



Growth and yield of saffron (*Crocus sativus* L.) affected by different levels of fulvic acid and cow manure in the second growing season

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ABSTRACT

Purpose: The experiment was conducted to evaluate the optimal amounts of cow manure and fulvic acid and the interaction effects of inputs on the yield of saffron in the second growing season.

Research methods: In this research, a factorial experiment based on Randomized Complete Block design (RCBD) with three replications was conducted in 2015-2016 and 2016-2017 growing seasons, in the University of Birjand, Iran. Experimental factors included cow manure in four levels (0, 10, 20, and 30 t.ha⁻¹) and fulvic acid plus some micronutrients in three levels (0, 5, and 10 kg.ha⁻¹).

Findings: The results in the second year showed that the effect of cow manure was significant on flower number per m² and total fresh flower yield and dry stigma yield so that the highest total fresh flower yield (32.081 g.m²) and dry stigma yield (0.325 g.m²) obtained in the treatment of 20 t.ha⁻¹ cow manure and the lowest amount of these traits observed in the control (no-fertilization). The application of 10 kg.ha⁻¹ fulvic acid led to the highest mean flower weight (0.512 g) and stigma yield (0.035 g.plant⁻¹). The results of interaction effects showed that simultaneous application of cow manure and fulvic acid had a significant effect on fresh flower yield and the highest amount of these traits (33.613 g.m²) obtained in the combined application of 20 t.ha⁻¹ cow manure and 10 kg.ha⁻¹ fulvic acid. In general, it seems that separate and simultaneous application of optimal amounts of cow manure and fulvic acid, while improving the reproductive characteristics and yield of saffron, can guarantee the healthy and sustainable production of this valuable medicinal.

Research limitations: There was no significant limitation to the research. **Originality/Value:** This research evaluates the simultaneous application of eco-friendly inputs of cow manure and fulvic acid on reproductive characteristics and yield of saffron which has been less studied.

INTRODUCTION

Saffron (*Crocus sativus L.*) as the most expensive agricultural and pharmaceutical product in the world, has special importance among Iran's industrial and export products and has several uses in food, health, cosmetics, and pharmaceutical industries (Mohammad Abadi et al., 2011). Although Iran is the largest producer of this product in the world, the maximum yield of saffron in Iran is significantly lower than the yield of this plant in countries such as Spain and Pakistan (Behdani et al., 2008). Therefore, it is necessary to provide optimal solutions to improve the yield of saffron in Iran by conducting appropriate research and extension works.

One of the important factors in this field is improving soil fertility and plant nutritional management through the use of organic and mineral fertilizers (Majidian et al., 2008).

Among organic fertilizers can be mentioned to fulvic acid and cow manure. Humic substances make up 85 to 90% of soil organic matter, so humic fertilizers have a significant role in improving soil fertility (Samavat & Malakuti, 2005). Fulvic acid is one of the humic substances that play an important role in soil fertility and increasing plant yield (Saffar Sabzevar & Jami Moeini, 2015). Fulvic acid is the most active humic compound that dissolves minerals in water and easily transports nutrients to the plant. The chelating ability of fulvic acid is unique in soil. Fulvic acid can also dissolve vitamins, isoenzymes, hormones, and natural antibiotics, thereby improving plant growth and development (Samavat & Malakuti, 2005). In one research, the positive effect of humic substances on the vegetative and reproductive characteristics of saffron was reported (Golzari, 2016). The study of the effect of humic substances on the yield of saffron stigma showed that during the two years of the experiment, the stigma yield increased by 154 and 92% compared to the control (Koocheki et al., 2016). In one research on Iranian Ox-Tangue (*Echium amoenum Fisch.*), the application of humic acid and fulvic acid increased flower yield by 36 and 27% compared to the control (Amiri et al., 2017).

The application of manure has a special place in agriculture since ancient times and today can play an effective role in sustainable and biological agriculture (Majidian et al., 2008). So that the researchers in South Khorasan climatic zone stated that up to 67% of saffron yield changes are affected by manure and phosphorous fertilizer (Behdani et al., 2005). In another study, the effective role of manure in increasing flower yield and stimulating corm production of saffron was mentioned (Koocheki et al., 2013). Hassanzadeh Aval et al. (2013) showed that cow manure in the second year had a significant effect on flower number and weight and stigma weight of saffron. In another study, simultaneous application of cow manure and humic acid caused an increase in dry stigma weight and fresh flower weight compared to the control (Mollafilabi & Khorramdel, 2016). Aghhavini Shajari et al. (2018) reported that the application of humic acid improved most of the criteria and yield of saffron replacement corms. In a research, humic acid improved the saffron flowering characteristics in the second growing season and the highest fresh and dry yield of stigma were obtained in plants treated with 10 kg.ha⁻¹ humic acid (Ahmadi et al., 2018). In another research, cow manure and fulvic acid improved the saffron flowering characteristics in the first year and the highest dry yield of stigma (0.085 g.m⁻²) was obtained with the simultaneous application of 10 t.ha⁻¹ cow manure and 5 kg.ha⁻¹ fulvic acid (Aminifard et al., 2020). Fallahi et al. (2020) reported that deficit irrigation treatment (3600 m³.ha⁻¹), associated with the use of humic acid had the highest flower and stigma yield. In another study on saffron, cow manure and humic acid increased the mean weight of corm by 9 and 10% compared to control, respectively (Rezaie et al., 2019).

Despite studies on the nutritional management of saffron, there is much disagreement about the nutritional needs of saffron and nutritional inputs that can be used in the production

of this valuable product (Rezvani Moghaddam et al., 2014). On the other hand, not many studies have been reported on the role of fulvic acid and its simultaneous application with cow manure on the flower and stigma yield of saffron. Therefore, this research aimed to evaluate the simultaneous application of different levels of fulvic acid and cow manure on the flowering of saffron.

MATERIALS AND METHODS

In this research, a factorial experiment based on RCBD design with three replications was conducted in 2015-2016 and 2016-2017 growing seasons, in the University of Birjand, Iran. Experimental factors included cow manure in four levels (0, 10, 20, and 30 t.ha⁻¹) and fulvic acid plus some micronutrients in three levels (0, 5, and 10 kg.ha⁻¹). Before preparing the soil to determine the physical and chemical characteristics of the soil, sampling was done from a depth of 0 to 30 cm (Table 1).

To prepare the land after plowing, discs, and leveling the soil, plots with dimensions of 2×2 m were created. To prevent water mixing of the experimental plots, a separate irrigation creek was used for each block and the distance between the plots and the blocks from each other was 1 and 2 m, respectively. To apply cow manure before planting, depending on the experimental treatment, the amount of cow manure was mixed with soil to a depth of 20 cm. The chemical characteristics of the studied cow manure are given in Table 2. The local corms of Birjand saffron with an average weight of 7-9 g were planted on September 7 of 2015 with a space on the row of 10 cm, space between a row of 20 cm, and a planting depth of 15 cm (50 corms.m²) (Rezvani Moghaddam et al., 2013). In each year, the fulvic acid was applied as a solution in irrigation water after planting with the first irrigation after waterlogging of the plot. Table 3 shows the chemical characteristics of the used fertilizer containing fulvic acid.

The first summer irrigation was done simultaneously with planting (September 7 of 2015 in the form of waterlogging) and the second irrigation was performed 10 days after the first one to facilitate the flower emergence. Next irrigations were done after the end of the flowering period according to the custom of the region with monthly intervals and by leakage method using a siphon. In the second year, fulvic acid was applied with irrigation water before flowering (pre-flowering irrigation). No pesticide, herbicide, or chemical fertilizers were used during the experiment. Due to low stigma yield and uncertainty about the effect of treatments in the first year, sampling was done in the second year to evaluate flower and stigma yield.

Table 1. Soil physical and chemical characteristics in the experimental field

Texture	EC (dS.m ⁻¹)	Total nitrogen (%)	Available potassium (mg.kg ⁻¹)	Available phosphorous (mg.kg ⁻¹)	pH	Organic matter (%)
Loamy	3.1	0.08	420	2.07	7.76	0.68

Table 2. Chemical characteristics of used cow manure

Nitrogen (%)	Available phosphorous (mg.kg ⁻¹)	Available potassium (mg.kg ⁻¹)
1.12	710	4125

Table 3. Characteristics of fertilizer containing fulvic acid

Trade name	Fulvic acid (%)	Humic acid (%)	Fe (%)	Mn (%)	Cu (%)	Zn (%)	Mg (%)	S (%)	pH
WGS75%	70	15	4	2.5	1	2.5	6-7	5-6	4-5

Simultaneous with the beginning of flowering, saffron flowers were harvested, counted, and weighed from the entire surface of the plots in the early morning hours of mid-November 2016. The studied traits included flower number per m², mean weight of fresh flower, the total yield of fresh flower, mean length of stigma, mean weight of fresh and dry stigma, fresh and dry yield of stigma, mean length of style and stamen, and mean weight of petal.

Data analysis was performed by using SAS 9.1 software. The mean comparison of treatments was done with Duncan's multiple range test at a 5% probability level.

RESULTS

Flower number

The simple effects of cow manure and fulvic acid on flower number per m² were significant, but the interaction effect of treatments had no significant effect on this trait (Table 4). Mean comparison of treatments showed that the application of 10 and 20 t.ha⁻¹ of cow manure increased flower number per m² by 9 and 10% compared to control, respectively (Table 5). Application of 5 kg.ha⁻¹ of fulvic acid produced the highest flower number per m² (66.4 numbers per m²), although the difference between this treatment and the level of 10 kg.ha⁻¹ of fulvic acid was not significant (Table 5).

Table 4. Analysis of variance (mean of squares) of flower and stigma yield of saffron affected by different levels of cow manure and fulvic acid.

S.O.V	Df	Flower number per m ²	Mean weight of the fresh flower	The total yield of fresh flower	Mean length of stigma	Mean weight of fresh stigma	Mean weight of dry stigma
Block	2	27.750ns	0.003ns	0.615ns	1.805ns	0.000ns	0.000ns
Cow manure	3	88.185**	0.009**	3.618*	2.139ns	0.003**	0.000*
Fulvic acid	2	96.083*	0.007**	11.277**	10.194**	0.003*	0.000**
Cow manure × Fulvic acid	6	27.490ns	0.001ns	3.630**	0.689ns	0.000ns	0.000ns
Error	22	17.537	0.001	0.815	0.706	0.000	0.000
CV (%)	-	6.492	6.950	2.862	2.640	12.701	10.967

ns, * and ** are non-significant and significant at a probability level of 5 and 1%, respectively

Table 4. Continued.

S.O.V	Df	The fresh yield of stigma	The dry yield of stigma	Mean length of style	Mean length of stamen	Mean weight of petal
Block	2	0.078ns	0.000*	14.034ns	51.706**	0.000ns
Cow manure	3	0.164*	0.000**	14.359ns	3.218ns	0.000ns
Fulvic acid	2	0.813**	0.024**	4.065ns	0.401ns	0.000ns
Cow manure × Fulvic acid	6	0.061ns	0.000ns	25.123ns	9.750ns	0.001ns
Error	22	0.053	0.000	36.893	7.710	0.000
CV (%)	-	12.264	4.412	22.456	17.896	8.632

Table 5. Mean comparison of flower and stigma yield of saffron affected by different levels of cow manure and fulvic acid.

S.O.V	Flower number per m ²	Mean weight of fresh flower (g)	The total yield of fresh flower (g.m ⁻²)	Mean length of stigma (mm)	Mean weight of three branched fresh stigmas (g)
Cow manure (t.ha ⁻¹)					
0	60.55b	0.44b	30.68b	31.14a	0.17b
10	66.66a	0.50a	31.48ab	32.27a	0.21a
20	67.33a	0.51a	32.08a	32.03a	0.22a
30	63.44ab	0.51a	31.97a	31.89a	0.21a
Fulvic acid (kg.ha ⁻¹)					
0	61.25b	0.464b	30.52b	30.77b	0.18b
5	66.41a	0.503a	31.70a	32.26a	0.22a
10	65.83a	0.512a	32.44a	32.46a	0.21a

Similar letters in each column indicate no significant difference at the 5% probability level (Duncan's multiple range test)

Table 5. Continued.

S.O.V	Mean weight of dry stigma (g)	The fresh yield of stigma (g.m ⁻²)	The dry yield of stigma (g.m ⁻²)	Mean length of style (mm)	Mean length of stamen (mm)	weight of petal (g.m ⁻²)
Cow manure (t.ha ⁻¹)						
0	0.030b	1.718b	0.302b	26.112a	14.984a	1.05a
10	0.034a	1.88ab	0.320a	28.066a	15.150a	1.08a
20	0.034a	2.04a	0.325a	28.206a	16.321a	1.09a
30	0.034a	1.89ab	0.323a	25.809a	15.606a	1.04a
Fulvic acid (kg.ha ⁻¹)						
0	0.030b	1.58b	0.265b	26.411a	15.304a	1.04a
5	0.034a	2.03a	0.345a	27.552a	15.618a	1.09a
10	0.035a	2.03a	0.343a	27.182a	15.624a	1.06a

Total yield and mean fresh flower weight

Total yield and mean fresh flower weight were affected by the application of different levels of cow manure and fulvic acid. The simultaneous application of these ecological inputs was also significant on the total fresh flower yield (Table 4). Simultaneous application of 20 t.ha⁻¹ of cow manure and 10 kg.ha⁻¹ of fulvic acid resulted in the highest total fresh flower yield (33.61 g.m⁻²) and total fresh flower yield in these conditions was 7% higher than when no cow manure and fulvic acid was used (Fig. 1). As shown in Figure 1, in conditions of non-application of cow manure there was no significant difference between different levels of fulvic acid, but when fulvic acid was used simultaneously with cow manure in all studied cow manure levels, the amounts of 5 and 10 kg.ha⁻¹ of fulvic acid significantly increased the total fresh flower yield compared to the control treatment of fulvic acid. The highest mean weight of fresh flower (0.512 g) was obtained at the level of 20 and 30 t.ha⁻¹ of cow manure and the lowest amount of this trait was observed in the control treatment (Table 5). Application of 10 t.ha⁻¹ of cow manure also caused a 12% increase in the mean weight of fresh flowers compared to the control (Table 5). Application of 5 and 10 kg.ha⁻¹ of fulvic acid increased the mean weight of fresh flowers by 8 and 9% compared to control, respectively (Table 5).

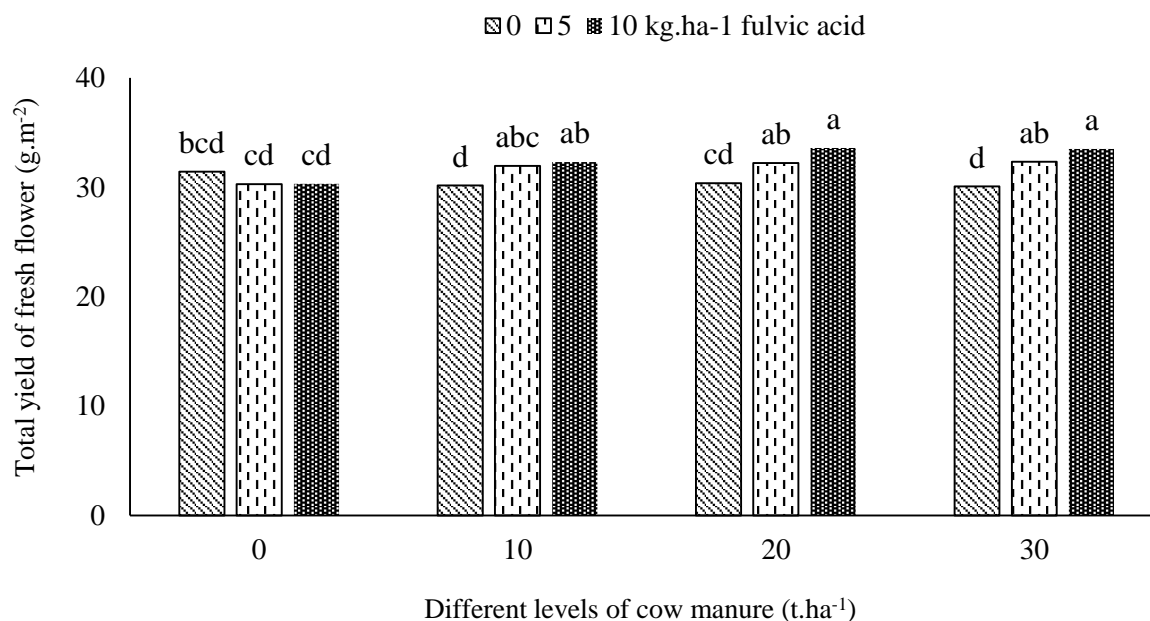


Fig. 1. Mean comparison of the total yield of the fresh flower of saffron affected by interaction effects of cow manure and fulvic acid

Mean length of stigma

As shown in table 4, the mean length of stigma in the second year was significantly affected by fulvic acid, while cow manure and interaction of two fertilizer types were not significant on this trait. The mean comparison of treatments showed that the highest mean length of stigma (32.4 mm) was obtained at the level of 10 kg.ha⁻¹ of fulvic acid and its lowest amount (30.7 mm) was observed in the control treatment (Table 5). Application of 5 kg.ha⁻¹ fulvic acid also led to a 5% increase in mean length of stigma compared to the control (Table 5).

Mean weight of fresh and dry stigma

The mean weight of fresh and dry stigma was significantly affected by different levels of cow manure and fulvic acid, but the interaction effect of experimental factors did not have a significant effect on these traits (Table 4). The highest mean weight of fresh and dry stigma was obtained at the level of 20 t.ha⁻¹ cow manure, which was 1.25 and 1.13 times higher than the control treatment, respectively (Table 5). Application of 10 and 30 t.ha⁻¹ of cow manure also improved the mean weight of fresh stigma by 12% and the mean weight of dry stigma by 18 and 19% compared to control, respectively (Table 5). Application of 5 and 10 kg.ha⁻¹ fulvic acid increased the mean weight of fresh stigma by 15 and 12% compared to control, respectively, and the mean weight of dry stigma by 12 and 14%, compared to control, respectively (Table 5).

Fresh and dry yield of stigma

Different levels of cow manure and fulvic acid had a significant effect on fresh and dry yield of stigma, but the simultaneous application of these inputs was not significant on these traits (Table 4). The highest fresh (2.04 g.m⁻²) and dry (0.32 g.m⁻²) yield of stigma was obtained at the level of 20 t.ha⁻¹ cow manure. However, in this regard, no significant difference was observed between the levels of cow manure (Table 5). Application of 10 and 30 t.ha⁻¹ cow manure also increased the dry yield of stigma by 6 and 7% compared to control, respectively (Table 5). The highest fresh (2.03 g.m⁻²) and dry (0.34 g.m⁻²) yield of stigma were observed at

the level of 10 and 5 kg.ha⁻¹ fulvic acid, respectively, which showed an increase of 29 and 30% compared to the control (Table 5). The fresh yield of stigma increased by 22% due to the application of 5 kg.ha⁻¹ fulvic acid compared to control and application of 10 kg.ha⁻¹ fulvic acid improved the dry yield of stigma by 23% compared to control (Table 5).

Mean length of style, mean length of stamen, and mean weight of petal

The results of analysis of variance showed that the treatments of cow manure, fulvic acid, and their interaction did not have a significant effect on mean length of style, mean length of stamen and mean weight of petal (Table 4).

DISCUSSION

The results showed that cow manure in this research had a significant effect on the flower number per m² (Table 4). The effective role of cow manure on most of the studied traits can be due to the improvement of nutritional conditions for effective growth of corms and also the improvement of physical conditions in the soil to facilitate flowering (Majidian et al., 2008). In this way, Behdani et al. (2005) stated that the gradual release of nutrients from cow manure, while providing plant nutritional needs can play an effective role in improving the physical and chemical structure of the soil. In research reported that the highest flower number and stigma yield was observed in 40 t.ha⁻¹ cow manure (Khorramdel et al., 2018). In another research, the highest fresh flower (72.3 kg.ha⁻¹) and stigma (5.38 kg.ha⁻¹) yield was achieved in 40 t.ha⁻¹ cow manure (Esmailian & Amiri, 2019).

Based on the results, fulvic acid also increased the flower number per m² (Table 5), which was similar to the results of other researchers on increasing flower number of saffron by consuming humic acid (Osmani Roudi et al., 2015). Similarly, one research showed that the application of humic acid during two successive years increased the flower number (Mollafilabi & Khorramdel, 2016). In another study, the highest amount of flower number (165 flowers.m⁻²) was obtained by using 40 kg.ha⁻¹ humic acid that was 22% higher than control (Fallahi et al., 2016). The results showed that the simple and interaction effect of cow manure and fulvic acid was significant on total fresh flower yield (Fig.1). Also, the mean flower weight was affected by the application of cow manure and fulvic acid (Table 4). The results showed that the level of 20 t.ha⁻¹ of cow manure created favorable conditions for the growth and yield of saffron, but its higher level (30 t.ha⁻¹) did not have a good effect on plant yield. Since the underground organ is the basis of the establishment and gravity center of the plant, especially in plants with underground storage organs, so change of the soil fertility management based on the use of organic fertilizers (such as cow manure) by improving soil characteristics, while increasing growth and yield of the plant, ensures ecosystem stability in long-term (Leithy et al., 2006).

The results showed a positive effect of fulvic acid on yield and mean flower weight (Table 5), which was similar to the results of Golzari (2016) on the effect of humic substances on saffron flower yield. The researchers also stated that the highest flower yield of saffron was obtained by application of humic acid during two years of experiment (Koocheki et al., 2016). Fulvic acid is a suitable natural tampon with high ion exchange power that increases the absorption of minerals in plants and thus increases the quality and quantity of saffron (Saffar Sabzevar & Jami Moeini, 2015). As the results showed, cow manure could not affect the mean length of stigma (Table 3), which its cause can be attributed to the low amount of soil organic matter (Table 1), but there was a significant difference between different levels of fulvic acid in the mean length of stigma (Table 4). In a study, the role of humic substances on

increasing the stigma length was reported (Ahmadi, 2017). Humic substances have different mechanisms for increasing plant growth, one of which is the direct effect of these compounds and the presence of hormone-like compounds, including auxin and auxin-like compounds, which can promote cell growth (Atiyeh et al., 2002) and consequently increase the stigma length.

In this research, the effect of cow manure was significant on the mean fresh and dry weight of stigma (Table 4). Similar to the results of this study, a significant increase in the mean fresh and dry weight of stigma with the application of cow manure has been reported (Hassanzadeh Aval et al., 2013). The results of Jahan and Jahani (2007) also confirmed the effective role of manure application in increasing the dry weight of stigma. It seems that cow manure by increasing the growth and activity of beneficial microorganisms in the rhizosphere (Mohammadi Aria et al., 2010) has increased nutrient uptake and thus increased stigma weight. In one research, the highest flower number (282.7 numbers.m⁻²), fresh flower weight (103.2 g.m⁻²), and dry stigma weight (1.73 g.m⁻²) were obtained in cow manure and once summer irrigation (Asadi et al., 2019).

As the results showed, the mean fresh and dry weight of stigma was affected by fulvic acid (Table 4). In one research, the positive effect of humic acid on stigma weight was reported (Ahmadi et al., 2017). In another study, humic acid increased stigma weight (Mollafilabi & Khorramdel, 2016). It seems that fulvic acid is the most active humic compound by dissolving minerals in water and their easy transfer to the plant (Samavat & Malakouti, 2005) increased photosynthesis and plant growth, and stigma weight. In this research, cow manure had a significant effect on the fresh and dry yield of stigma (Table 4). Similar to the results of this research, improvement of flower and stigma yield as a result of cow manure application has been reported (Osmani Roudi et al., 2015). According to the results, fulvic acid increased the fresh and dry yield of stigma (Table 5). Investigating the effect of humic substances on the stigma yield of saffron showed that during the two years of the experiment, the stigma yield increased by 154 and 92% compared to the control treatment (Koocheki et al., 2016), which was similar to the results of this experiment. In another research, the application of humic substances increased the stigma yield, so that the highest and the lowest dry stigma yield were obtained in treatments of humic acid and control, respectively (Golzari, 2016). It seems that the application of organic fertilizers (cow manure and fulvic acid) by increasing organic matter, moisture storage content, and improving the availability and absorption of essential micronutrients in the soil increase flower and stigma yield. In one research, deficit irrigation treatment (3600 m³.ha⁻¹), associated with the use of humic acid, had the highest flower and stigma yield (Fallahi et al., 2020). In another study, fulvic acid increased seed and biological yield of sesame (*Sesamum indicum* L.) by 33 and 23% compared to control, respectively (Ayoubzadeh et al., 2020). The highest plant dry weight, number of flowers per plant, and biological yield in basil (*Ocimum basilicum* L.) were obtained in conditions of 10 kg.ha⁻¹ fulvic acid (Askarian et al., 2020). Cow manure and fulvic acid had a significant impact on antioxidant compounds and active ingredients of saffron and the highest rate of anthocyanin and total phenol were obtained in the treatment of 5 kg.ha⁻¹ fulvic acid (Aminifard & Ahmadi, 2019).

CONCLUSION

The results showed that all amounts of cow manure and fulvic acid increased flower number per m², mean weight of fresh flower, the total yield of fresh flower, mean weight of dry stigma, and fresh and dry yield of stigma compared to the control, but there was no significant difference between the levels of 10, 20 and 30 t.ha⁻¹ of cow manure and between the levels of

5 and 10 kg.ha⁻¹ of fulvic acid. In general, the results of this research indicated the positive effect of ecological inputs of cow manure and fulvic acid in improving the quantitative characteristics and yield of saffron, but because the simultaneous application of cow manure and fulvic acid did not have a significant effect on most traits compared to their separate use and due to between the levels of cow manure and fulvic acid was not observed significant difference, a separate application of 10 t.ha⁻¹ cow manure and 5 kg.ha⁻¹ fulvic acid is recommended to achieve maximum yield of saffron flower and stigma.

Conflict of interest

The authors have no conflict of interest to report.

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