, although no significant difference was observed between this treatment and the nitroxin and biophosphate treatments. Overall, the results of this three-year experiment show a very high response of the saffron plant to the simultaneous use of manure and biological fertilizers and, therefore, it is possible to replace chemical fertilizers with organic and biological fertilizers in saffron cultivation to implement organic agriculture and achieve acceptable quantitative and qualitative yields in areas similar to the experiment location.

1. Introduction

Nowadays, the global agricultural management tends to an organic farming system, which may be derived from traditional agriculture forms or implemented after installing new plantations (Delmotte et al., 2016; Eyhorn et al., 2019). The main principle is to try to not use inputs of chemical origin, reduce soil pollution, and avoid the use of machinery damaging the fertile horizons and compacting the layers of the soil (Eyhorn et al., 2019; Henneron et al., 2015; Reganold et al., 1987). Such a system is also able to reduce the damage caused by pests and diseases due to biological conditions, soil fertility, and proper soil and plant production (Flieβbach et al., 2007), which will help to reach the next important goal, i.e. the protection of natural resources and soil ecosystem, achieving land degradation neutrality (Kapović Solomun et al., 2018; Keesstra et al., 2018; Smetanová et al., 2019) and favoring the regeneration or protection of the ecosystem services. In organic agriculture systems, soil management should be designed based on maintaining its health and biological activity as well as providing a suitable environment for plant growth (Cerdà et al., 2021a, 2021b, 2021a). In some places, organic and biological fertilizers should be used to improve the soil, strengthen the land, and promote crop quality. Undoubtedly, in addition to the positive effects on all soil properties, the use of organic and biological fertilizers is useful for economic, environmental, and social aspects and can be a suitable and ideal alternative to chemical fertilizers (Mehraban, 2013).

The use of organic and biological inputs has been shown to increase the stability of production while reducing the use of chemical fertilizers and related environmental hazards (Negi et al., 2021; Hosseinzadeh et al., 2021). Some authors demonstrated that the most important growth-promoting bacteria are *Azotobacter*, *Azospirillium*, and phosphate-solubilizing bacteria (PSB, *Pseudomonas*), which use soil organic residues through heterotrophy. PSB forms an integral component of the P soil cycle. They convert insoluble, inorganic, and organic P forms into the bioavailable orthophosphate form which is the only form that can be taken up by plant roots. PSB releases several organic acids, including citric, oxalic, fumaric, malic, formic, lactic, and succinic acid. These organic acids can reduce the pH of surrounding soils and contribute to solubilizing phosphate in the rhizosphere. In addition, PSB can produce Indole acetic acid (IAA), which stimulates the production of longer roots and increases the number of root hairs and root laterals (Nacoon et al., 2020; Khan et al., 2022). *Azotobacter* is a free-living nitrogen-fixing bacterium and among its merits include the ability to produce auxin and growth-promoting hormones, various vitamins, and amino acids, as well as the synthesis of antifungal agents (Mishra and Dash, 2014). *Azospirillium* is one of the major nitrogen-fixing microorganisms in temperate, cold, and tropical regions of the world (Pathania et al., 2020). Biofertilizers have a high potential as a renewable nutrient supplement for crops that are compatible with natural ecosystems and represent one of the most important components of the combined nutrition method in crops (Atieno et al., 2020; Asadu et al., 2020), playing an important role in sustainable agricultural development (Bhattacharjee and Dey, 2014) and recovering infected soils (Liu et al., 2018).

Saffron (*Crocus sativus* L.) is a perennial herbaceous plant whose economic part is the three-lobed stigma. It can be considered one of the most valuable agricultural and medicinal species worldwide. In addition to its nutritional uses, this plant is used in various industries, dyeing fabrics, decorative fibers, and colored papers, as well as a remedy in traditional medicine (Koocheki and Khajeh-Hosseini, 2020). The major producer of saffron in the world is Iran, where saffron cultivation is important from various economic aspects, including new aspects of medicine, low water demand, high water productivity compared to other crops, rural employment, and income development (Koocheki, 2013).

To improve saffron growth and yield, fertilization, and proper nutrition to balance the vegetative and reproductive growth of the saffron plant has special importance (Koocheki, 2004). Several investigations have shown that physicochemical and biological properties of soil are key indicators and can serve as criteria in determining crop yield plan design and, therefore, help to select the use of manure to improve crop yield by improving soil properties (Ma et al., 2021). Some studies confirm that from 16 to 80% of changes in flower yield of this plant depend on soil properties (Aghhavani Shajari et al., 2018; Cardone et al., 2020), therefore soil conservation is key to obtaining a high-quality production. In the last three decades, the area under cultivation of saffron has grown dramatically in eastern and northeastern Iran, owing to its importance and various medicinal, food, and industrial applications, high economic value, low water requirements, and adaptation to climatic conditions in these areas and even other areas with suitable climatic conditions for the cultivation of this plant. Saffron accounts for a major part of non-oil exports in Iran and is the main source of income for farmers in many rural areas of the country (Golmohammadi, 2014). As such, the main area under cultivation of agricultural products is dedicated to saffron cultivation in eastern and northeastern Iran. Given as saffron cultivation has changed from traditional and smallholder to commercial cultivation on a larger scale, saffron growers tend to increasingly use chemical inputs, leading to a sharp reduction in the use of organic and biological fertilizers. Unfortunately, Iran is one of the countries facing the crisis of overconsumption of chemical fertilizers. Published researches and statistics indicate a significantly decreased yield of saffron in Iran in recent years. The main reasons for this include the reduction of soil fertility and the degradation of its physical, chemical, and biological quality (Mohtashami and Zandi Daregharibi, 2018; Ramezani et al., 2022). Evidence indicates the increasing growth of soil and water pollution in developing countries due to excessive use of chemical fertilizers (Guo et al., 2020). In addition, chemical fertilizers are a source of trace and heavy elements that cause the accumulation of these elements in the soil and plant system. Their destructive environmental effects, including pollution of water, soil, and air sources and environments (Savci, 2012; Khan et al., 2017; Guo et al., 2020; Haghnazar et al., 2021), and increasing greenhouse gas emissions, affect global warming caused by the use of fossil fuels to produce these chemicals (Bhattacharjee et al., 2008) should not be overlooked. Soil biota can be affected by the use of chemical fertilizers, and the biodiversity of soil microorganisms is reduced by the impact of these substances (Pahalvi et al., 2021). The use of chemical fertilizers has many direct and indirect harmful effects on humans and other living organisms, in addition to reducing the quality of agricultural products

(Zhang et al., 2018). Therefore, it is necessary and inevitable to redefine soil nutrition and fertility management systems based on the principles of agroecology and sustainable agriculture to reduce the consumption and adverse effects of chemical fertilizers on the health of living organisms and the environment. In addition to the benefits of organic fertilizers in improving soil's physical, chemical, and biological properties (Li et al., 2011), increasing water absorption, holding in the soil, and providing plant nutrients (Tang et al., 2015), they are also known as an effective, low-cost, and environmentally friendly amendments for the control and reduction of soil contaminants and their effects on plants (Filipe et al., 2010; Aragón et al., 2019). Furthermore, soil microorganisms, especially plant growth-promoting bacteria (PGPB), have been found to significantly influence soil remediation and dissolution of trace elements, in addition to improving the plant growth environment through various means (Rajkumar et al., 2012).

In the present study, we aimed to investigate the growth and the functional and qualitative responses of saffron to organic nutrition systems for the feasibility of replacing organic and biological nutrition systems with chemical nutrition in saffron production in an arid climate in one of the major saffron cultivation regions in Iran.

2. Materials and methods

2.1. Study area

This experiment was conducted at the Research Farm of the University of Gonabad (58° 43′ E, 34° 20′ N), Khorasan Razavi Province, Iran, during three growing seasons (2015–2018). It is situated at an altitude of 1085 m a.s.l. (Fig. 1). Based on Iran Meteorological Organization, the average and the minimum and maximum temperatures of the region are 18, 10, and 23 °C, respectively. The average annual rainfall is 146 mm and the regional climate is arid and warm based on the Köppen climate classification (Köpppen and Geiger, 1954; Peel et al., 2007). The specific meteorological data registered in the experimental site during the saffron growth period are shown in Fig. 2.

2.2. Experimental design and crop management

The experiment was performed as split plots based on a randomized complete blocks design with three replications. The first factor comprised of control (no manure application) and manure (60 t ha^{-1}). The second factor included control (no fertilizer application), bio-sulfur (5 kg ha^{-1}), bio-phosphate (3 L ha^{-1}), nitroxin (3 L ha^{-1}), and chemical fertilizer (150, 100, and 100 kg ha^{-1} of urea, triple superphosphate, and potassium sulfate, respectively) (see Table 1).

Before planting, the soil was sampled randomly from 0 to 30 cm depth and sent to the laboratory. The physicochemical properties of the experimental field focused on soil and manure applied are presented in Table 2. To prepare the planting bed, the land was first plowed to a depth of 25 cm, using a disk plowed, and the soil surface was leveled by a land leveler. Each plot consisted of 10 planting rows with a distance of 20 cm. Before planting, furrows were made with a depth of 15 cm and three corms were planted in each place on each row based on planting distances of 10 cm. The planted corms (6–8 g corm weight) were prepared from the native saffron ecotype of the region.

The planting (September 25, 2015) was immediately followed by

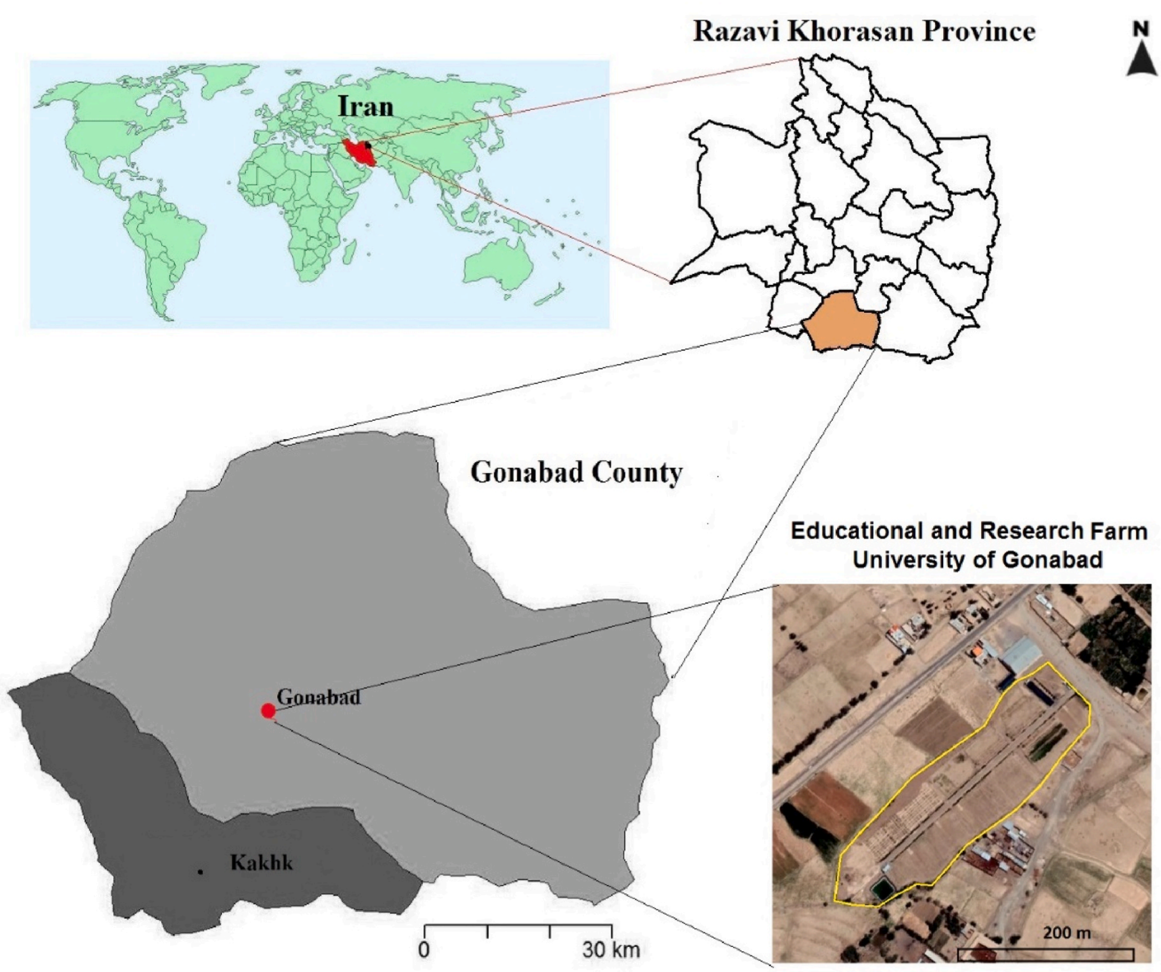

Fig. 1. Experimental site location.

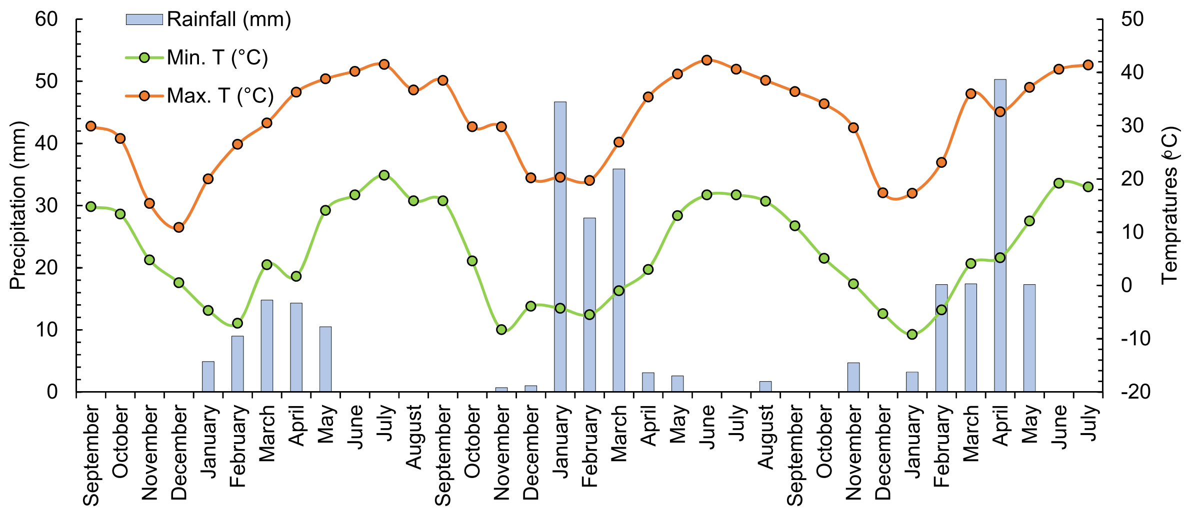

Fig. 2. Meteorological data of the experimental site during the saffron growth periods.

Table 1
Physical and chemical properties of the experimental site soils and manure.

	Texture	Organic Carbon	N	P	K	Fe	Zn	Cu	pH	Electrical conductivity
		%				mg kg^{-1}				dS m^{-1}
Soils	Silty loam	0.56	0.14	0.003	0.049	3.42	0.52	0.65	7.7	2.3
Manure	–	17.30	4.12	0.271	0.913	840	9.50	30	8.4	7.9

irrigation operations. The second irrigation was done slightly at a 5-day interval to ensure uniform emergence. The used biofertilizers, including bio-phosphate, bio-sulfur, and nitroxin, were applied to the respective plots along with the first irrigation water each year. Biophosphate (containing *Pseudomonas* sp. and *Enterobacter* sp.) with a CFU (number of living cells mL^{-1}) of 10^8, biosulfur (containing *Thiobacillus* sp.) with CFU = 10^7, and nitroxin (containing *Azotobacter chroococcum* and *Azospirillum brasilense*) with CFU = 10^8 were used in this experiment.

During the crop growing period, crust breaking, weeding, and irrigation operations were carried out according to local farming practices. No specific pests or diseases were observed on the farm during the experiment period.

M0, and M1 will be used for no manure, and manure application, respectively; F0 to F4: No fertilizer, biosulfur, nitroxin, biophosphate, and chemical fertilizer application, respectively.

2.3. Measurements and data collection

At the end of the leaf growth stage (March), dry leaf weight was measured by harvesting 1 m^2 of plant leaves, drying in an oven at 65 °C for 48 h and, then, weighted. To measure weed dry weight, weeds were sampled randomly from 1 m^2 of each plot, the samples were dried in an oven and dry weight was calculated.

Flowers were harvested daily from each plot during the saffron flowering period in November every year and the flowers were immediately weighed in the laboratory. Stigmas were separated manually from the flowers and dry stigma yield was determined after air drying of stigmas at room temperature for 4 days. Flower dry weight was determined after air-drying of flower for 8 days.

To determine the quality parameters of saffron, the content of crocin (color agent), picrocrocin (flavor agent), and safranal (aroma agent), which are secondary metabolites of dried stigmas, were measured from the sample of stigmas. The samples were analyzed in the laboratory of the Saffron Institute of the University of Torbat Heydarieh. According to ISO/TS 3632–2 (2013) standard method, extraction of dried stigmas was done with distilled water. About 500 mg of saffron stigma was weighed from each treatment and poured into Erlenmeyer with a volume of 1000 ml. The balloons were completely covered with aluminum foil to prevent light from reaching the samples. Then, about 900 ml of distilled water was added to the balloons, and the samples were placed on a magnetic mixer for 1 h. The volumetric balloon was increased to the target line with distilled water and shaken again to obtain a uniform solution. Then, using a pipette, transferred 20 ml of the solution to a 200 ml volumetric balloon and increased to volume. The solution was remixed to obtain a uniform solution, and it was filtered with an air vacuum pump and silicate filter paper. The soluble light absorption was measured using a spectrophotometer (WPA model, S2000 UV/Vis Spectrophotometer) at 254, 330, and 440 nm for picrocrocin, safranal, and crocin, respectively. The results were expressed based on the maximum absorption of one percent aqueous solution at the mentioned wavelengths based on minimum dry matter, according to Eq. (1) (Manzo et al., 2015; Aghhavani Shajari et al., 2022):

$$E_{1cm}^{1\%} = \frac{D \times 10000}{m \times (100 - H)} \quad (1)$$

In this equation, $E_{1cm}^{1\%}$ is the absorbance of aqueous saffron extract; D is the read number from the spectrophotometer, m is the weight of the saffron stigmas in grams and H is the moisture content of the samples, which is considered 6.45.

At the end of each growing season and during the dormancy period of corms (June), the corms were randomly harvested from 0.5 m^2 of each experimental plot. The recorded traits of corms were replacement corm weight, replacement corm size (volume measurement with graduated cylinder), and the number of buds in replacement corms.

2.4. Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA), using SAS software version 9.1 (SAS, Cary, NC, USA). Data means were compared using Duncan's multiple range test at a 5% probability level.

Table 2
Dry leaf yield, flower number, fresh flower yield, dry flower yield, dry stigma yield, and weeds dry weight of saffron as affected by manure, and bio- and chemical fertilizers.

Treatment	Dry leaf yield ($g\ m^{-2}$)			Flower number ($flower\ m^{-2}$)			Fresh flower yield ($g\ m^{-2}$)			Dry flower yield ($g\ m^{-2}$)			Dry stigma yield ($g\ m^{-2}$)			Weeds dry weight ($g\ m^{-2}$)		
	2015–16	2016–17	2017–18	2015–16	2016–17	2017–18	2015–16	2016–17	2017–18	2015–16	2016–17	2017–18	2015–16	2016–17	2017–18	2015–16	2016–17	2017–18
Manure																		
M0	108.9b	251.5b	253.7b	10.1b	10.3b	22.9b	6.8b	8.8b	11.4b	1.07b	1.27b	1.48b	0.12b	0.14b	0.18b	127.8b	319.4b	298.7b
M1	125.7a	300.7a	287.9a	13.9a	12.4a	29.3a	8.0a	12.1a	13.4a	1.45b	1.62a	1.97a	0.16a	0.22a	0.21a	151.7a	350.9a	348.3a
Bio- and chemical fertilizers																		
F0	98.0c	212.7b	219.8b	8.5c	8.0c	16.7c	5.1c	6.2b	8.8c	0.87c	0.92b	1.22c	0.10c	0.10d	0.14b	134.3a	320.3a	336.7a
F1	108.5bc	281.5a	270.8a	11.3b	10.1b	23.7b	6.7bc	8.2b	12.3b	1.17b	1.19b	1.63b	0.13b	0.14c	0.17 ab	133.7a	330.8a	338.2a
F2	120.2b	297.5a	283.8a	12.7 ab	11.8a	28.8a	7.8 ab	11.4a	14.1 ab	1.37 ab	1.69a	1.69b	0.15 ab	0.17b	0.20a	139.7a	330.3a	345.8a
F3	126.8b	290.5a	291.3a	12.8 ab	12.0a	30.0a	8.4a	12.5a	14.3 ab	1.47a	1.85a	1.88b	0.16a	0.22a	0.23a	140.5a	345.0a	351.0a
F4	142.0a	298.3a	288.3a	14.8a	12.3a	31.2a	8.8a	14.2a	15.1a	1.43a	1.58a	2.20a	0.17a	0.22a	0.23a	150.7a	349.2a	355.8a
F-value																		
M	5.60*	15.46***	10.53**	27.8***	12.5**	49.5***	5.81*	14.8***	11.1**	32.26***	15.17***	32.33***	6.93*	6.01*	6.91*	6.93*	6.01*	6.91*
F	3.13*	6.68***	6.29**	8.42***	3.94*	34.6***	6.98***	11.7***	15.0***	11.37***	14.51***	14.51***	0.77ns	0.62ns	3.66ns	0.77ns	0.62ns	3.66ns
M × F	0.38ns	2.96*	3.93*	11.5*	4.06*	3.19*	0.90*	2.72*	3.2*	3.89*	0.70*	3.02*	0.87*	3.01*	1.02*	0.87*	3.01*	1.02*

M0, and M1: No manure, and manure application, respectively; F0 to F4: No fertilizer, biosulfur, nitroxin, biophosphate, and chemical fertilizer application, respectively.
Statistically significant at (*$p \le 0.05$; **$p \le 0.01$; ***$p \le 0.001$; ns, not significant).
Column means with the same letter are not significantly different by Duncan's multiple range test ($p < 0.05$).

3. Results

3.1. Dry leaf yield

Table 2 presents the results of the ANOVA considering the obtained experimental data for dry leaf yield (DLY) of saffron. It shows that manure and various sources of bio- and chemical fertilizers significantly affected the DLY of saffron in the first year of the experiment, but the interaction effect of these two factors was not significant. In the second and third years of the experiment, however, significant effects of manure and various sources of bio- and chemical fertilizers, as well as their interaction, were observed on DLY. According to the mean comparisons for the DLY data in the first experiment year, the application of manure caused a 13.3% increase in DLY compared to control. Among different sources of bio- and chemical fertilizers, the highest DLY (142 $g\ m^{-2}$) was obtained by the chemical fertilizer treatment. After this treatment, biofertilizer treatments, including nitroxin, bio-phosphate, and bio-sulfur, were placed in second place. The lowest value of DLY (98 $g\ m^{-2}$) was observed in the control. Mean comparison of the interaction effect of manure and different sources of bio- and chemical fertilizers in the second experiment year also showed the superiority of the manure plus chemical fertilizer treatment (M2F5) with a DLY of 361.7 $g\ m^{-2}$. Simultaneous use of manure with biofertilizers (nitoxin or bio-phosphate) was also in the next place. In the second year, the lowest DLY (206.3 $g\ m^{-2}$) was found in the control plot (M_0F_0) (Fig. 4a). The results for the third experiment year also revealed that the highest DLY (319.7 $g\ m^{-2}$) belonged to manure and nitroxin applications (M_1F_3), showing a 78.3% increase in this parameter compared to the control (Fig. 3a).

3.2. Number of flowers per unit area

According to the ANOVA, the effects of manure, different sources of bio- and chemical fertilizers, and their interaction were significant considering the number of flowers (NF) in all three experiment years (Table 2). The mean comparison indicated that the highest value (15 and 18 flower m^{-2}) for the first and second year, respectively, was obtained under manure plus chemical fertilizer application (M_1F_4). There was no significant difference between the mentioned treatments and applications of manure plus nitroxin (M_1F_3) or biophosphate (M_1F_2). In the third year, however, the manure plus nitroxin treatment (M_1F_3) with the value of 32.7 flower m^{-2} caused better results than the other fertilizer treatments, but this treatment was not significantly different paying attention to the interaction of manure and chemical fertilizers as well as from the manure plus biophosphate treatment (M_1F_2). The lowest value of NF (8.3, 8.7, and 0.15 flower m^{-2}) was recorded for the no-fertilizer treatment (M_0F_0) for the first, second, and third years, respectively (Fig. 4b).

3.3. Fresh flower yield

The fresh flower yield (FFY) of saffron showed a significant difference as affected by the experiment treatments (Table 2). The results showed that the highest value (9.3 and 15.3 $g\ m^{-2}$) for the first and second year, respectively, belonged to the manure plus chemical fertilizer treatment (M_1F_4). However, no statistically significant differences were observed between the mentioned treatment and the manure plus nitroxin (M_1F_3) or biophosphate (M_1F_2). In the third year, the highest value (17.2 $g\ m^{-2}$) was obtained by the application of manure plus nitroxin (M_1F_3). However, there was no statistically significant difference between this treatment and the manure plus chemical fertilizer (M_1F_4) or biophosphate (M_1F_2). While, the lowest values (3.8, 5.8, and 0.8 $g\ m^{-2}$) for the first, second, and third year, respectively, were recorded for the control (M_0F_0) (Fig. 4c).

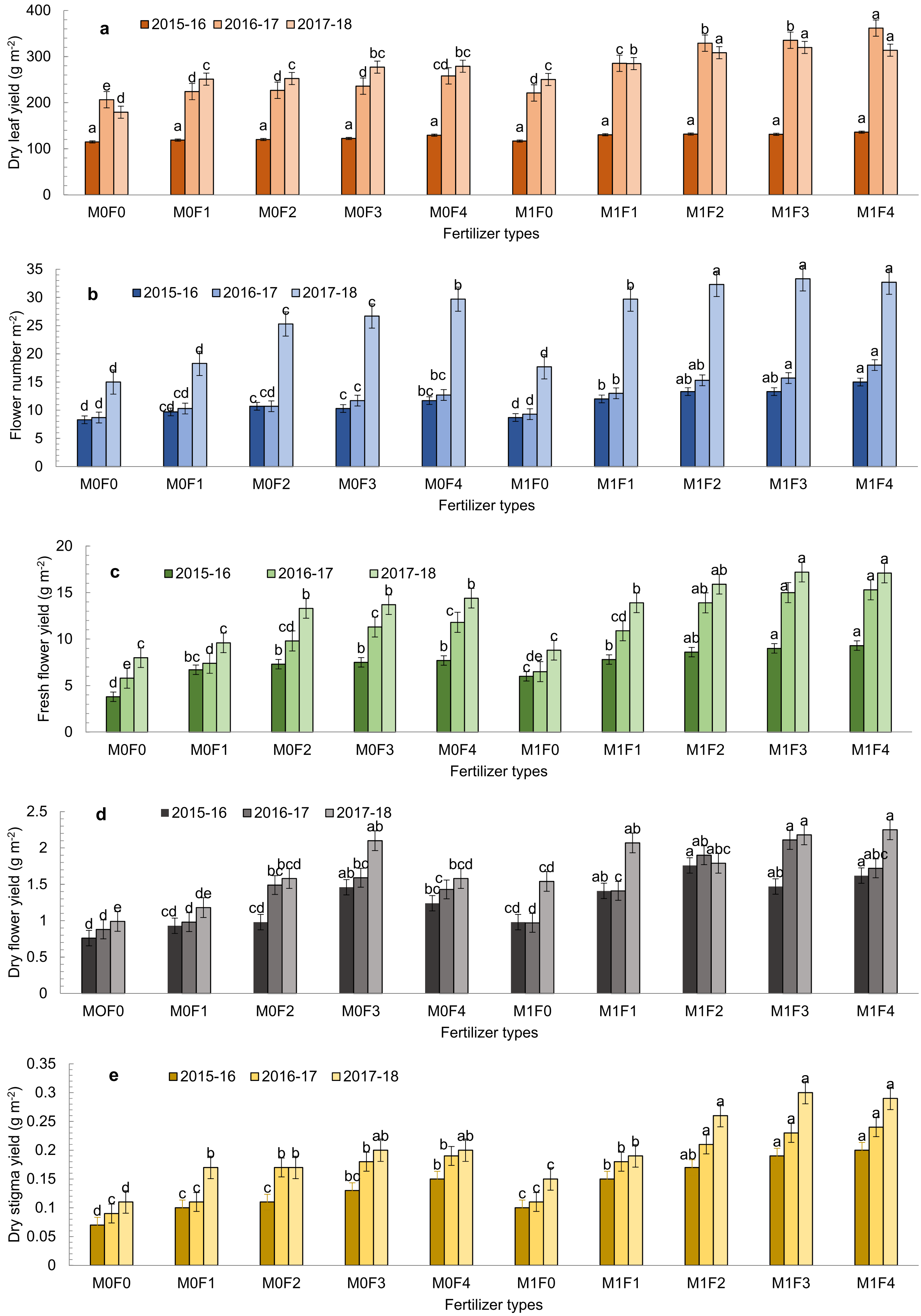

Fig. 3. Interaction effects of manure × bio- and chemical fertilizers on dry leaf yield (a), flower number (b), fresh flower yield (c), dry flower yield (d), and dry stigma yield (e) of saffron. M0, and M1 will be used for no manure, and manure application, respectively; F0 to F4: No fertilizer, biosulfur, nitroxin, biophosphate, and chemical fertilizer application, respectively.

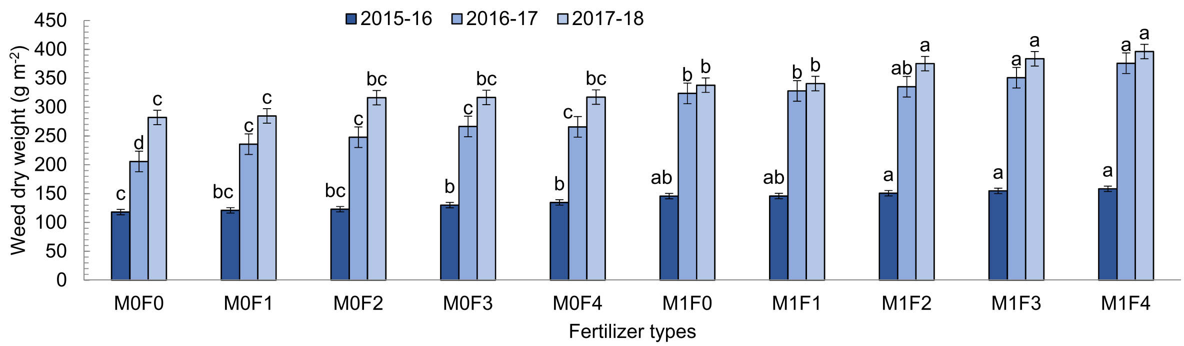

Fig. 4. Interaction effects of manure × bio- and chemical fertilizers on weeds dry weight. M0, and M1 will be used for no manure, and manure application, respectively; F0 to F4: No fertilizer, biosulfur, nitroxin, biophosphate, and chemical fertilizer application, respectively.

3.4. Dry flower yield

The Dry flower yield (DFY) was significantly affected by manure, bio- and chemical fertilizers, and the interaction of manure × fertilizer types (Table 2). Experimental treatments achieved almost different results during the experimental years, so the highest value of DFY in the first (1.76 g m^{-2}), second (2.11 g m^{-2}), and third year (2.25 g m^{-2}) was obtained from the M_1F_2, M_1F_3, and M_1F_4 treatments, respectively. However, no significant difference was observed between the M_1F_2 and M_1F_4, and M_1F_4 and M_1F_3 in the first and third years of the experiment. The difference between the fertilized plots and no fertilized plots in the case of DFY increased from the first to the third year, so the highest increase (127%) was observed in the third experiment year (Fig. 3d).

3.5. Dry stigma yield

ANOVA results showed significant variations in the dry stigma yield (DSY) in response to the application of different fertilizer sources, which had a similar trend to the fresh flower yield, indicating a strong correlation between these two traits. According to the results (Table 2), under the interaction effect of manure and chemical fertilizer (M_1F_4), the DSY presented the highest value (0.20 and 0.23 g m^{-2}) for the first and second growing seasons, respectively. No significant differences were found between the mentioned treatment and manure plus nitroxin (M_1F_3) or biophosphate application (M_1F_2). In the third year of the experiment, no significant differences were observed in the SDY between manure application along with chemical fertilizer (M_1F_4), nitroxin (M_1F_3), and biophosphate (M_1F_2). Therefore, the dry stigma yield showed a 65, 63, and 58% increase due to the simultaneous use of manure and chemical fertilizer (M_1F_4), nitroxin (M_1F_3), or biophosphate (M_1F_2), respectively, compared to the control (M_0F_0). The yield increases for the second and the third years were 62, 60, and 57% and 62, 63, and 0.57%, respectively (Fig. 3e).

3.6. Replacement corm weight and size, number of buds per corm

The ANOVA of the experimental data for the replacement corm weight (RCW) and replacement corm size (RCS) showed that only the main effects of manure and different sources of bio- and chemical fertilizers on these parameters were significant (Table 3). According to the

Table 3
Replacement corm weight, replacement corm size, and bud number per corm of saffron as affected by manure, and bio- and chemical fertilizers.

Treatment	Replacement corm weight (g)			Replacement corm size (cm^3)			Bud number per corm		
	2015–16	2016–17	2017–18	2015–16	2016–17	2017–18	2015–16	2016–17	2017–18
Manure									
M0	1.17b	1.64b	1.91b	1.18b	1.42b	1.68b	2.49b	1.78b	1.66b
M1	1.42a	2.04a	2.39a	1.34a	1.83a	1.96a	3.17a	2.23a	1.89a
Bio- and chemical fertilizers									
F0	1.08c	1.29c	1.74c	0.98b	1.08c	1.49c	2.03c	1.67b	1.46b
F1	1.17b	1.63b	2.10b	1.22 ab	1.47b	1.79b	2.88b	1.97 ab	1.88a
F2	1.35a	1.99a	2.31a	1.22 ab	1.74 ab	1.99 ab	3.00 ab	2.20a	1.83a
F3	1.40a	2.00a	2.36a	1.39a	1.90a	2.23a	2.90b	2.15a	1.82a
F4	1.43a	2.11a	2.44a	1.46a	1.96a	2.32a	3.37a	2.03a	1.87a
Interaction									
M0F0	1.22a	1.55a	1.86a	0.97a	0.97a	1.94a	1.62d	1.57c	1.31c
M0F1	1.22a	1.53a	1.98a	1.13a	1.45a	2.10a	2.23c	1.65bc	1.68b
M0F2	1.24a	1.92a	2.02a	1.31a	1.62a	2.26a	2.63b	1.70bc	1.68b
M0F3	1.26a	2.04a	2.08a	1.10a	1.54a	2.27a	2.68b	1.92b	1.70b
M0F4	1.36a	2.09a	2.22a	1.39a	1.54a	2.26a	2.71b	2.04b	1.75b
M1F0	1.22a	1.84a	1.92a	1.00a	1.20a	1.95a	2.14c	1.75bc	1.61b
M1F1	1.38a	2.04a	2.11a	1.32a	1.48a	2.27a	3.06b	2.01b	1.76b
M1F2	1.39a	2.12a	2.29a	1.33a	1.86a	2.39a	3.72a	2.54a	1.91a
M1F3	1.41a	2.17a	2.31a	1.34a	1.86a	2.32a	3.87a	2.66a	2.07a
M1F4	1.49a	2.18a	2.36a	1.37a	1.97a	2.37a	4.09a	2.69a	1.97a
F-value									
M	23.7***	11.09**	17.4***	4.43*	22.2***	14.3***	27.7***	17.4***	6.84*
F	5.88**	8.24***	4.07*	4.88**	13.45***	7.38***	11.2***	3.00*	3.40*
M × F	3.88ns	0.20ns	3.26ns	0.22ns	3.15ns	3.57ns	3.10*	4.81**	0.59**

M0, and M1: No manure, and manure application, respectively; F0 to F4: No fertilizer, biosulfur, nitroxin, biophosphate, and chemical fertilizer application, respectively.
Statistically significant at (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ns, not significant).
Column means with the same letter are not significantly different by Duncan's multiple range test (p < 0.05).

mean comparison, the use of manure caused a 17.6, 19.6, and 20.0% increase in RCW in the first (1.4%), second (2.1%), and third (2.4%) year as compared to the control, respectively. This increase in RCS was calculated as 11.9, 22.4, and 14.2% for the first, second, and third years, respectively. Mean comparison for different sources of bio- and chemical fertilizers revealed that the greatest RCW and RCS were achieved by chemical fertilizer application in all three experiment years. The highest RCW were 1.43, 2.11, and 2.44 g, with values of 1.46, 1.96, and 2.32 cm^3 for RCS in the first, second, and the third year, respectively. Our results also showed that no significant differences were observed between the chemical fertilizer and biofertilizers (nitroxin or biophosphate) treatments in all three experiment years. The lowest values of RCW (1.08, 1.29, and 1.74 g, respectively) and RCS (0.98, 1.08, and 1.49 cm^3) were measured in non-fertilized plants during the experiment years.

Statistical analysis showed that the tested experiment factors and their interaction effect significantly influenced the number of buds per corm (NBC) of saffron. According to the mean comparison of the interaction effects (Table 3), the highest values (4.09 and 2.69) in the first and second year, respectively, were obtained by simultaneous application of manure and chemical fertilizer (M_1F_4). However, no significant differences were found between this treatment and manure × nitroxin (M_1F_3) or biophosphate (M_1F_2). In the third year of the experiment, the highest value (2.07) was obtained by manure × nitroxin (M_1F_3), which had no difference from M_1F_4 or M_1F_2. The lowest NBC (1.62, 1.57, and 1.31 buds per corm) for the first, second, and third year, respectively, were recorded for M_0F_0 (Table 3).

3.7. Weeds dry weight

Results revealed that weeds' dry weight (WDW) was significantly affected by experiment treatments (Table 2). According to mean comparisons, manure application significantly increased WDW than the control in all three years of the experiment. The highest WDW was obtained by application of manure plus chemical fertilizer (M_1F_4), nitroxin (M_1F_3), or biophosphate (M_1F_2) and there were not any significant differences between these treatments in all three experiment years. The WDW values obtained by the application of chemical fertilizer, nitroxin, or biophosphate without using manure were much less than that of manure application. The lowest WDW values (0.118, 205.7, and 0.282 g m^{-2}) were measured for the first, second, and third years, respectively, in the control treatment (M_0F_0) (Fig. 4).

3.8. Stigma volatile compounds

The results presented in indicate that the effect of manure on Stigma volatile compounds (SVC) was not significant in all three years of the experiment. Nevertheless, the effect of different sources of bio- and chemical fertilizers was significant on variations of SVC (crocin, picrocrocin, and safranal) in all three experiment years. Also, the interaction effect of manure and different sources of bio- and chemical fertilizers was significant only on the crocin content among all three experiment years. According to the mean comparison results (Table 4), the highest picrocrocin content was obtained by application of chemical fertilizer in all three years of the experiment, with a 16.3, 0.19, and 17.6% increase compared to the control. The safranal content increased by 23.6, 19.2, and 16.7% for the first, second, and third years, respectively, due to chemical fertilizer application. For both the above qualitative parameters, no significant differences were observed between the chemical fertilizer and nitroxin, and biophosphate treatment in all three years of the experiment. The lowest values of picrocrocin and safranal in all the experiment years were observed in the control (Table 4). The results of the mean comparison of interaction effects of the experimental factors on the crocin content revealed that the highest increases (17.9, 17.3, and 17.1% for the first, second, and third experiment year, respectively) belonged to manure × chemical fertilizer interaction (M_1F_4). For all three years of the experiment, however, no statistically significant differences were detected between the mentioned treatment and M_1F_3 or M_1F_2. The lowest value was obtained by the control (M_0F_0) for the first, second, and third experiment years (Table 4).

Table 4
Crocin, picrocrocin and safranal of the saffron stigma as affected by manure, and bi- and chemical fertilizers.

Treatment	Crocin ($E^{1\%}_{1cm}$ 440nm)			Picrocrocin ($E^{1\%}_{1cm}$ 257nm)			Safranal ($E^{1\%}_{1cm}$ 330nm)		
	2015–16	2016–17	2017–18	2015–16	2016–17	2017–18	2015–16	2016–17	2017–18
Manure									
M0	193a	205a	205a	77a	70a	78a	35a	70a	78a
M1	200a	207a	218a	83a	76a	82a	36a	76a	80a
Bio- and chemical fertilizers									
F0	151c	199b	204b	72b	64b	70b	29b	63c	70c
F1	177b	201 ab	205 ab	77 ab	71 ab	79 ab	30b	71b	75b
F2	193a	209a	213a	82a	75a	82a	35a	75 ab	81a
F3	198a	209a	214a	83a	75a	83a	37a	75 ab	83a
F4	202a	211a	216a	86a	79a	85a	38a	78a	84a
Interaction									
M0F0	190bc	197c	199c	69a	62a	66a	32a	65a	76a
M0F1	192bc	204bc	203c	73a	68a	79a	33a	68a	80a
M0F2	195b	205bc	212b	80a	71a	80a	35a	71a	80a
M0F3	198b	210b	216b	82a	73a	81a	35a	73a	81a
M0F4	200b	210b	216b	83a	76a	84a	36a	76a	84a
M1F0	183c	211b	202c	75a	66a	74a	33a	66a	76a
M1F1	187c	214b	216b	82a	75a	79a	36a	75a	77a
M1F2	211 ab	224a	225a	83a	77a	84a	36a	77a	82a
M1F3	223a	226a	227a	87a	79a	85a	37a	79a	83a
M1F4	224a	231a	233a	89a	82a	86a	38a	82a	86a
F-value									
M	3.31ns	11.55***	22.8***	8.75**	17.8***	7.63**	6.51ns	17.8***	5.40*
F	0.98**	11.95***	2.95*	6.67**	13.9***	13.0***	1.98ns	13.9***	21.8***
M × F	1.21*	4.16*	3.48*	0..34ns	0.40ns	1.01ns	0.37ns	0.40ns	3.66ns

M0, and M1: No manure, and manure application, respectively; F0 to F4: No fertilizer, biosulfure, nitroxin, biophosphate, and chemical fertilizer application, respectively.
Statistically significant at (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ns, not significant).
Column means with the same letter are not significantly different by Duncan's multiple range test ($p < 0.05$).

4. Discussion

The findings of this research show an increasing trend of values obtained for saffron traits related to leaves, flowers, and corm during the three-year-lasting experiment (Tables 2 and 3). Ghanbari et al. (2019), in a study on the effects of nutritional regimes on saffron traits, reported improvements in the parameters related to saffron flowers and corm during the first to third year of their experiment. Given that the condition of corms determines the vegetative and reproductive growth conditions of saffron, the number of corms per unit area and, consequently, the corn yield will usually be increased during the growth periods of this perennial plant with the production of alternative (daughter) corms on old (mother) corms. This trend usually continues until the fourth to sixth year, when the yield decreases due to the increased number of corms and other factors related to the environmental conditions of the plant growth (Khazaei et al., 2013; Koocheki and Khajeh-Hosseini, 2020).

Considering that different organs of the saffron plant, including leaves, flowers, and corm, are used economically, the saffron requirement to nutrition supply will vary depending on the parts that will be used and, therefore, nutrition management in saffron cultivation is very complex and important. The results of this experiment indicated that the chemical fertilizer improved all the quantitative traits of saffron (Tables 2 and 3). In various studies, the supply of essential elements required by the plant, especially N and P, has been shown to improve the yields of saffron corms and flowers (Chaji et al., 2013) by affecting plant growth and production of daughter corms (Koocheki et al., 2014; Koocheki and Seyyedi, 2015). The research results of Behzad et al. (1992a,b) and Behnia et al. (1999) showed a significant increase in saffron yield due to chemical fertilizer application.

Various reports demonstrate the high importance of soil quality parameters in improving the growth and yield of saffron. Given that saffron is a perennial plant, the appropriate agronomic operation is to maintain the soil's physical, chemical, and biological properties for the optimal growth of roots and corm and, ultimately, good vegetative and reproductive growth of the plant is very important. The continuous use of chemical fertilizers in such crops as saffron can lead to contamination of water and soil resources and the incidence of environmental and health hazards, in addition to reduced soil quality and fertility. The findings of this experiment reveal the excellent response of saffron to the application of manure. The utilization of this organic matter in the soil significantly increased the dry leaf yield, the number of flowers, fresh flower yield, dry flower yield, dry stigma yield, daughter corm weight, daughter corm size, and the number of buds per corm in all the experiment years (Tables 2 and 3). The results of other studies show that saffron is a special plant and suitable for the implementation of organic agriculture principles. Moreover, yield and yield components of this plant favorably respond to the use of organic fertilizer sources, especially manure, due to its relatively low fertilizer requirements and perennially (Jahan and Jahani, 2007; Koocheki and Seyyedi, 2015; Ghanbari et al., 2019). It has frequently been reported that manure is considered a suitable alternative to chemical fertilizers due to its beneficial properties that balance plant nutrition and allowed for improved crop growth and yield, as other authors demonstrated (Huang, 2020; Wang et al., 2020; Wang et al., 2021; Abou-Sreea et al., 2021). Manure can be considered a key factor in improving soil fertility and due to its positive effects on the physical, chemical, and biological properties of soil (Zhuang et al., 2019), can reduce soil pollution and provide suitable growing conditions in the soil rhizosphere (Haghnazar et al., 2021). Manure also increases crop yield and productivity due to the improvement of soil chemical properties, such as pH, cation exchange capacity (CEC), enhancing soil microorganisms, and nutrient availability (Babaeian et al., 2011; Esmaeilian et al., 2011). Other authors highlighted that manure can reduce even soil erosion and retain water within the fertile horizon (Antoneli et al., 2019; Peng et al., 2016; Ramos and Martínez-Casasnovas, 2006). Increasing soil organic matter leads to the formation and stabilization of soil aggregates and improves water holding capacity (WHC), hydraulic conductivity, bulk density, degree of compaction, soil fertility, and resistance to water and wind erosion (Choudhary et al., 2021). The potential of manure to improve the rhizosphere environment and supply nutrients needed for the plant during growing periods will lead to more and better growth of roots and shoots and, ultimately, improve quantitative and qualitative traits of the crop (Agbede, 2021). As a result of another research, it was reported that the application of 60 t ha^{-1} manure significantly increased the yield and harvest index (dry style and stigmas weight divided by leaf dry weight) of saffron. The authors stated that manure increased saffron yield by improving soil fertility and nutrient availability for the plant (Yarami and Sepaskhah, 2015).

The results of our experiment showed a significant effect of the interaction between manure and chemical/biological fertilizers on the dry leaf yield, the number of flowers, fresh flower yield, dry flower yield, dry stigma yield, and weed dry weight (Tables 2 and 3). In almost all the experiment years, the results showed that the combined use of manure and chemical fertilizer led to the greatest positive effect on the studied traits. According to various studies, the combined use of manure and chemical fertilizers can reduce the limitations and shortcomings of individual fertilizers and is an appropriate agronomic method to improve soil fertility and quality and achieve higher yields (Cui et al., 2018). Similarly, some studies show a higher effect of combined use of manure and chemical fertilizers than their application alone on different traits of saffron (Sadeghi et al., 1992; Turhan et al., 2007; Amiri, 2008).

In addition to the very significant response of the saffron plant to the application of manure, the results of this experiment showed that a result equivalent to the chemical fertilizer in most of the studied traits was obtained using nitroxin and biophosphate biofertilizers. These fertilizers known as plant growth stimulants not only cause the release and availability of nutrients but also improve the growth and the crop quantity and quality by improving soil properties and the physiological functions of the plant. Similar to the results of this study, Omidi et al. (2009) reported that the effect of nitroxin on the vegetative and reproductive traits of saffron was to be equivalent to the chemical fertilizer and even nitroxin had a more positive effect on the quantitative properties of the saffron corm than the chemical fertilizer. It has been reported that nitroxin fertilizer increased the secretion of plant growth-promoting hormones and some vitamins and organic acids by biological nitrogen fixation and balancing the absorption of macronutrients and therefore, improving the vegetative and reproductive growth of the saffron plant (Gutierrez-Manero et al., 2001; Mrkovacki and Milic, 2001). In another experiment, increases in the number of leaves, stigma length, corm weight, and yield of saffron were reported by the use of PGPB. The authors introduced PGPB-containing biofertilizers as the best fertilizer to increase growth and stimulate the physiological cycles of the saffron plant (Rasouli et al., 2013).

The results of this experiment properly fulfilled the hypotheses and objectives of the research to evaluate the feasibility of replacement of chemical fertilizers with organic and biological fertilizers in order to change the tendency of saffron cultivation from conventional to organic farming so that the combined use of manure and biofertilizers was more superior to the use of manure or chemical fertilizers alone. In addition, the effect of the combined application of manure and biofertilizers (nitroxin and biophosphate) on the studied quantitative traits of saffron was equal to that of the combined application of manure and chemical fertilizers, suggesting an excellent response of saffron to the implementation of the organic/biological fertilization system. It is necessary to mention that most saffron cultivation in Iran is limited to arid and semi-arid regions of this country, where the soil has low organic matter and low quality, in addition to water resource limitations. Hence, this has made it inevitable to implement the organic/biological nutrition system in saffron cultivation in these areas. Due to the perennially of saffron, the use of chemical fertilizers results in soil degradation and reduces both water use efficiency and crop yield in the long term. From the results, it is inferred that manure provided a suitable environment for the growth and activity of these microorganisms by providing a nutrient source for bacteria (Mohammadi Aria et al., 2010), and on the other hand, bacteria in the applied biofertilizers have increased access to macro- and microelements, hormones, enzymes, and vitamins in manure, thereby improving the growth environment and ultimately increasing the vegetative and reproductive growth parameters of the plant (Alizadeh et al., 2019). In this regard, Nehvi et al. (2010) achieved the highest saffron flower yield by the combined application of vermicompost and *Azotobacter*. The results of another experiment demonstrated a significant increase in the traits related to saffron flowers by the interaction of organic and biological fertilizers (Alizadeh et al., 2019). Aytekin and Acikgoz (2008) also experimented with the effects of different fertilizer types on saffron and concluded that the best fertilizer composition for saffron cultivation was growth-promoting microorganisms and biohumus.

During the 3 experiment years, the measurement of weed dry weight showed an increase in this index with increasing the field age. Due to the growth form of the saffron plant, the distances between the rows, and the spacing between adjacent plants in the row, as well as the conventional method of irrigation in this crop (flood irrigation) in Iran, this plant usually is not very able to compete with weeds that grow with high biodiversity with this plant. Thus, weeds are one of the important factors in the reduction of saffron yield in important areas of saffron farming in Iran (Ghorbani and Koocheki, 2007; Zare Hosseini et al., 2014). Furthermore, fertilization and the application of nutritional regimes in the saffron (especially manure) increased the dry weight of weeds, suggesting that weeds compatible with the saffron field benefited from the improved soil fertility and nutritional conditions. A high significant increase in the dry weight of weeds was observed due to manure fertilization, which can be one of the main challenges and limitations of saffron organic farming in the study area.

Our results showed that the combined use of nitroxin (M_1F_3) and biophosphate (M_1F_2) biofertilizers with manure led to equal values under manure × chemical fertilizer (M_1F_4) for almost all studied traits in this experiment. Biofertilizers are substances containing different types of free-living microorganisms (Nofal and Rezk, 2009) that can convert essential nutrients from unavailable to available forms during biological processes (Stavros et al., 2012) and lead to better root system development (Mandal et al., 2007). Free-living bacteria also participate in some key ecosystem processes, including those involved in the biological control of plant pathogens, nutrient cycles, and seedling establishment (Benabdellah et al., 2011). Bacterial and fungal microorganisms, in particular rhizobacteria, are among the most important bio-fertilizers that stimulate plant growth and induce changes in the content of plant hormones, production of volatile compounds and increase the availability of nutrients. Bacteria in biofertilizers can cause biological fixation of nitrogen, release phosphate and potassium ions from insoluble soil compounds, and help the plant enhance nutrient uptake (Ruzzi and Aroca, 2015).

In recent years, many studies have been performed on PSB, indicating that the use of PSB not only improves the quantitative and qualitative traits of crops but also helps to reduce the use of chemical fertilizers (Seif; Sharma et al., 2020). The use of these bacteria has been reported to improve P uptake and reduce the use of chemical fertilizers, ultimately improving crop yields such as saffron (Díez-Méndez and Rivas, 2017). It has been reported that PSB plays an effective role in the availability and uptake of P by plants and plays a role as a growth stimulant in plants. Researchers demonstrated that P is one of the main elements required by plants involved in all biochemical processes, energy-carrying compounds, and energy transfer mechanisms. Additionally, P is a component of cellular proteins and plays a special role as a component of cellular enzymes, cell membranes, and nucleoids that are associated to plant growth and reproduction processes (Rodríguez and Fraga, 1999).

Measurements of the saffron volatile compounds (crocin, picrocrocin, and safranal) showed changes in the content of crocin, safranal, and picrocrocin during the experiment years. Overall, the contents of these compounds generally improved in saffron stigma with increasing the age of the saffron farm, so the greatest effect of saffron age on stigma volatile compounds was observed on safranal (Table 4). However, a study on the effects of different nutritional regimes on saffron quality reported no significant differences in the content of saffron volatile compounds between three experiment years (Ghanbari et al., 2019).

In our experiment, the stigma of volatile compounds significantly improved due to the utilization of chemical fertilizer as well as nitroxin and biophosphate biofertilizers. Furthermore, simultaneous application of manure and bio- and chemical fertilizers enhanced saffron quality. It seems that any factor that enhances the concentrations of plant carbohydrates will have a direct effect on increasing the volatile compounds in saffron. Alizadeh et al. (2019) stated that the picrocrocin, safranal, and crocin content of saffron was 73, 77, and 83% higher, respectively, due to the combined application of manure and biofertilizers treatment than control. Other researchers showed that biofertilizers improved saffron essential oil more than chemical fertilizers. For example, Omidi et al. (2009) reported that the application of biofertilizers increased picrocrocin and safranal of saffron stigma more than chemical fertilizer.

5. Conclusions

The results of this study showed that the traits related to saffron leaves, flowers, and corm improved during the first to third year of the experiment. Also, the quality parameters of saffron (crocin, picrocrocin, and safranal) improved with increasing the age of the saffron plant. The application of manure had a significant and positive effect on the studied traits of saffron and overall caused an increase of 13.4–37.5% of the quantitative traits of saffron, while it had no statistically significant effect on the qualitative parameters of saffron. However, the application of manure increased saffron weed (based on dry weight) and the WDW increased by 9.8–16.6% during the experimental years. The results also revealed that although in most of the studied traits chemical fertilizer caused the highest increase in the value of these traits, biological fertilizer treatments (nitroxin and biophosphate) achieved results equivalent to chemical fertilizer. The interaction of manure with chemical and biological fertilizers was significant on leaf dry weight, the number of flowers, fresh and dry flower yield, and dry stigma yield. In this case, although in most of the studied traits, the simultaneous use of manure and chemical fertilizers was superior, the combined use of manure with nitroxin or biophosphate in most traits was equal to the mentioned treatment. The interaction effect of manure with chemical fertilizers and biological fertilizers had a significant effect just on the crocin content of saffron stigma and the highest values were obtained due to the simultaneous application of manure and chemical fertilizer, although there was no significant difference between the interaction treatments of manure with nitroxin/biophosphate and mentioned treatment. Based on the research results, it is concluded that by combined use of manure and biofertilizers in climatic regions similar to the experiment site, equivalent and even higher yields from chemical fertilizer can be achieved, and, in addition, to obtain saffron economic yields, its quality Improved and by reducing or eliminating chemical fertilizers in saffron cultivation, took an effective step towards organic and sustainable agriculture and maintaining the health of the agro-ecosystem.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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