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Assessment of the Impacts of Humic Acid Application and Mycorrhizal Fungi Inoculation on Flowering and Stigma Quality of Saffron under Two Irrigation Regimes

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Abstract

The shift toward sustainable medicinal plant production underscores the importance of biological inputs. This study evaluated the effects of humic acid application, mycorrhizal inoculation, and two irrigation regimes on reproductive growth and quality of saffron (*Crocus sativus* L.). The experiment was conducted as a split-split plot based on a randomized complete block design (RCBD) with three replications, where irrigation intervals (20 and 40 days) were the main plot, humic acid (0 and 5 $kg.ha^{-1}$) was the sub-plot, and mycorrhizal inoculation (non-inoculated, *Glomus mossea*, and *G. intraradices*) was the sub-sub plot. The studied traits were the number of flowers, flowering rate, flower yield, petal yield, style yield, and stigma yield, as well as stigma quality parameters (crocin, picrocrocin, and safranal content). Results indicated that the triple interaction of experimental factors was significant on all studied traits. The shortest irrigation interval (20 days) combined with humic acid application and *G. mossea* inoculation significantly increased fresh flower yield (79.63 $g.m^{-2}$), dry stigma yield (0.88 $g.m^{-2}$), style yield (0.22 $g.m^{2}$), and petal yield (9.88 g.m ^{2}). Crocin content was highest (274.5, absorbance of 1% aqueous solution at 440 nm) under 40-day irrigation intervals with humic acid and *G. mossea* inoculation, while safranal (35.80, absorbance at 330 nm) and picrocrocin (124.5, absorbance at 257 nm) improved under 20-day irrigation intervals with humic acid and *G. mossea*. Conversely, extended irrigation intervals (40 days) without humic acid and without mycorrhizal inoculation resulted in the lowest crocin and safranal content. Overall, the findings demonstrate that combining humic acid with *G. mossea* inoculation under frequent irrigation optimizes both the quantity and quality of saffron. It was concluded that although the use of organic inputs is beneficial, the results of their single application may differ from those of their combined application. On the other hand, the interaction among two or more of these inputs can also vary depending on conditions, including water availability and the type of mycorrhizal species.

Keywords: Crocin*, Crocus sativus*, Flowering rate, *Glomus mossea*, Picrocrocin, safranal.

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Introduction

Saffron (*Crocus sativus* L.) is botanically an annual plant of the Iridaceae family, but is primarily cultivated as a perennial crop. Its cultivation is expanding in various regions of the world, although Iran remains the largest producer, accounting for about 90% of global saffron production (Behdani & Fallahi, 2024). The unique secondary metabolites of saffron make it highly valuable for medicinal, culinary, nutritional, and cosmetic applications (Aghhavani Shajari & Rezvani Moghaddam, 2022). However, its global production is increasingly challenged by limited water resources and the expanding use of chemical inputs in some fields. Therefore, ensuring sustainable production of this valuable plant requires strategies that reduce reliance on chemical inputs while promoting the use of biological inputs such as humic acid and mycorrhizal fungi.

Previous studies have shown that irrigation regime and fertilization can influence saffron growth and flowering. For example, Fallahi and Mahmoodi (2018) showed that applying organic fertilizer significantly increased flower and stigma yields compared with chemical fertilization, especially under longer irrigation intervals. Their results suggest that, at least in the first growing season, combining organic fertilizer with moderate irrigation (3600 $m^3.ha^{-1}$) is beneficial for saffron production. Humic acid, a well-known biological soil amendment, has also been shown to improve saffron yield and quality. Koocheki et al. (2016) reported that application of humic acid enhanced both vegetative growth and yield of saffron. Feizi et al. (2025) also found that humic acid significantly affected the growth parameters and macronutrient uptake in saffron. Similarly, Zakaria et al. (2025) found that both humic and fulvic acids improve saffron stigma quality. In a related study, Aghavani Shajari et al. (2022) found that nutrient and irrigation management, including humic acid and mycorrhiza, significantly alters not only flowering but also quality-related parameters such as crocin, picrocrocin, and safranal content. Another study found that, in the first growing season, the use of 3,600 $m^3.ha^{-1}$ of irrigation water (compared with 4,200 and 4,800 $m^3.ha^{-1}$) combined with the application of humic acid improved saffron yield, whereas mycorrhiza had an inhibitory effect (Fallahi et al., 2021). Another previous study indicated that mycorrhizal inoculation significantly enhanced key quantitative traits of saffron, including flower number, leaf biomass, and stigma yield (Jami et al., 2020). Habibi et al. (2022) also found that corm inoculation by *Rhizophagus irregularis* improved saffron growth traits, including leaf number, biomass, and corm size. Similarly, Caser et al. (2019) reported that inoculated corms of saffron developed about 50% larger replacement corms. *R. intraradices* alone led to higher spice quality and antioxidant activity, while the combination of *R. intraradices* and *F. mosseae* increased polyphenol levels. Despite previous studies on the effects of mycorrhizal fungi on saffron, their impact can vary considerably depending on environmental properties, fungal species, and whether the inoculum is applied singly or in combination (Behdani & Fallahi, 2024). Therefore, the intricate interactions among fungal type, environment, and saffron plants need further investigation.

Despite some of the above-mentioned advances, there is still a gap in understanding the interaction effects of humic acid, mycorrhizal inoculation, and irrigation frequency on saffron's growth, yield, and quality. Therefore, this study was designed to evaluate the combined effects of these biological inputs and irrigation regimes on both the quantitative and qualitative traits of saffron. The study aims to provide practical insights into improving saffron productivity through eco-friendly inputs across two irrigation management systems.

Materials and methods

Study area

This experiment was conducted during 2016–2017 at the research field of the Faculty of Agriculture at the University of Birjand. Sarayan County is located at 58°31′ E longitude and 33°51′ N latitude, at an elevation of 1450 meters above sea level. The main climatic characteristics of Sarayan during the experimental period are presented in Table 1.

Table 1. The main climatic parameters of the research site during the growing of saffron

Year	Month	Minimum temperatures (°C)	Maximum temperatures (°C)	Rainfall (mm)
2016	October	9.6	37.6	0.3
	November	3.7	28.5	0
	December	-8.7	23.7	0.7
	January	-4	21.6	17.4
	February	-5.9	18.7	70.5
	March	-2.7	25.3	11.3
2017	April	2.4	35.2	10.8
	May	9	37.4	7.7
	June	16.3	41.5	0
	July	15.4	43.5	0
	August	16.2	38.1	0
	September	11.9	35.5	0
	October	3.8	34.1	0

Experimental Treatments

The experiment was conducted as a split–split plot arrangement based on a randomized complete block design (RCBD) with three replications. Irrigation interval was considered the main factor, with two levels: every 20 days and every 40 days. After the flowering period (early December), all plots were uniformly irrigated (post-flowering irrigation), and then the irrigation treatments were initiated and continued as flood irrigation until the end of the growing season. Accordingly, the 20- and 40-day irrigation intervals resulted in 8 and 4 irrigation events, respectively, during the growing season. Considering that approximately 500 m^3 ha-1 water was applied at each irrigation time, total water consumption during the saffron growing season was 4000 and 2000 $m^3.ha^{-1}$ for the 20- and 40-day intervals, respectively. The irrigation dates in a 20-day interval were 31-Dec, 20-Jan, 9-Feb, 1-March, 21-March, 20-April, and 10-May. The irrigation dates for 40-day irrigation intervals were 20-Jan, 1-March, and 20-April.

Humic acid application (0 and 5 $kg.ha^{-1}$) and mycorrhizal inoculation (*Glomus mosseae* and *G. intraradices* and no-inoculation as control) were assigned to the sub-plot and sub-sub-plot, respectively. For the mycorrhizal inoculation treatment, a soil-based inoculum containing arbuscular mycorrhizal fungi species (number of live spores: ~50-150 per g of soil), supplied by Zist Fanavaran Tooran Co. (Semnan, Iran), was used. The inoculum was placed below the planting rows simultaneously with the corm planting (~15g per corm)(Aghhavani-Shajari et al., 2017). Humic acid, obtained from Aria Shimi Co. (Zahedan, Iran), was applied through the pre-flowering irrigation as fertigation and repeated in three post-flowering irrigations. The humic acid formulation contained 30% humic acid, 10% fulvic acid, 8% phosphate, 11% nitrogen, and 17% potassium.

Agronomic operations

For field preparation, a 400 m^2 area was ploughed with a moldboard plough on 23 September 2016; afterwards, the land was levelled. To determine the selected physical and chemical properties of the soil, a composite soil sample was collected from 0-30 cm depth before the start of the experiment, and the results are presented in Table 2.

Table 2. Some **physical and chemical properties of soil in the experimental site**

Organic carbon	Organic matter	Total nitrogen	Phosphorous (mg.kg^{-1})	Electrical conductivity (dS m^{-1})	pH	Texture
	%					
0.234	0.4	0.02	3.8	3.95	7.98	Sandy loam

The corms used (Sarayan landrace) were free of fungal and mite infestation, physically undamaged, and those weighing 8-10 g were selected for planting. In each experimental plot (1.5 × 1.5 m), the corms were planted in seven rows spaced 20 cm apart, on 29 September 2016, at a depth of 15 cm in a bead-like arrangement, resulting in a planting density of approximately 100 corms m^{-2}. Immediately after planting, the plots were irrigated, and humic acid was applied to the corresponding plots. To facilitate flower emergence, five days after the first irrigation, the soil crust was broken to a depth of approximately 5 cm using a hand cultivator. Hand weeding was performed twice in 2017, in mid-March and early-April.

Measured parameters

Flowering traits were not measured in the first experimental year (autumn 2016) because irrigation treatments had not yet begun, and mycorrhiza and humic acid had been applied only a few days earlier, before flower emergence. Therefore, flowering was evaluated at the beginning of the second growing season (autumn 2017). Saffron flowering started approximately two weeks after the first autumn irrigation in 2017. The two side rows in each plot were considered as the marginal effect, and flowers were harvested from the five middle rows of each plot. The newly emerged flowers were hand-harvested each morning and transferred to the laboratory. After counting and weighing the flowers in each plot, the stigmas and styles were manually separated from the petals. The floral components were air-dried in the shade at room temperature, and at the end of the flowering period, their dry weight was determined using an analytical balance with 0.001 g precision. To measure the relative flowering rate, the equation proposed by Fallahi and Mahmoudi (2017) was used. The relative rate is usually lower than 1, but here we report it as a percentage by multiplying the relative flowering rate by 100 to show the percentage of flowers that appeared each day during the flowering period.

To determine the key quality indices of saffron stigmas, the method described by the Iranian National Standards Organization (INSO, Standard No. 259-2: 2013) was used. Initially, 500 mg of the ground saffron stigmas was weighed and transferred to a 1000 mL volumetric flask, to which approximately 900 mL of distilled water was added. The resulting solution was stirred in the dark using a magnetic stirrer at 1000 rpm for one hour. The flask was then filled to the calibration mark with distilled water, capped, and stirred to obtain a homogeneous solution. Next, 20 mL of this solution was pipetted into a 200 mL volumetric flask and diluted to the mark with distilled water. The resulting solution was stirred to be homogeneous and then quickly filtered, protected from light, to obtain a clear solution. The prepared solution was used to measure absorbance at 330 nm for safranal (aroma), 257 nm for picrocrocin (taste), and 440 nm for crocin (color), with distilled water as the blank. The content of the key quality compounds in the stigmas was calculated using the following equation, where D represents the absorbance of crocin, picrocrocin, or safranal, m is the sample mass in grams, and Wmv is the moisture content and volatile matter of the sample expressed as a percentage by weight (INSO, 2013).

$$A^{1\%}_{1cm}\,(\lambda max)\,\frac{D \times 10000}{m \times (100 - Wmw)} \qquad (1)$$

Statistical analysis

Prior to data analysis, the normality of the residuals was verified. Analysis of variance (ANOVA) was then performed to evaluate the effects of the factors and their interactions. Statistical analyses were conducted using SAS software, version 9.1. Mean comparisons were performed using Duncan's multiple range test at 5% level of probability ($p \leq 0.05$).

Results and Discussion

Relative flowering rate

The triple interaction of irrigation regimes, mycorrhizal inoculation, and humic acid application was significant on saffron relative flowering rate (RFR) (Table 3). Shorter irrigation intervals (20 days) combined with humic acid and *G. intraradices* inoculation showed the highest RFR, while *G. mosseae* inoculation had a tendency to reduce RFR regardless of humic acid level. With longer irrigation intervals (40 days), RFR improved with mycorrhizal inoculation, particularly with *G. intraradices*, mitigating water-stress effects. Under the 20-day irrigation regime, only the combined application of humic acid and *G. intraradices* enhanced RFR. In the 40-day irrigation regime without humic acid, only *G. intraradices* was effective; with humic acid application, both fungal species positively affected RFR (Table 4). This indicates that the synergistic application of mycorrhizal fungi and humic acid is more effective under water stress conditions, helping to improve RFR, likely due to enhanced nutrient uptake and stress alleviation mechanisms. The two mycorrhiza species had distinct effects on saffron RFR: only one accelerated flowering, while the other reduced RFR in most treatment combinations. This species-specific response is consistent with previous studies (Caser et al., 2019; Fallahi et al., 2021), which show that mycorrhizal effects depend on species. In addition, environmental conditions can even negatively affect the fungi's impact on plant growth under certain water and nutrient conditions (Wang et al., 2023). Such species/environmental-dependent behavior suggests that the effective species likely improved root function or substances signaling related to flowering, whereas the other species did not have such an effect. Overall, mycorrhizal fungi do not have a fixed, always beneficial effect on plants, and their impact varies depending on the species and the specific aspect of plant performance (growth vs. defense) (Wang et al., 2023).

Flower yield

The fresh flower yield of saffron was significantly affected by the triple interaction of experimental factors ($p<0.01$) (Table 3). The highest flower yield (79.63 $g.m^{-2}$) was obtained under the 20-day irrigation interval (4000 $m^3.ha^{-1}$, water), corm inoculation with *G. mossea* combined with humic acid application (Table 4). In a similar study, the application of 3600 $m^3.ha^{-1}$ water, combined with humic acid, improved saffron yield (Fallahi et al., 2021). Under the 20-day irrigation regime, the use of both mycorrhizal fungi species, especially *G. mosseae*, increased flower yield in both levels of humic acid. The beneficial effect was slightly higher when humic acid was applied (Table 4), indicating that the combined use of mycorrhiza and humic acid synergistically enhances flowering by improving nutrient uptake and plant growth conditions under this irrigation regime.

At the 40-day irrigation interval (2000 $m^3.ha^{-1}$, water), a higher fresh flower yield was observed without humic acid and no inoculation (74.73 $g.m^{-2}$). In the 40-day irrigation regime and without humic acid application, both mycorrhizal fungi species, especially *G. mosseae*, reduced flower yield. However, when humic acid was applied, both fungi, particularly *G. mosseae*, led to a relative increase in flower yield (Table 4). It seems that in the symbiotic relationships between mycorrhizal fungi and saffron plants under water-stress conditions and without humic acid application, the energy cost the plant invests in supporting the fungi often exceeds the benefit the fungi provide to the plant. However, with the application of humic acid, this interaction is modulated in favor of the plant, likely by improving nutrient availability and reducing the energy required to sustain the fungi. Thereby, the mutualistic balance becomes optimized in favor of the plant's growth and stress tolerance.

Overall, under sufficient water availability, both mycorrhizal species were beneficial regardless of humic acid application. However, under reduced water availability, mycorrhizal fungi were only beneficial when applied with humic acid (Table 4). This suggests that humic acid may play a crucial role in modulating the symbiotic relationship under water stress. The average saffron flower yield across the six treatments irrigated every 20 days was 71 $g.m^{-2}$, while in the six treatments irrigated every 40 days it averaged 69.3 $g.m^{-2}$, representing a 2.45% difference. This small difference indicates that, consistent with the findings of Koocheki et al. (2020), saffron exhibits a considerable capacity to tolerate deficit irrigation.

Flower components yield

The interaction effect of three experimental factors was significant on the dry yield of all flower components, including stigma, style, and petals (Table 3). The highest stigma yield (0.88 $g.m^{-2}$), style yield (0.22 $g.m^{-2}$), and petal yield (9.88 $g.m^{-2}$) were obtained with lower irrigation intervals (20-day), humic acid application, and inoculation with *G. mosseae* (Table 4). Under the 20-day irrigation regime, dry stigma yield increased significantly with *G. mosseae* inoculation at both levels of humic acid. In this level of irrigation, inoculation with *G. intraradices* was beneficial only with no-humic acid application. Under this irrigation level, *G. mosseae* increased petal yield at both humic acid levels, whereas *G. intraradices* reduced it at both humic acid levels. A similar trend was observed for style yield at this irrigation level, where only *G. mosseae* increased yield at both humic acid levels, particularly under humic acid application (Table 4). It has been reported that applying any mycorrhizal species does not necessarily enhance saffron growth or yield (Fallahi et al., 2021). In this context, research has shown that the type of inoculum (single or combined inoculation with mycorrhizal species) and environmental growth conditions can significantly influence the effectiveness of mycorrhizal fungi in saffron (Caser et al., 2019).

Under 40-day irrigation intervals, dry stigma yield was generally lower, and only *G. intraradices* increased stigma yield at both humic acid levels compared with the non-inoculated control. At this irrigation level, the application of humic acid slightly decreased stigma and style yield under all mycorrhizal inoculation treatments, but it slightly increased petal yield (Table 4). These results indicate that the synergistic use of mycorrhizal fungi and humic acid improves the dry matter production of saffron flower parts, particularly stigma and petals, under optimal irrigation conditions, while under water stress, benefits are limited or variable. In a similar study, the application of humic acid reduced root colonization by mycorrhizal fungi. This effect was attributed to the ability of humic acid to modify soil pH and nutrient availability, particularly phosphorus, which can reduce the plant's reliance on mycorrhiza for phosphorus uptake and thereby diminish the necessity for fungi colonization (Lahbouki et al., 2023).

Apocarotenoids content

The triple interaction of irrigation regime, humic acid application, and mycorrhizal inoculation had a significant effect on all measured quality traits of saffron stigma, including crocin, picrocrocin, and safranal (Table 5). The response patterns of the three compounds varied across different treatment combinations, indicating that the simultaneous management of water supply, soil bio-stimulants, and symbiotic fungi strongly affects saffron's secondary metabolite profile. Among all treatment combinations, the highest crocin content (274.50 units) was obtained with a 40-day irrigation interval when humic acid was applied with *G. mosseae* (Table 6). In the study by Fallahi et al. (2021), the application of humic acid increased stigma crocin content by 11%, a result consistent with those reported by Golzari Jahan Abadi et al. (2017). The use of organic and biological inputs is known to stimulate the production of plant hormones and water-soluble vitamins, which in turn may influence the biosynthesis of secondary metabolites (Heidari et al., 2014).

Table 3. The mean squares of traits associated with the reproductive growth of saffron as affected by mycorrhizal inoculation, humic acid application, and irrigation regimes

S.O.V	Df	Flowering rate	Fresh flower yield	Dry stigma yield	Dry petal yield	Dry style yield
Replication	2	0.121	0.64	0.005	2.71	0.00008
Irrigation (A)	1	0.804^{ns}	27.72^{*}	0.12^{**}	37.74^{**}	0.00022
Error a	2	0.814	12.5	0.004	2.42	0.0008
Humic acid (B)	1	1.224^{ns}	23.34^{*}	0.023^{*}	2.43^{*}	0.001002^{**}
A×B	1	1.246^{ns}	155.34^{**}	0.054^{**}	2.60^{*}	0.00266
Error b	4	0.125	5.50	0.006	0.38	0.00018
Mycorrhizal inoculation (C)	2	$^{**}10.819$	15.27^{ns}	0.0134^{*}	5.37^{**}	0.0006^{*}
A×C	2	18.203^{**}	1.62^{ns}	0.0073^{ns}	0.05^{ns}	0.0001^{ns}
B×C	2	4.659^{**}	88.40^{**}	0.0168^{*}	1.22^{ns}	0.0010^{**}
A×B×C	2	3.102^{**}	323.59^{**}	0.040^{**}	5.01^{**}	0.0015^{**}
Error c	16	0.47	4.92	0.003	0.52	0.0001
C.V (%)	-	4.74	3.15	8.25	9.31	6.57

ns: non-significant; * and **: significant at the 5% and 1% probability levels, respectively.

Table 4. The mean comparison of traits associated with the reproductive growth of saffron as affected by mycorrhizal inoculation, humic acid application, and irrigation regimes

Irrigation intervals (Days)	Humic acid ($kg.ha^{-1}$)	Mycorrhizal inoculation	Relative flowering rate (%)	Fresh flower yield ($g.m^{-2}$)	Dry stigma yield ($g.m^{-2}$)	Dry petal yield ($g.m^{-2}$)	Dry style yield ($g.m^{-2}$)
20	No-application	No-innoculation	15.86^{a}	61.55^{g}	0.66^{def}	5.52^{d}	0.17^{cde}
		G. mossea	12.29^{c}	76.83^{ab}	0.87^{ab}	6.97^{cd}	0.20^{ab}
		G. intradiaces	13.94^{b}	71.15^{cd}	0.74^{bcd}	5.14^{d}	0.17^{cde}
	Application	No-innoculation	15.53^{a}	63.63^{fg}	0.86^{ab}	8.87^{ab}	0.21^{ab}
		G. mossea	12.52^{c}	79.63^{a}	0.88^{a}	9.88^{a}	0.22^{a}
		G. intradiaces	16.27^{a}	73.20^{bc}	0.81^{abc}	6.92^{cd}	0.19^{bcd}
40	No-application	No-innoculation	14.20^{b}	74.73^{b}	0.70^{cde}	7.02^{cd}	0.21^{a}
		G. mossea	13.96^{b}	68.53^{de}	0.63^{efg}	8.01^{bc}	0.19^{bcd}
		G. intradiaces	15.94^{a}	73.45^{bc}	0.79^{abc}	8.49^{abc}	0.19^{bc}
	Application	No-innoculation	12.40^{c}	64.41^{fg}	0.56^{g}	9.25^{ab}	0.17^{de}
		G. mossea	15.74^{a}	68.09^{de}	0.57^{fg}	8.69^{ab}	0.16^{e}
		G. intradiaces	15.96^{a}	66.92^{ef}	0.63^{efg}	8.40^{abc}	0.17^{de}

In each column, the means that have at least one similar letter are not significantly different at the 5% probability level, based on Duncan's test.

Under the 20-day irrigation regime, the maximum crocin level (268.13 units) was recorded in the non-inoculated treatment with humic acid application. At this irrigation interval, humic acid enhanced crocin content in the absence of mycorrhizal inoculation; however, when both mycorrhizal species were applied, the non-application of humic acid resulted in higher crocin values (Table 6). Some studies have shown that applying humic acid can alter soil bacterial and fungal communities (Li et al., 2019). Accordingly, an increase in the population of certain microorganisms may deprive mycorrhizal fungi of nutrient resources, thereby reducing their effectiveness. Under no humic acid application, inoculation, particularly with *G. mosseae*, improved crocin content, whereas with humic acid application, inoculation with both species reduced crocin compared with the non-inoculated control (Table 6). At the 40-day irrigation interval, inoculation with *G. mosseae* increased crocin content at both humic acid levels, especially when humic acid was applied. Under this irrigation regime, humic acid application increased crocin content in both non-inoculated and G. mosseae-inoculated plants (Table 6). The mean crocin content across the six treatments for each irrigation regime at the 20- and 40-day irrigation intervals was 240.1 and 238 units, respectively (Table 6). This shows that even with a 50% reduction in water consumption (4000 vs. 2000 $m^3.ha^{-1}$), the stigma quality, as measured by crocin content, remained unchanged.

Among the 12 treatment combinations, the highest (124.50 units) and the lowest (96.43 units) picrocrocin contents were recorded under the 20-day irrigation interval with humic acid application in plants inoculated with *G. mosseae* and *G. intraradices*, respectively, representing a 29.1% difference. Under this irrigation regime, *G. mosseae* increased picrocrocin content at both humic acid levels, particularly when humic acid was applied, whereas *G. intraradices* was beneficial only in the absence of humic acid and showed a negative effect when humic acid was applied. Within the 20-day interval, humic acid was advantageous only when combined with *G. mosseae* (Table 6). Under the 40-day irrigation interval, the highest picrocrocin content (119.53 units) was obtained in the non-humic treatment inoculated with *G. mosseae*. At this level of water availability, *G. intraradices* reduced picrocrocin content at both humic acid levels. Moreover, humic acid application under no-inoculation increased picrocrocin content from 105.86 to 113.33 units, corresponding to a 6.7% increase, whereas its application under inoculation with mycorrhizal fungi had a negative effect (with *G. mosseae*) or only a slight positive effect (with *G. intraradices*) (Table 6). If a suitable mycorrhizal species is used for symbiosis with saffron, it is possible to increase its secondary metabolites. However, precisely interpreting the plant-fungus relationships is complicated (Fallahi et al., 2021). In inoculated plants, certain cytological changes occur, including an increase in the number of plastids and mitochondria. These changes activate the tricarboxylic acid cycle and plastid biosynthetic pathways, thereby enhancing the production of both primary and secondary metabolites. Additionally, the host plant exhibits increased photosynthetic activity, leading to greater synthesis of primary metabolites that serve as precursors for secondary metabolites (Pedone-Bonfim et al., 2015).

The mean picrocrocin value for the 20- and 40-day irrigation intervals was 102.43 and 109.26 units, respectively (Table 6). In a similar study, lower water availability was found to increase safranal and picrocrocin content (Fallahi et al., 2021). The increase in the proportion of certain secondary metabolites in saffron under controlled drought stress has been suggested as a potential mechanism for stress tolerance (Koocheki & Seyyedi, 2016).

Across all experimental treatments, safranal content of stigma ranged from 24.30 units (under the 40-day irrigation interval, no-mycorrhizal inoculation and no-humic acid application) to 35.80 units (under the 20-day irrigation interval with humic acid application and *G. mosseae* inoculation), representing a 47.3% difference between these two treatments (Table 6). The use of compatible mycorrhizal species can enhance stigma safranal content, whereas incompatible species may inhibit this trait (Caser et al., 2019; Fallahi et al., 2021). The biosynthesis of terpenoid compounds, such as safranal, is highly dependent on the availability of key nutrients, including nitrogen and phosphorus. Given the effective role of mycorrhizal fungi in improving plant access to essential nutrients, especially phosphorus, an increase in safranal content under inoculation with appropriate fungal species is fully expected (Alizadeh, 2018). The results of the present experiment also showed that *G. mosseae* increased soil available phosphorus content by about 77% (data not shown), whereas *G. intraradices* had no effect on improving the plant's access to phosphorus. Therefore, the improvement in stigma quality under *G. mosseae* inoculation may be due to enhanced nutrient availability to the plant.

Under the 20-day irrigation interval, *G. mosseae* increased safranal content at both humic acid levels, with the greatest enhancement observed when humic acid was applied. In contrast, the combined application of humic acid and *G. intraradices* reduced safranal and resulted in the lowest value among the six treatments of this irrigation regime (Table 6). At the 40-day irrigation interval, *G. mosseae*, particularly in the absence of humic acid, produced the highest safranal content among the treatments at this irrigation level. At this irrigation interval, *G. intraradices* inoculation was beneficial only in the absence of humic acid, whereas its combination with humic acid reduced safranal content (Table 6). This finding is consistent with reports by Thielicke et al. (2023), which show that mycorrhizal inoculation can, under certain conditions, shift from beneficial to detrimental. They hypothesized that if the naturally occurring soil microbiome is already optimal, introducing additional microorganisms via mycorrhiza cannot confer further benefits and could even reduce plant performance. They also stated that in nutrient-rich soils where plants have sufficient access to nutrients such as phosphorus, the mycorrhizal symbiosis cannot confer substantial advantages and, in some cases, may negatively affect yield or quality. In the present experiment, the combined application of mycorrhiza and humic acid under a 40-day irrigation interval was not beneficial. On average, the application of humic acid increased soil-soluble phosphorus content by about 66% compared with the control without humic acid. Consequently, leaf phosphorus content in the humic-acid treatment increased by 46% (data not shown). It is possible that in soils already rich in nutrients -particularly phosphorus- the carbon cost that the plant must allocate to the fungus (for maintenance and mycorrhizal activity) may outweigh the benefits. In addition, water deficit (a 40-day irrigation interval) may reduce the plant's ability to supply the carbon required by the fungus, potentially shifting the plant-fungus relationship toward temporary parasitism.

Table 5. The mean squares of traits associated with the stigma quality of saffron as affected by mycorrhizal inoculation, humic acid application, and irrigation regimes

S.O.V	Df	Crocin	Picrocrocin	Safranal
Replication	2	69.53	1.42	0.004
Irrigation (A)	1	35.80	9.81^{*}	5.21^{**}
Error a	2	18.59	4.95	0.01
Humic acid (B)	1	470.16^{**}	0.134^{ns}	9.10^{**}
A×B	1	751.67^{**}	2.667^{ns}	42.46^{**}
Error b	4	14.94	2.008	0.39
Mycorrhizal inoculation (C)	2	3234.66^{**}	254.92^{**}	98.06^{**}
A×C	2	1756.10^{**}	90.19^{**}	15.33^{**}
B×C	2	2503.37^{**}	545.58^{**}	79.39^{**}
A×B×C	2	153.93^{**}	204.37^{**}	0.85^{*}
Error c	16	21.43	1.90	0.17
C.V (%)	-	1.93	1.26	1.33

ns: non-significant; * and **: significant at the 5% and 1% probability levels, respectively.

Table 6. The mean comparison of traits associated with the quality of saffron stigma as affected by mycorrhizal inoculation, humic acid application, and irrigation regimes

Irrigation intervals (Days)	Humic acid (kg.ha^{-1})	Mycorrhizal inoculation	Crocin (Absorption of a 1% aqueous solution at 440 nm)	Picrocrocin (Absorption of a 1% aqueous solution at 257 nm)	Safranal (Absorption of a 1% aqueous solution at 330 nm)
20	No-application	No-innoculation	232.50^{e}	101.33^{g}	30.16^{d}
		G. mossea	254.86^{cd}	113.16^{c}	31.03^{c}
		G. intradiaces	235.86^{e}	111.16^{cd}	30.16^{d}
	Application	No-innoculation	268.13^{ab}	102.73^{fg}	34.20^{b}
		G. mossea	232.83^{e}	124.50^{a}	35.80^{a}
		G. intradiaces	216.46^{fg}	96.43^{h}	27.40^{f}
40	No-application	No-innoculation	204.16^{h}	105.86^{e}	24.30^{g}
		G. mossea	261.33^{bc}	119.53^{b}	35.43^{a}
		G. intradiaces	224.10^{f}	101.76^{g}	27.60^{f}
	Application	No-innoculation	248.93^{d}	113.33^{c}	33.90^{b}
		G. mossea	274.50^{a}	110.76^{d}	34.26^{b}
		G. intradiaces	215.43^{g}	104.33^{ef}	28.70^{e}

In each column, the means that have at least one similar letter are not significantly different at the 5% probability level, based on Duncan's test.

Conclusion

The integrated management of irrigation regime, mycorrhizal inoculation, and humic acid significantly influenced saffron flowering, yield, and stigma quality. *G. mosseae* enhanced flower and stigma production, as well as crocin, picrocrocin, and safranal contents, particularly under short irrigation intervals and humic acid application, while *G. intraradices* showed benefits mainly under no-humic conditions or specific water regimes. Humic acid improved metabolite accumulation mainly in non-inoculated plants and modulated plant–fungi interactions under water stress. Overall, the combination of *G. mosseae*, humic acid, and sufficient water availability was most effective for maximizing both yield and bioactive compound content, highlighting the importance of coordinated irrigation and bio-stimulant strategies in saffron cultivation. The effects of experimental factors on stigma yield and stigma quality characteristics were somewhat different. For example, *G. mossea* had a greater effect on crocin and safranal content in 40-day irrigation intervals, and the same species increased picrocrocin content in 20-day irrigation intervals. While *G. mossea* increased stigma yield at 20-day irrigation intervals, *G. intradiaces* increased stigma yield at 40-day irrigation intervals.

In this experiment, mycorrhiza was used simultaneously with the planting of saffron corms. To ensure the establishment of this microorganism in the soil and the initiation of the symbiotic relationship between the fungus and the plant, corm growth indicators were measured after about 6 months, and flowering indicators after about 1 year. After establishing in the soil, these microorganisms can persist for a long time and remain productive if various stress factors, such as non-host plants, the use of some chemical fertilizers and pesticides, etc., do not occur in the field. Humic acid was also used during the first growing season along with irrigation water. The beneficial effects of using this substance during the first growing season can affect the growth of saffron corms at the end of the first growing season, and since the flowering of the plant depends on the growth status of the corms in the previous growing season, it can also affect the flowering parameters at the beginning of the second growing season. However, in order to fully benefit from the benefits of this substance in subsequent years, it will be necessary to use it again in the soil. It is recommended that future studies evaluate the percentage of root colonization by mycorrhizal fungi, the effects of symbiosis on nutrient absorption, particularly phosphorus, and the dynamics of the soil microbiome across different climates.

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Conflict of interest

There is no conflict of interest to be declared.

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