The possibility for improvement of flowering, corm properties, bioactive compounds, and antioxidant activity in saffron (Crocus sativus L.) by different nutritional regimes

Ghanbari, J., Khajoei-Nejad, G., van Ruth, S. M., & Aghighi, S.

Please cite this publication as follows:

Ghanbari, J., Khajoei-Nejad, G., van Ruth, S. M., & Aghighi, S. (2019). The possibility for improvement of flowering, corm properties, bioactive compounds, and antioxidant activity in saffron (Crocus sativus L.) by different nutritional regimes. Industrial Crops and Products, 135, 301-310. https://doi.org/10.1016/j.indcrop.2019.04.064

The possibility for improvement of flowering, corm properties, bioactive compounds, and antioxidant activity in saffron (*Crocus sativus* L.) by different nutritional regimes

Jalal Ghanbari [a,b], Gholamreza Khajoei-Nejad [a,c,*], Saskia M. van Ruth [d,e], Sonia Aghighi [c]

[a] Department of Agronomy and Plant Breeding, Shahid Bahonar University, P.O. box 76169-133, Kerman, Iran

[*]Corresponding author: khajoei@uk.ac.ir

[b] Young Researchers Society, Shahid Bahonar University of Kerman, Kerman, Iran. jalalghanbari@agr.uk.ac.ir

[c] Research and Technology Institute of Plant Production (RTIPP), Shahid Bahonar University of Kerman, Kerman, Iran. aghighis@uk.ac.ir

[d] Food Quality and Design Group, Wageningen University and Research, P.O. box 17, 6700 AA Wageningen, The Netherlands. saskia.vanruth@wur.nl

[e] Institute for Global Food Security, School of Biological Sciences, Queen's University, Belfast, Northern Ireland, UK.

Abstract

Saffron as one of the most precious spices and medicinal plants, is highly valued for its bioactive compounds. Quantity and quality in spices and medicinal plants can be improved by the plant nutrition. In this field study the sole and integrated application of various fertilizers types and arbuscular-mycorrhizal fungus (AM), *Glomus mosseae* with respect to the flower-related traits, corm properties, quality, bioactive compounds and antioxidant activity of saffron at Kerman region, Iran was examined over a three years period. The fertilizer treatments comprised control (non-amended soil); 20 Mg ha^{-1} compost; 10 Mg ha^{-1} compost+ 8 Mg ha^{-1} biochar and chemical fertilizers. In each fertilizer treatment, planting bed was inoculated or non-inoculated with AM. The results showed that during the first flowering period (2015-16), neither AM nor fertilizer types affected flowering. Inoculation with AM particularly in the application of fertilizer treatments

through positive effects on different corm properties during the vegetative growing seasons of 2015-16 and 2017-18, improved flower-related traits in the next flowering periods of 2016-17 and 2017-18. Picrocrocin and safranal content as well as total phenolic content and total flavonoid content in tepals were considerably enhanced by organic amendments and chemical fertilizers compared with the control. While the total phenolic content in stigmata was reduced by AM-inoculation, the total flavonoid content and antioxidant activity of stigmata and tepals were not significantly influenced. Principal Component Analysis clearly discriminated the integrated nutritional treatments from the sole ones based on flower-related traits and corm properties which were positively related with integrated treatments. Organic amendments were characterized by a higher total phenolic content and antioxidant activity in stigmata. Chemical fertilizers alone or in AM-inoculation associated with quality attributes and total flavonoid content in tepals. Research findings confirmed that the integrated application of mycorrhizal fungus, organic, and chemical fertilizers significantly influences the overall production of saffron.

Keywords: Apo-carotenoids, Arbuscular-mycorrhizal fungus, Chemical fertilizer, Corm, Organic amendments, Saffron.

1. Introduction

Saffron (*Crocus sativus* L.) as a perennial crop is commonly grown in arid and semi-arid regions of Iran (Behzad et al., 1992a; Behnia et al., 1999; Kafi et al., 2002). The dried stigmata of saffron are considered as the world's most precious spice. It is commonly used as a seasoning, fragrant, flavouring, and colouring agent because of its unique colour, taste, and aroma (Gresta et al., 2008b; Melnyk et al., 2010; Zeka et al., 2015). In addition to the seasoning properties, saffron

has also been demonstrated to have different health benefits due to its bioactive compounds and antioxidant potential (Melnyk et al., 2010; Karimi et al., 2010; Baba et al., 2015).

Considering the biological and agricultural aspects of saffron such as the flowering in autumn, well adaptability to harsh-environmental conditions and low water and fertilizer requirements, this valuable plant has been introduced for low-input farming systems and sustainable agriculture (Gresta et al., 2008b). Moreover, saffron is considered as a particular crop for organic farming since mineral fertilizers and chemical weed control are usually not applied in growing areas (Siracusa et al., 2011).

Saffron is grown as a perennial crop, therefore maintaining long-term soil fertility is necessary to gain the high production and profitability per unit area. Saffron yield is highly affected by organic and chemical fertilization (Behzad et al., 1992a, b; Behnia et al., 1999; Jahan and Jahani, 2007; Koocheki and Seyyedi, 2015) and beneficial micro-organisms (Aimo et al., 2010). Carbon sequestration through increase in soil organic matter and improved nutrient retention capacity, along with the increment of under-ground biomass, can enhance saffron productivity (Husaini, 2014). Compost, biochar, and arbuscular-mycorrhizal fungi as well as different types of organic amendments are widely used for nutrition of organically grown crops to improve soil health, fertility, agronomic performance and increase agricultural productivity (Fischer and Glaser, 2012; Cavagnaro, 2015; Agegnehu et al., 2017).

Although the application of manure and composted manure is more common in saffron cultivation areas (Gresta et al., 2008b), chemical fertilizers, especially N fertilizers, have also led to significant increase in corm and stigmata yield (Behzad et al., 1992a, b). In order to compare organic and chemical fertilizer types, Koocheki and Seyyedi (2015) applied different types of fertilizers (cattle manure compost 25 Mg ha^{-1} and chemical fertilizer (N 300 kg ha^{-1} + P 100 kg ha^{-}

69 [1]). They examined nutrient use efficiency and yield of saffron and stated that applied organic and
70 chemical fertilizers increased the flower number, dry stigmata yield, and corm properties
71 significantly. However, the manure application was more efficient.

72 Biochar is a carbon-rich organic substance produced by pyrolysis from biomass under
73 minimal presence of oxygen. Biochar is generally beneficial to soil and enhances the availability
74 of nutrients and consequently improves crop growth and health (Schulz and Glaser, 2012;
75 Agegnehu et al., 2016). A number of studies show the combined application of compost and
76 biochar resulted in C sequestration, soil fertility, and improvement of the plant growth and yield,
77 compared with biochar, alone (Fischer and Glaser, 2012; Schulz and Glaser, 2012; Agegnehu et
78 al., 2016). For instance, Schulz and Glaser (2012) compared the effects of biochar, compost, and
79 inorganic fertilizers on infertile sandy soil and reported that the effects of compost (5% by weight)
80 and the combination of compost and biochar (2.5% + 2.5% by weight) on oat (*Avena sativa* L.)
81 plant growth and soil fertility in greenhouse production was significantly higher than biochar.
82 Agegnehu et al. (2015 and 2016) indicated that addition of organic treatments 10 Mg ha^{-1} biochar;
83 25 Mg ha^{-1} compost; 2.5 Mg ha^{-1} biochar + 25 Mg ha^{-1} compost on-site mixed, and 25 Mg ha^{-1} co-
84 composted biochar and compost along with fertilizers significantly improved plant nutrient
85 availability as well as peanut and maize yield.

86 Arbuscular mycorrhizal fungi have revealed a mutualistic symbiotic association with most of
87 the plant species which play a crucial role in host-plant growth and productivity via improving
88 nutrient acquisition (Cavagnaro, 2015). For instance, Aimo et al. (2010) investigated the saffron
89 growth inoculated with *Glomus* and revealed that inoculated soil significantly enhanced saffron
90 yield and corm diameter. Mycorrhizal fungi-inoculated ornamental plants have been reported to
91 stimulate flowering and increase the flower number (Perner et al., 2007; Gaur and Adholeya,

2005). Biomass and bulb dry weight in onion were also positively affected by inoculation with mycorrhiza (Charron et al., 2001; Mohamed et al., 2014).

Saffron quality depends fully on the content and composition of the metabolites responsible for red colour, bitter taste, and aroma (crocin, picrocrocin, and safranal, respectively). These traits are influenced by fertilization with organic (Rezaian and Paseban, 2006) and foliar application of (Rabani-Foroutagheh et al., 2013) fertilizers and inoculation with the beneficial microorganisms (Aimo et al., 2010).

In addition to the apo-carotenoids, some scientific studies have found that saffron stigmata and tepals contain other bioactive compounds such as phenolic and flavonoid compounds and also exhibit antioxidant activity (Karimi et al., 2010; Baba et al., 2015; Sánchez-Vioque et al., 2012; Zeka et al., 2015; Tuberoso et al., 2016). In particular tepals of saffron, as a major saffron by-product, are potentially new sources of phytochemical and bioactive compounds since they are usually discarded as useless floral bio-residue in saffron growing areas.

The current study was aimed to evaluate the effects of different organic and chemical fertilizers along with arbuscular-mycorrhizal fungi inoculation on the flowering, yield, quality, bioactive compound content, and antioxidant activity of saffron.

2. Materials and methods

2.1. Site description and experimental design

A field examination was established at the research field of Shahid Bahonar University (30.1440˚ N; 57.0715˚E and 1774m altitude) of Kerman, Iran, during three growing seasons of 2015-16 (first year), 2016-17 (second year), and 2017-18 (third year). Regional meteorological records (precipitation and air temperature data) of the research site was obtained from Meteorological Laboratory of Kerman, Iran (Fig. 1). Pre-planting composite soil samples were

115 randomly taken based on Estefan et al. (2013) from 10 points across the trial site from the depth
116 of 0-10 cm, sieved (2 mm mesh), air dried, and analysed. The soil texture was sandy-loam (55%
117 sand, 32.2% silt and 12.8% clay). The chemical characteristics of the soil are given in Table 1.
118 The experiment evaluated eight treatments comprising inoculated (+AM) and non-inoculated
119 (-AM) planting bed with mycorrhizal fungus (*G. mosseae*) with four different fertilizer treatments.
120 The latter included a control (C), cattle manure compost (COM) applied at 20 Mg ha^{-1}, cattle
121 manure compost + biochar (COM+B) applied at 10 + 8 Mg ha^{-1}, and chemical fertilizers (CF)
122 which involved the application of 250 kg urea and 66 kg triple superphosphate during the 2015-
123 16 to 2016-17 growing seasons. During the 2017-18, flower-related traits were measured and
124 measurements for the vegetative growth was not a part of the aims of this experiment, therefore
125 fertilizers didn't apply during this year.

126 *2.2. Compost, biochar, and mycorrhizal fungus inoculum*

127 Cattle manure compost was provided by the organic farm of Zahra Rosewater Co (EA11075),
128 Kerman, Iran. Biochar was purchased from Beshel Activated Carbon Industry,
129 Qaemshahr, Mazandaran, Iran. The preparation procedure of applied biochar was followed by the
130 method explained by Ghafourian (2016). Briefly, the feedstock (cellulosic raw materials of forest
131 biomass and wood wastes) converted to the biochar under anaerobic conditions as follows: (i)
132 evaporation of existing water of feedstock at 110°C for 2 hours; Increasing the temperature to
133 270°C for 4 hours for removing of volatile compounds; carbonization and formation of a porous
134 structure; (ii) nano-materials production at a temperature of 900-1050°C using 4 bar steam pressure
135 in a rotary kiln; (iii) finally, biochar was mixed with nanoporous carbon (9:1 w/w) in an electric
136 mixer for 20 minutes. Some chemical characteristics of compost and biochar are provided in Table

1. Commercially available mycorrhizal fungus (*Glomus mosseae*) inoculum was added to the planting rows in each +AM treatment (250 g m^{-2}; 120 spores g^{-1}) at planting time.

2.3. Experimental set-up

During the first growing season, after plowing and field leveling, the soil was amended with organic and chemical fertilizers and mixed into the upper 10 cm of the soil profile. Chemical fertilizers were applied five times for urea (10 December 2015; 4 March 2016; 8 October 2016; 7 December 2016, and 5 March 2017) in equal split doses of 50 kg urea ha^{-1} and for P (triple superphosphate) totally at a rate of 66 kg ha^{-1} at the beginning of the first two growing seasons.

Saffron mother corms (4-8 g weight) were planted manually in 10-15 cm depth at 50 corms m^{-2} density on 18 October 2015, in each experimental plot (14.4 m^2, 0.2 m row-spacing and 0.1 m corm-spacing). Daughter corms were maintained during the experiment to evaluate the annual increasing in flowering and corm properties and providing conditions exactly similar to the saffron fields for more valid recommendation for growing areas of saffron. First irrigation was applied immediately after planting. Irrigation was performed 5 times after first irrigation, on 1 November 2015 (to improve bud emergence), 10 December 2015 (after flowering), 26 January 2016, 4 March 2016 (after weeding), 16 April 2016 (supplementary irrigation) during the first growing season. In the second growing season, due to suitable rainfall distribution, a four interval irrigation was conducted: on 8 October 2016 (first irrigation), 7 December 2016 (after flowering), 5 March 2017 (after weeding and chemical fertilizers application), and on 9 April 2017, respectively. The first irrigation at the beginning of the third growing season was performed on 7 October 2017. Other agronomic practices were conducted uniformly for each plot during the growth period.

2.4. Plant sampling and measurements

159 Flowering of saffron started 23, 20, and 18 days after first irrigation in the first, second, and
160 third growing season, respectively. In all three-growing seasons, whole flowers per plot were
161 manually picked up daily after sunrise, during the flowering period. Stigmata were manually
162 separated from the flowers. Flower samples were air-dried in the shade afterwards and flower-
163 related traits including flower number (FN), flower dry weight (FDW), dry stigmata weight to
164 determine stigmata yield (SY), and stigma length (SL) (including the red parts) were measured
165 immediately by a Scale (AEL-40SM, Shimadzu, Japan; 10^{-5} g accuracy). Daughter corms were
166 harvested in a 1.8 m^{-2} for each nutritional treatment on 4 May 2016 and 20 April 2017, for the first
167 and second growing seasons, respectively. Corm-related properties such as corm number (CN),
168 unitary corm weight (UCW), and corm yield (CY) (on the basis of dry weight) were determined.
169 Dry stigmata were kept in the dark at room temperature until further analyses.

170 *2.5. Root mycorrhizal fungus colonization*

171 The percentage of mycorrhizal root colonization was determined based on the method
172 described by Phillips and Hayman (1970) with slight modification. Briefly, after washing root
173 samples with tap water and rinsing by distilled water, roots were cleared in KOH 10% (w/v) for 4
174 days at room temperature and thereafter acidified in 15% HCl for 5 minutes. Samples were then
175 stained 15 minutes with 0.05% trypan blue and finally de-stained and stored in lactoglycerol (1: 1:
176 1 glycerol, lactic acid, distilled water). Twenty-five randomly selected root segments (1-cm length)
177 were examined for root colonization intensity (%) according to the grid-line intersect method
178 (McGonigle et al., 1990).

179 *2.6. UV–vis spectrophotometry analysis*

180 Saffron's colour, bitter taste, and aroma strength were measured based on the ISO 3632 trade
181 standard (ISO/TS 3632, 2003). The absorbance of an aqueous extract ($E^{1\%}$ w/v) was read at 440,

257, and 330 nm, using a SPUV-26 UV/Vis spectrophotometer (SCO Tech, Germany) with a 1 cm cuvette. In each nutritional treatment and year, the expressed values of the crocin, picrocrocin, and safranal are the average values of three replications and two reads per replication. The results were expressed according to the following equation (Lage and Cantrell, 2009):

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

Where: D is the absorbance values at 257, 330, and 440 nm; m is the initial weight of the used sample (in grams); H is the mass fraction (moisture and volatile content of the sample).

2.7. Total phenolic and flavonoid contents

Extraction was performed using 250 mg of dried and ground samples of tepals and stigmata and 10 mL of 80% (v/v) methanol and shaken slowly (110 rpm) for 8 h. After filtration, the resulting extract was stored in the dark condition at a temperature of 4ºC until further analyses.

The Folin-Ciocalteu method was adapted for determination of total phenolic content (TPC) in the extract (Tohidi et al., 2017). In summary, 0.5 mL of each sample was mixed with 2.5 mL of diluted Folin-Ciocalteu's reagent (1:10) and incubated for 5 min at room temperature. Then, 2 mL of 7.5% sodium carbonate solution (w/v) was added. The mixture after shake incubated in a hot water bath at 45°C for 15 min. Finally, absorbance was measured at 765 nm using a SPUV-26 UV/Vis spectrophotometer (SCO Tech, Germany), against a blank. The results were expressed as mg of gallic acid equivalent (GAE)/g sample dry weight (DW).

The total flavonoid content (TFC) was assessed according to the aluminum chloride colorimetric assay described by Tohidi et al. (2017). Briefly, 0.5 mL of methanolic extract was mixed with 0.3 mL of 5% $NaNO_2$ solution. The mixture was incubated in the dark for 6 min at room temperature. Thereafter, 0.6 mL of 10% $AlCl_3$ was added and stored for 5 min. Afterward, 3 mL of NaOH 1M was added and the final volume was made to 10 mL with distilled water. The

absorbance was read at 510 nm after 15 min incubation. The TFC values were expressed as mg of quercetin equivalent (QE) per g DW.

2.8. DPPH radical scavenging activity

Methanolic DPPH solution 0.5 mM (1.5 mL) was added to 0.75 mL of prepared 50, 100, and 300 µg/ mL extract concentrations (Parejo et al., 2003). The same concentrations of ascorbic acid were used as a positive control. After 20 min of reaction, absorbance of the solution was measured at 517 nm against the 80% methanol as the blank. After calculation of the percentage of inhibition (Eq. 1), a linear regression model was established based on decolorization and sample extract concentration in order to determine the IC_{50} (50% inhibitory concentration).

$$\text{Inhibition (\%)} = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100 \quad (2)$$

Where A_{Sample} is the absorbance values of the plant extract/ascorbic acid and $A_{Control}$ is the absorbance values of the control.

2.9. Statistical analysis

Collected data were subjected to analysis of variance (ANOVA) followed by the Least Significant Difference test (LSD; $P<0.05$ probability level) using SAS software version 9.1 (SAS, Cary, NC, USA). Results were presented as mean values ± standard error (SE). Principal component analysis (PCA) was performed to determine the relationship between studied traits and nutritional treatments as well as to check for similarities among the nutritional treatments, using XLSTAT 2016 (Addinsoft, New York, NY, USA).

3. Results

3.1. Root mycorrhizal colonization

The root colonization rate was significantly affected by AM-inoculation, fertilizer types, and interaction effect of AM-inoculation and fertilizer types. In non-inoculation plots, colonization rate was not significantly altered by different fertilizer types while, application of compost and on-site mixed compost and biochar contributed to enhancement in root colonization rate, significantly in AM-inoculated plots (Fig. 2).

3.2. Flower-related traits

Significant fertilizer × mycorrhiza × year interaction was recorded in flower number (FN), flower dry weight (FDW), and stigmata yield (SY) (Table 2). All fertilizer treatments significantly increased the flower-related traits in the second and third year of the experiment when inoculated with AM whereas in the first year neither AM nor fertilizer types affected the flower-related traits significantly (Table 2). In the second and third year, the higher average of FDW and SY per m^2 were recorded in COM+AM and resulted from a considerably greater FN per m^2 (on average 1.23 and 4.33 g m^{-2}, 128.3 and 493.5 mg m^{-2}, and 33 and 116.1 FN m^{-2}, respectively). Saffron plants inoculated with AM especially when integrated with COM, COM+B, and CF showed higher flower-related traits compared with the control (e.g., for SY: 52, 66, 61, and 60% in AM, COM+AM, COM+B+AM, and CF+AM, respectively) and non-inoculated plants, resulted in higher performance in inoculated plots (Table 3). In non-inoculated treatments, differences in FN and SY traits were observed only in the third year (Table 2).

Opposed to the first year results which revealed no significant differences between the inoculated and non-inoculated plants, mycorrhizal fungus inoculation significantly increased all flower-related traits compared with non-inoculation in the second and third years (Tables 2 and 3). Inoculation with AM significantly increased SL (Table 3). In addition to the FN, SY, and FDW,

248 year induced a significant increase in SL by 14.3 and 24.3% for 2016-17 and 2017-18 compared
249 with the 2015-16, respectively (Table 3).

250 *3.3. Corm properties*

251 Fertilizer type × year interaction was statistically significant (P<0.01) for the corm number
252 (CN) m^{-2}. During the first year, although significant differences were observed for organic
253 amendments in comparison with no amended soil, the differences were more evident in 2016-17
254 when compost treatment produced significantly greater CN (more than 1000 corm m^{-2}), followed
255 by COM+B and CF on average 37.5, 26.6, and 8.8%, respectively compared with the control (Fig.
256 3).

257 The interaction effect of fertilizer types, mycorrhizal fungus inoculation and year on corm
258 yield of saffron is presented in Fig. 4. Results showed that CY responded differently to studied
259 experimental factors. Despite gaining the highest CN under application of compost in both years,
260 the highest CY (4.6 kg m^{-2}) were obtained from CF inoculated by AM at the end of the second
261 growing season (Fig. 4) mainly due to increase in unitary corm weight (UCW) (Table 3). Amended
262 plots with COM, COM+B, increased CY by about 60% compared with the control in AM-
263 inoculated plots (Fig. 4). Chemical fertilizers application significantly increased UCW by 25%
264 than to control while COM and COM+B did not show any significant differences with control due
265 to higher CN per unit area (Table 3).

266 Results of ANOVA showed that no significant interaction between AM-inoculation and year
267 (results not shown). As seen in Table 3, although no positive effect of AM inoculation was
268 observed in CN, through significant raise (24%) of UCW it showed enhanced CY per m^2 by 33%.
269 Moreover, UCW was increased by 36% in 2016-17, than 2015-17 (Table 3).

270 *3.4. Picrocrocin, safranal, and crocin contents*

Fertilizer types had a significant influence on the picrocrocin and safranal content while these quality aspects were not affected by mycorrhizal inoculation, years, and their interaction effects (Table 4). Picrocrocin content ranged between 68 and 93. The results generally indicated that picrocrocin content was significantly higher in CF that in COM, COM+B, and control. There was no statistically significant difference between the organically grown stigmata and control with respect to the picrocrocin content (Table 4).

Table 4 shows that significant increases (13-15%) in safranal content were found for organic and chemical fertilizer treatments as compared with the control. Crocin content ranged from 218-219 across experimental treatments that resulted in non-statistically significant differences between experimental factors (Table 4).

3.5. TPC, TFC, and antioxidant activity

Results revealed that total phenolic content of stigmata was significantly affected by mycorrhizal inoculation × fertilizer type and mycorrhizal inoculation × year interaction effects (Fig. 5). Inoculation with mycorrhiza significantly decreased TPC by 13, 11, and 6.5 percent for COM, COM+B, and CF, respectively. Inoculation of unamended plots didn't affect significantly the TPC (Fig. 5A). Generally, TPC of stigmata was higher in control compared with COM+B and CF in which a reduction of 12% and 23% respectively (Table 5). Whilst inoculation had no effect on TPC in 2016-17, reduced TPC (19%) in 2017-18, significantly (Fig. 5B) which resulted in significant decrease of TPC by 9% in 2017-18 and 8.5% in inoculated plants (Table 5).

Stigmata TFC varied with different fertilizer treatments, year as well as their interaction at $P \leq 0.01$ (Table 5). The highest value of TFC was observed in fertilizer treatments in 2016-17 while in 2017-18, COM+B and control were higher. The maximum mean value of TFC in stigmata was observed for the COM+B treatment (Table 5). Except for the control condition which showed no

294 difference for both growing seasons, TFC was significantly higher (21%) in the first growing
295 season in fertilizer treatments than in the second year (see Table 5).

296 Significant fertilizer type × year interaction (P<0.05) was found for TPC, TFC, and
297 antioxidant activity of tepals extract (Table 5). Whereas, there was no significant difference in IC_{50}
298 mean values in the 2017-18, fertilizer treatments led to an increase in antioxidant activity compared
299 with control in 2016-17 (Table 5). This trend was similar for TFC in tepals. Total flavonoid content
300 was not affected by amending the fertilizers in 2017-18 while chemical fertilizer treatment resulted
301 in higher TFC levels in 2016-17 (Table 5). Organic and chemical fertilizer treatments in spite of
302 improving the TPC in 2016-17, showed no significant difference compared with control in 2017-
303 18 (except for CF that showed significantly lower TPC compared with control) (Table 5). In
304 general, improving effects of fertilizer treatments were observed on TPC and TFC of tepals and
305 stigmata.

306 Stigmata and tepals IC_{50} were not statistically different between the fertilizer types, AM-
307 inoculation, nor was the interaction effect (Table 5). Furthermore, fertilizer types did not influence
308 scavenging activity which might be due to variation in TPC and TFC in fertilizer treatments in
309 interaction with other factors such as years and mycorrhizal inoculation (Table 5 and Fig. 5).
310 Nonetheless, antioxidant ability was significantly affected by the growing season (Table 5). IC_{50}
311 values for stigmata and tepals in 2017-18 were, approximately 10% and 18% higher than in 2016-
312 17, respectively. The higher amounts of TPC and TFC in 2016-17 is possibly the reason for the
313 differences between years (Table 5).

314 *3.6. Principal component analysis*

315 Standardized data were subjected to PCA to clarify the relationship between the studied
316 attributes and nutritional treatments. A two-dimensional PCA scatter plot (based on two first PCs)

was constructed (Fig. 6). The two first PCs comprised 74% of the total variance. As depicted, the first PC explained 51% of the total variation and is positively and strongly correlated with flower and corm related traits as well as safranal content. High positive scores in the first dimension were observed for integration treatment of fertilizers and mycorrhizal fungus inoculation. They represent a high agronomic performance area. The first PC was negatively correlated with TPC and antioxidant activity in stigmata. Compost and compost+ biochar was characterized by higher TPC and antioxidant activity in stigmata. Therefore, PC1 discriminated the integrated nutritional treatments from non-integrated, on the basis of most of the traits. The second PC, which explained 23% of the variance, was positively and strongly correlated with tepals TFC as well as picrocrocin and crocin contents. Treatments in this area (CF and CF+AM) present the highest apo-carotenoids contents and tepals TFC (Tables 4 and 5).

4. Discussion

Saffron roots were colonized to a higher level in AM-inoculation plots under organic nutritional treatments (Fig. 2) which can be due to different properties as well as slow rates of nutrient mineralization of organic amendments (Cavagnaro, 2015; Lehmann et al., 2011). These results are supported by literature which documented that root colonization rate can be positively affected by different types of organic amendments, biochar (Blackwell et al., 2010; Vanek and Lehmann, 2015) and compost (Roldán et al., 2006; Cavagnaro, 2015).

This experiment clearly showed that the highest variations in flower-related traits and corm properties in saffron were found among the growing seasons. During the corms-formation at first growing season of saffron, new daughter corms will be produced and grow and thereby caused an increment in flower production in the next year (Gresta et al., 2008a; de Juan et al., 2009). In the first growing season, flower-related traits were not affected by fertilizer types and AM-inoculation

(Tables 2 and 3). Flowering occurs in saffron before vegetative growth or with leaf emergence simultaneously, in Iranian cultivation areas (Kafi et al., 2002). At this time, the vegetative growth and new corm production have not yet been started. Accordingly, growth and corm formation have not affected by different nutritional treatments. Therefore, any difference can be due to the variation in the mother corms behaviour. According to the obtained results, application of organic amendments and chemical fertilizers in this study had a positive effect on the flower production and yield of saffron in the second and third years, especially when incorporated together with mycorrhiza (Table 3). Such results can be due to the positive effects of the nutritional regimes on growth and daughter corms development during the vegetative growing seasons of 2015-16 and 2017-18 (Figs. 3 and 4). The application of farmyard manure has been reported to enhance the saffron stigmata yield in comparison with chemical fertilizer (Jahan and Jahani, 2007; Koocheki and Seyyedi, 2015). The increase in saffron yield by application of chemical fertilizers has been also reported (Behzad et al., 1992a, b; Behnia et al., 1999).

While quality attributes were not affected by the years, the highest antioxidant ability was measured in 2016-17 as revealed by DPPH-radical scavenging activity. This can be mainly due to a higher amount of total phenolic and flavonoid compounds (Table 5).

Results indicated an increase of SY by 111% in 2016-17 and 78% in 2017-18 in AM-inoculation alone (AM) while AM-inoculation in COM, COM+B, and CF fertilizer treatments induced increase of SY by 194, 154, and 152% in 2016-17 and 228, 188, and 169% in 2017-18, respectively, compared with the control (Table 2). This improvement in flower-related traits could be explained by the improvement of corm properties at the end of the previous years (e.g. for CY: 21, 68, 51, and 50% in the end of 2015-16 and 9, 60, 57, and 83% in the end of 2016-17, for AM, COM, COM+B, and CF compared with control, respectively) (Fig. 4). Higher CN, UCW, and

363 thereby CY resulted in higher flower production that increases FDW and SY per unit of the surface
364 as discussed above. These relationships also clearly explained by PCA analysis (Fig. 6). It has
365 been reported that flower production in saffron was increased by 168% in the inoculated with M,
366 compared with non-inoculated (Aimo et al., 2010).

367 Synergistic effects of AM fungi and organic amendments as well as chemical fertilizers on
368 the flower and corm related traits of saffron have not been well investigated. Some studies have
369 reported improvement of plant performance in combined either application of organic amendments
370 or chemical fertilizers and AM-inoculation. Improvement of lettuce yield with combined
371 application of biochar and mycorrhiza fungi compared with each individually has been
372 documented by Hammer et al. (2015). Organic amendment (cattle manure) together with *Glomus*
373 *intraradices* also resulted in higher yield in tobacco (Wang et al., 2012). Furthermore, it has also
374 been reported that N fertilizer enhances root and shoot biomass of alfalfa (*Medicago sativa*) plants
375 inoculated with different *Glomus* species (Liu et al., 2017). Similarly, another study reported that
376 the dry weight of onion bulbs and maize were obtained from NPK and NK respectively when
377 inoculated with mycorrhizal fungi (Mohamed et al., 2014).

378 The response in flower related traits depend mainly on FN m^2 (Tables 2 and 3), therefore FN
379 is arguably the most important trait which directly affects FDW and SY m^2. Principal component
380 analysis (Fig. 6) points out that the strongest positive linear relationship was observed between FN
381 and SY which confirms the above results. Similar relationships have been documented by Gresta
382 et al. (2009).

383 Improvement in FN and therefore FDW and SY of saffron in integrated nutritional treatments
384 are directly related to the positive effects of organic amendments on saffron CN as well as AM-
385 inoculation on UCW which produces more CY (Table 3 and Figs. 3 and 4). Corm yield per unit

area involves multiplying the number of corms and unitary corm weight. Fertilizer treatment and AM-inoculation positively affected CN per area unit and UCW, respectively, supporting the CY improvement (Table 3 and Figs. 3 and 4). Compost and compost + biochar gave the highest CN and CY in the first year whereas, in the second year, maximum CY was related to CF due to increasing the UCW. In the first year, both physical and nutritional properties of compost resulted in increasing the number and growth of corms. In the second year split and adequate values of chemical fertilizers although were not led to the maximum CN but with a rise in UCW, increased CY per unit area (Table 3 and Figs. 3 and 4). Higher CN in organic amendments is related to improvements in soil physical properties which led to significant improvements in buds emergence and early growth of saffron (Ghanbari and Khajoei-Nejad, 2017). Since buds are meristematic points which produce new replacement corms, the enhancement of bud emergence of saffron mother corm leads to more number of replacement corms (Behdani et al., 2016). Furthermore, AM-inoculation influenced significantly (24%) the UCW in both years of the experiment caused a greater CY by 33%, as compared with the non-inoculation (Table 3). Aimo et al. (2010) stated that a significant increase in the diameter of the saffron corm in inoculation with mycorrhizal fungi (genus *Glomus*). Similar results were also reported in inoculated bulbs of onion plants (Charron et al., 2001; Mohamed et al., 2014).

Saffron quality greatly depends on the variety, growing conditions and nutritional status (Lage and Cantrell, 2009; Gresta et al., 2009; Siracusa et al., 2011; Rezaian and Paseban, 2006; Rabani-Foroutagheh et al., 2013). The average values for picrocrocin, safranal, and crocin were in the range of 68-93, 39-45, and 217-219 (category I), respectively (Table 4). These ranges are similar to other studies. For instance, the values of picrocrocin, safranal, and crocin in saffron stigmata were reported between 52-78, 36-50, and 117-350, respectively, depending on corm provenances

409 and environmental conditions (Gresta et al., 2009; Lage and Cantrell, 2009). In another study
410 picrocrocin, safranal, and crocin values of saffron in different fertilizer treatments were between
411 65-69, 38-42, and 161-178 (category II) (Rezaian and Paseban, 2006).

412 Except for a few number of studies, the roles of organic and chemical plant nutrition on saffron
413 quality have not been very well studied. According to the current results, fertilizer types improved
414 the picrocrocin and safranal content but did not cause any significant effect on crocin content in
415 saffron stigmata (Table 4). These observations are suggesting that nutritional status in parallel with
416 increased plant growth, play an important role in the improvement of saffron quality. Rezaian and
417 Paseban (2006) reported that crocin and picrocrocin contents increased by application of 25 Mg
418 ha^{-1} cow manure compared with the control while the safranal concentration was decreased.
419 According to the findings of Rabani-Foroutagheh et al. (2013), the crocins content of saffron can
420 be improved by bio-fertilizers but safranal and picrocrocin contents were decreased with
421 application of bio-fertilizers and concluded that crocin increase could be due to higher elements
422 amounts of bio-fertilizers. It seems that changes in apo-carotenoids content in saffron in addition
423 to the fertilizer type, depended on other factors in each experiment.

424 Total phenolic content ranged in this study between 2.5-5.7 and 4-6 mg GAE/g DW in tepals
425 and stigmata, respectively (Table 5). Total flavonoid content ranged between 0.48-1.3 and 2.9-4.5
426 mg QE/g DW in tepals and stigmata, respectively. Values of IC_{50} varied from 223 to 271 and 235
427 to 263 µg/ mL for tepals and stigmata, respectively (Table 5). These ranges are similar to other
428 studies conducted regarding saffron tepal (Sánchez-Vioque et al., 2012; Tuberoso et al., 2016) and
429 stigmata (Karimi et al., 2010; Baba et al., 2015).

430 AM-inoculation reduced the TPC in stigmata in different fertilizer treatments, as well as in
431 2017-18 compared with 2016-17 (Fig. 5). Additionally, all the agronomic-related traits negatively

correlated with TPC, denoting that an increase in corm yield and FN, SY, and SL negatively affected the TPC (Fig. 6). These relationships might be due to the dilution effect (Onofrei et al., 2017). The increase in FN, SY, and SL in inoculated plots led to reduction of bioactive compounds per unit weight. Principal component analysis (Fig. 6) clearly confirmed the negative relationships between flower-related traits and bioactive compounds particularly with TPC in stigmata. Onofrei et al. (2017) reported that addition of nitrogen-based fertilizers due to increase in plant growth cause a decrease in phenolic compounds in *Calendula officinalis* L.

Strong fertilizer type × year variation was uncovered in TFC in stigmata which indicated the different effects of the fertilizer treatments in studied years. These results finally led to significantly higher TFC in COM+B as well as in 2016-17 compared with 2017-18 (Table 5). In tepals, the highest TPC and TFC were reached in organic amendments and chemical fertilizers, respectively, in 2016-17 while in 2017-18, bioactive compounds were not influenced by the fertilization (Table 5). Likewise, as a result of these trends, antioxidant activity in tepals was only improved by the fertilization in 2016-17, while no effect was observed for 2017-18 (Table 5). Principal component analysis results established positive and direct associations between bioactive compounds and antioxidant activity (Fig. 6). Therefore, the results confirmed that the antioxidant activity in saffron tepals and stigmata may be influenced by the bioactive compounds. Positive relationships between total phenolic and flavonoid contents with antioxidant potential are well documented (Karimi et al., 2010; Zeka et al., 2015; Baba et al., 2015).

In the current study distinct effects of organic and chemical fertilizers in combination with AM-inoculation on the levels of bioactive compounds and antioxidant activity levels in saffron were determined. In some other industrial and medicinal plants, the effects of different types of fertilizers have been studied on bioactive compounds and antioxidant activity. For instance, the

application of different organic and chemical fertilizers alone and in combination resulted in higher antioxidant activity in *Pelargonium graveolens* L'Hér. leaf extracts (Pandey and Patra, 2015). Pandey et al. (2016) reported a similar finding on the antioxidant activity in basil (*Ocimum basilicum* L.). Similarly, Emami Bistgani et al. (2018) reported that antioxidant activity in *Thymus daenensis* improved by different types of organic amendments and chemical fertilizers. In another study conducted by Onofrei et al. (2017), the highest of total phenolic, total flavonoid contents and antioxidant activity were obtained in plants fertilized by different ecological foliar fertilizers depending on the harvest date. Oloyede et al. (2014) recommended the application of a moderate rate of chemical fertilizers (135-180 kg ha^{-1} NPK) than those that higher supplied for producing the highest amounts of total phenolic and total flavonoid contents which provided optimal antioxidant activity in pumpkin fruit.

5. Conclusion

Obtained data clearly point out that variations on almost all saffron flower and corm parameters were observed among the years. Results also demonstrated that saffron stigmata and corm yield are strongly affected by AM-inoculation particularly when combined with organic amendments and chemical fertilizers. Integrated fertilization treatments through improving plant growth and daughter corm development in the first year had a significant positive effect on flower number and thereby stigmata yield in the following years. Moreover, organic and chemical fertilizer application positively influenced the aroma and flavour indicators of saffron. Furthermore, the treatments can be partly effective in improving the production of bioactive components in saffron particularly in tepals. The PCA based on the flower-related traits and corm characteristics provided criteria to discriminate amongst sole and integrated nutritional treatments

477 depending on the required improvements. These approaches will help to enhance the production
478 of saffron.

479 **Acknowledgments**

480 This research was financially supported by a grant (No: P900/106) from the Research and
481 Technology Institute of Plant Production (RTIPP), Shahid Bahonar University of Kerman,
482 Kerman, Iran. Authors are thankful to the Zahra Rosewater Company for providing organic
483 compost.

484 **References**

485 Agegnehu, G., Bass, A.M., Nelson, P.N., Bird, M.I., 2016. Benefits of biochar, compost and
486 biochar -compost for soil quality, maize yield and greenhouse gas emissions in a tropical
487 agricultural soil. Sci. Total Environ. 543, 295-306.
488 https://doi.org/10.1016/j.scitotenv.2015.11.054
489 Agegnehu, G., Bass, A.M., Nelson, P.N., Muirhead, B., Wright, G., Bird, M.I., 2015. Biochar and
490 biochar-compost as soil amendments: Effects on peanut yield, soil properties and greenhouse
491 gas emissions in tropical North Queensland, Australia. Agric. Ecosyst. Environ. 213, 72-85.
492 https://doi.org/10.1016/j.agee.2015.07.027
493 Agegnehu, G., Srivastava, A.K., Bird, M.I., 2017. The role of biochar and biochar-compost in
494 improving soil quality and crop performance: A review. Appl. Soil Ecol. 119, 156-170.
495 https://doi.org/10.1016/j.apsoil.2017.06.008
496 Aimo, S., Gosetti, F., D'Agostino, G., Gamalero, E., Gianotti, V., Bottaro, M., Gennaro, M.C.,
497 Berta, G., 2010. Use of arbuscular mycorrhizal fungi and beneficial soil bacteria to improve

498 yield and quality of saffron (*Crocus sativus* L.). Acta Hortic. 850, 159-164.
499 https://doi.org/10.17660/ActaHortic.2010.850.25
500 Baba, S.A., Malik, A.H., Wani, Z.A., Mohiuddin, T., Shah, Z., Abbas, N., Ashraf, N., 2015.
501 Phytochemical analysis and antioxidant activity of different tissue types of *Crocus sativus* and
502 oxidative stress alleviating potential of saffron extract in plants, bacteria, and yeast. S. Afr. J.
503 Bot. 99, 80-87. https://doi.org/10.1016/j.sajb.2015.03.194
504 Behdani, M.A., Jami Al-Ahmadi, M., Fallahi, H.R., 2016. Biomass partitioning during the life
505 cycle of saffron (*Crocus sativus* L.) using regression models. J. Crop Sci. Biotech. 19 (1), 71-
506 76. https://doi.org/10.1007/s12892-015-0082-0
507 Behnia, M.R., Estilai, A., Ehdaie, B., 1999. Application of Fertilizers for Increased Saffron Yield.
508 J. Agron. Crop Sci. 182, 9-15. https://doi.org/10.1046/j.1439-037x.1999.00274.x
509 Behzad, S., Razavi, M., Mahajeri, M., 1992a. The effect of mineral nutrients (N.P.K.) on saffron
510 production. Acta Hortic. 306, 426-430. https://doi.org/10.17660/ActaHortic.1992.306.56
511 Behzad, S., Razavi, M., Mahajeri, M., 1992b. The effect of various amount of ammonium
512 phosphate and urea on saffron production. Acta Hortic. 306, 337-339.
513 https://doi.org/10.17660/ActaHortic.1992.306.44
514 Blackwell, P., Krull, E., Butler, G., Herbert, A., Solaiman, Z., 2010. Effect of banded biochar on
515 dryland wheat production and fertiliser use in south-western Australia: an agronomic and
516 economic perspective. Aust. J. Soil Res. 48, 531-545. https://doi.org/10.1071/SR10014
517 Cavagnaro, T.R., 2015. Biologically Regulated Nutrient Supply Systems: Compost
518 and Arbuscular Mycorrhizas-A Review. Adv. Agron. 129, 293-321.
519 https://doi.org/10.1016/bs.agron.2014.09.005

520 Charron, G., Furlan, V., Bernier-Cardou, M., Doyon, G., 2001. Response of onion plants to
521 arbuscular mycorrhizae 2. Effects of nitrogen fertilization on biomass and bulb firmness.
522 Mycorrhiza 11, 145-150. https://doi.org/10.1007/s005720100122
523 de Juan, J.A., Córcoles, H.L., Muñoz, R.M., Picornell, M.R., 2009. Yield and yield components
524 of saffron under different cropping systems. Ind. Crop Prod. 30, 212-219.
525 https://doi.org/10.1016/j.indcrop.2009.03.011
526 Emami Bistgani, Z., Siadat, S.A., Bakhshandeh, A., Ghasemi Pirbalouti, A., Hashemi, M., Maggi,
527 F., Morshedloo, M.R., 2018. Application of combined fertilizers improves biomass, essential
528 oil yield, aroma profile, and antioxidant properties of *Thymus daenensis* Celak. Ind. Crop Prod.
529 121, 434-440. https://doi.org/10.1016/j.indcrop.2018.05.048
530 Estefan, G., Sommer, R., Ryan, J., 2013. Methods of Soil, Plant, and Water Analysis: A manual
531 for the West Asia and North Africa region, third ed. International Center for Agricultural
532 Research in the Dry Areas, Beirut, Lebanon.
533 Fischer, D., Glaser, B., 2012. Synergisms between compost and biochar for sustainable soil
534 amelioration, in: Sunil, K., Bharti, A. (Eds.), Management of organic waste. InTech, Rijeka,
535 Croatia, pp. 167-198. https://doi.org/10.5772/31200
536 Gaur, A., Adholeya, A., 2005. Diverse response of five ornamental plant species to mixed
537 indigenous and single isolate arbuscular mycorrhizal inocula in marginal soil amended with
538 organic matter. J. Plant Nutr. 28:707-723. https://doi.org/10.1081/PLN-200052647
539 Ghafourian, H., 2016. Nano-Biochar, Biochar, Charcoal & Coal Based Biochar, Design of
540 Pyrolysis Reactors Production Methods & Application, first ed. Amazon Kindle, Pp. 116.
541 Ghanbari, J., Khajoei-Nejad, G.R., 2018. Effect of compost and combination of compost and
542 biochar application on soil bulk density of planting bed and seedling emergence rate and early

growth of saffron ecotypes. J. Saffron Agron. Tech. 19, 17-33. https://doi.org/10.22048/JSAT.2017.57414.1178

Gresta, F., Avola, G., Lombardo, G.M., Siracusa, L., Ruberto, G., 2009. Analysis of flowering, stigmas yield and qualitative traits of saffron (*Crocus sativus* L.) as affected by environmental conditions. Sci. Hortic. 119, 320-324. https://doi.org/10.1016/j.scienta.2008.08.008

Gresta, F., Lombardo, G.M., Siracusa, L., Ruberto, G., 2008a. Effect of mother corm dimension and sowing time on stigma yield, daughter corms and qualitative aspects of saffron (*Crocus sativus* L.) in a Mediterranean environment. J. Sci. Food Agric. 88, 1144-1150. https://doi.org/10.1002/jsfa.3177

Gresta, F., Lombardo, G.M., Siracusa, L., Ruberto, G., 2008b. Saffron, an alternative crop for sustainable agricultural systems. A review. Agron. Sustain. Dev. 28, 95-112. https://doi.org/10.1051/agro:2007030

Hammer, E.C., Forstreuter, M., Rillig, M.C., Kohler, J., 2015. Biochar increases arbuscular mycorrhizal plant growth enhancement and ameliorates salinity stress. Appl. Soil Ecol. 96, 114-121. https://doi.org/10.1016/j.apsoil.2015.07.014

Husaini, A.M., 2014. Challenges of climate change: Omics-based biology of saffron plants and organic agricultural biotechnology for sustainable saffron production. GM Crops Food. 5(2), 97-105. https://doi.org/10.4161/gmcr.29436

ISO/TS 3632-1/2., 2003. Technical Specification. *Crocus sativus* L. Saffron. Ed. ISO, Geneva, Switzerland.

Jahan, M., Jahani, M., 2007. The Effects of Chemical and Organic Fertilizers on Saffron Flowering. Acta Hortic. 739, 81-86. https://doi.org/10.17660/ActaHortic.2007.739.9

565 Kafi, M., Koocheki, A., Molafilabi, M., 2002. Saffron Production and Processing. Ferdowsi
566 University of Mashhad Press, Iran.
567 Karimi, E., Oskoueian, E., Hendra, R., Jaafar, H.Z.E., 2010. Evaluation of *Crocus sativus* L.
568 stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules 15, 6244-
569 6256. https://doi.org/10.3390/molecules15096244
570 Lage, M., Cantrell, C.L., 2009. Quantification of saffron (*Crocus sativus* L.) metabolites crocins,
571 picrocrocin and safranal for quality determination of the spice grown under different
572 environmental Moroccan conditions. Sci. Hortic. 121, 366-373.
573 https://doi.org/10.1016/j.scienta.2009.02.017
574 Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar
575 effects on soil biota—a review. Soil Biol. Biochem. 43, 1812-1836.
576 https://doi.org/10.1016/j.soilbio.2011.04.022
577 Liu, M., Sun, J., Li, Y., Xiao, Y., 2017. Nitrogen fertilizer enhances growth and nutrient uptake of
578 *Medicago sativa* inoculated with *Glomus tortuosum* grown in Cd-contaminated acidic soil.
579 Chemosphere 167, 204-211. https://doi.org/10.1016/j.chemosphere.2016.09.145
580 McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method
581 which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal
582 fungi. New Phytol. 115, 495-501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x
583 Melnyk, J.P., Wang, S., Marcone, M.F., 2010. Chemical and biological properties of the world's
584 most expensive spice: Saffron. Food Res. Int. 43, 1981-1989.
585 https://doi.org/10.1016/j.foodres.2010.07.033
586 Mohamed, A.A., Eweda, W.E.E., Heggo, A.M., Hassan, E.A., 2014. Effect of dual inoculation
587 with arbuscular mycorrhizal fungi and sulphur-oxidising bacteria on onion (*Allium cepa* L.)

588 and maize (*Zea mays* L.) grown in sandy soil under greenhouse conditions. Ann. Agric Sci.
589 59(1), 109-118. https://doi.org/10.1016/j.aoas.2014.06.015
590 Oloyede, F.M., Adebooye, O.C., Obuotor, E.M., 2014. Planting date and fertilizer affect
591 antioxidants in pumpkin fruit. Sci. Hortic. 168, 46-50.
592 https://doi.org/10.1016/j.scienta.2014.01.012
593 Onofrei, V., Teliban, G., Burducea, M., Lobiuc, A., Sandu, C.B., Tocai, M., Robu, T., 2017.
594 Organic foliar fertilization increases polyphenol content of *Calendula officinalis* L. Ind. Crop
595 Prod. 109, 509-513. https://doi.org/10.1016/j.indcrop.2017.08.055
596 Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Saavedra, G., Murcia, M.A., Jiménezd,
597 A.M., Codina, C., 2003. Investigation of Bolivian plant extracts for their radical scavenging
598 activity and antioxidant activity. Life Sci. 73, 1667-1681. https://doi.org/10.1016/S0024-
599 3205(03)00488-0
600 Pandey, V., Patel, A., Patra, D.D., 2016. Integrated nutrient regimes ameliorate crop productivity,
601 nutritive value, antioxidant activity and volatiles in basil (*Ocimum basilicum* L.). Ind. Crop
602 Prod. 87, 124-131. https://doi.org/10.1016/j.indcrop.2016.04.035
603 Pandey, V., Patra, D.D., 2015. Crop productivity, aroma profile and antioxidant activity in
604 *Pelargonium graveolens* L'Hér. under integrated supply of various organic and chemical
605 fertilizers. Ind. Crop Prod. 67, 257-263. https://doi.org/10.1016/j.indcrop.2015.01.042
606 Perner, H., Schwarz, D., Bruns, C., Mäder, P., George, E., 2007. Effect of arbuscular mycorrhizal
607 colonization and two levels of compost supply on nutrient uptake and flowering of
608 pelargonium plants Mycorrhiza 17, 469-474. https://doi.org/10.1007/s00572-007-0116-7

609 Phillips, J.M., Hayman, D.S., 1970. Improved procedure of clearing roots and staining parasitic
610 and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br.
611 Mycol. Soc. 55, 159-161. https://doi.org/10.1016/S0007-1536(70)80110-3
612 Rabani-Foroutagheh, M., Hamidoghlia, Y., Mohajeri, S.A., 2013. Effect of split foliar fertilisation
613 on the quality and quantity of active constituents in saffron (*Crocus sativus* L.). J. Sci. Food
614 Agric. 94:1872-1878. https://doi.org/10.1002/jsfa.6506
615 Rezaian, S., Paseban, M., 2006. The effect of micronutrients and manure fertilizers on the quantity
616 and quality of Khorasan saffron. Acta Hortic. 739, 25-33.
617 https://doi.org/10.17660/ActaHortic.2007.739.20
618 Roldán, A., Carrasco, L., Caravaca, F., 2006. Stability of desiccated rhizosphere soil aggregates
619 of mycorrhizal *Juniperus oxycedrus* grown in a desertified soil amended with a composted
620 organic residue. Soil Biol. Biochem. 38, 2722-2730.
621 https://doi.org/10.1016/j.soilbio.2006.04.024
622 Sánchez-Vioque, R., Rodríguez-Conde, M.F., Reina-Ureña, J.V., Escolano-Tercero, M.A.,
623 Herraiz-Peñalver, D., Santana-Méridas, O., 2012. In vitro antioxidant and metal chelating
624 properties of corm, tepal and leaf from saffron (*Crocus sativus* L.). Ind. Crop Prod. 39, 149-
625 153. https://doi.org/10.1016/j.indcrop.2012.02.028
626 Schulz, H., Glaser, B., 2012. Effects of biochar compared to organic and inorganic fertilizers on
627 soil quality and plant growth in a greenhouse experiment. J. Plant Nutr. Soil Sci. 175, 410-
628 422. https://doi.org/10.1002/jpln.201100143
629 Siracusa, L., Gresta, F., Ruberto, G., 2011. Saffron (*Crocus sativus* L.) apocarotenoids: A review
630 of their biomolecular features and biological activity perspectives, in: Yamaguchi, M. (Eds.),
631 Carotenoids: Properties, Effects and Diseases, Nova Science Publishers, Inc., UK, pp. 1-33.

632 Tohidi, B., Rahimmalek, M., Arzani, A., 2017. Essential oil composition, total phenolic, flavonoid
633 contents, and antioxidant activity of *Thymus* species collected from different regions of Iran.
634 Food Chem. 220, 153-161. https://doi.org/10.1016/j.foodchem.2016.09.203
635 Tuberoso, C.I.G., Rosa, A., Montoro, P., Fenu, M.A., Pizza, C., 2016. Antioxidant activity,
636 cytotoxic activity and metabolic profiling of juices obtained from saffron (*Crocus sativus* L.)
637 floral by-products. Food Chem. 199, 18-27. https://doi.org/10.1016/j.foodchem.2015.11.115
638 Vanek, S.J., Lehmann, J., 2015. Phosphorus availability to beans via interactions between
639 mycorrhizas and biochar. Plant Soil 395, 105-123. https://doi.org/10.1007/s11104-014-2246-
640 y
641 Wang, F.Y., Wang, L., Shi, Z.Y., Li, Y.J., Song, Z.M., 2012. Effects of AM inoculation and
642 organic amendment, alone or in combination, on growth, P nutrition, and heavy-metal uptake
643 of tobacco in Pb-Cd-contaminated soil. J. Plant Growth Regul. 31, 549-559.
644 https://doi.org/10.1007/s00344-012-9265-9
645 Zeka, K., Ruparelia, K.C., Continenza, M.A., Stagos, D., Vegliò, F., Arroo, R.R.J., 2015. Petals
646 of *Crocus sativus* L. as a potential source of the antioxidants crocin and kaempferol.
647 Fitoterapia 107, 128-134. https://doi.org/10.1016/j.fitote.2015.05.014

Table 1

Chemical analysis of the soil and used compost and biochar

	Soil	Compost	Biochar
pH (H_2O)	7.34 (1:5)	8.83 (1:5)	8.75 (1:10)
pH ($CaCl_2$)	7.27 (1:5)	8.2 (1:5)	7.75 (1:10)
EC (dS m^{-1})	1.18 (1:5)	5.38 (1:5)	0.001 (1:10)
Organic carbon (%)	0.4	8.8	19.1
Organic matter (%)	-	15.1	32.9
$CaCO_3$ (%)	22.5	-	-
CEC (Cmol(+) kg^{-1})	20	-	-
N (%)	0.053	0.8	0.3
Available P (mg kg^{-1})	6.2	0.19	0.05

pH: Potential of Hydrogen; EC: Electrical Conductivity; CEC: Cation Exchange Capacity

Table 2

Effect of different fertilizer types (F) and mycorrhizal fungus inoculation (AM) interaction on flower number, flower dry weight, and stigmata yield of saffron in growing seasons of 2015-16, 2016-17, and 2017-18.

Fertilizer types	Inoculation	Flower number (m^2)			Stigmata yield (mg m^{-2})			Flower dry weight (g m^{-2})		
		2015-16	2016-17	2017-18	2015-16	2016-17	2017-18	2015-16	2016-17	2017-18
Control	-AM	2.13± 0.14 [a]	11.7± 0.39 [d]	39.1± 1.27 [e]	6.25± 0.23 [a]	43.6± 1.33 [d]	151± 8.1 [f]	0.059± 0.0046 [a]	0.40± 0.027 [d]	1.46± 0.035 [e]
	+AM	1.99± 0.06 [a]	23.0± 1.89 [c]	70.2± 2.70 [c]	6.14± 0.57 [a]	92.0± 9.89 [c]	268± 12.2 [d]	0.056± 0.0031 [a]	0.85± 0.054 [c]	2.69± 0.174 [c]
COM	-AM	2.36± 0.16 [a]	13.1± 0.67 [d]	47.5± 0.32 [d]	6.76± 0.38 [a]	49.5± 2.09 [d]	193± 3.0 [e]	0.064± 0.0073 [a]	0.49± 0.024 [d]	1.72± 0.035 [d]
	+AM	2.39± 0.14 [a]	33.0± 1.86 [a]	116± 0.87 [a]	7.12± 0.22 [a]	128.3± 6.8 [a]	493± 7.3 [a]	0.070± 0.0057 [a]	1.23± 0.047 [a]	4.33± 0.070 [a]
COM+B	-AM	2.39± 0.20 [a]	13.5± 1.19 [d]	45.5± 3.80 [d]	7.42± 0.31 [a]	54.3± 6.66 [d]	191± 16.4 [e]	0.074± 0.0083 [a]	0.46± 0.048 [d]	1.74± 0.137 [d]
	+AM	2.34± 0.20 [a]	27.9± 0.10 [b]	94.9± 3.49 [b]	7.15± 0.45 [a]	110.8± 1.69 [b]	434± 23.9 [b]	0.072± 0.0083 [a]	0.98± 0.019 [b]	3.68± 0.174 [b]
CF	-AM	2.11± 0.14 [a]	15.3± 0.74 [d]	48.3± 1.21 [d]	6.41± 0.38 [a]	59.1± 1.81 [d]	206± 10.7 [e]	0.061± 0.0055 [a]	0.51± 0.004 [d]	1.82± 0.091 [d]
	+AM	2.16± 0.20 [a]	26.6± 2.1 [bc]	94.1± 4.04 [b]	6.46± 0.69 [a]	110.0± 2.5 [b]	406± 18.2 [c]	0.069± 0.0045 [a]	0.94± 0.041 [bc]	3.55± 0.215 [b]
Source of variation										
F×AM×Y			P< 0.0001			P< 0.0001			P= 0.0001	

For each trait in each year, means ± standard errors (n = 3) with the same letter are not significantly different at P<0.05 according to LSD test.

COM: compost; COM+B: compost + biochar; CF: chemical fertilizers; +AM: inoculated and –AM: non-inoculated with mycorrhizal fungus; Y: Year

Table 3

Flower-related traits and corm characteristics of saffron as affected by main effects of experimental factors

Experimental factors	Flower number (m^2)	Flower dry weight ($g\ m^{-2}$)	Stigmata yield ($mg\ m^{-2}$)	Stigma length (cm)	Corm number (m^2)	Corm yield ($kg\ m^{-2}$)	Unitary corm weight (g)
Fertilizer types							
Control	24.7± 5.86 ^{c}	0.921± 0.227 ^{c}	94± 22.6 ^{b}	2.04± 0.046 ^{a}	467± 86 ^{d}	1.52± 0.331 ^{c}	3.02± 0.209 ^{b}
COM	35.7± 9.58 ^{a}	1.32± 0.358 ^{a}	146± 41.0 ^{a}	2.11± 0.045 ^{a}	623± 124 ^{a}	1.98± 0.445 ^{b}	3.18± 0.257 ^{ab}
COM+B	31.1± 7.86 ^{b}	1.17± 0.308 ^{b}	134± 36.2 ^{a}	2.10± 0.046 ^{a}	580± 112 ^{b}	1.97± 0.450 ^{b}	3.20± 0.232 ^{ab}
CF	31.4± 7.83 ^{b}	1.16± 0.299 ^{b}	132± 34.1 ^{a}	2.11± 0.054 ^{a}	508± 94 ^{c}	2.22± 0.530 ^{a}	3.78± 0.347 ^{a}
P-value	< 0.0001	< 0.001	< 0.001	ns	< 0.0001	< 0.0001	ns
AM-inoculation							
-AM	20.2± 3.11 ^{b}	0.738± 0.118 ^{b}	81± 13.1 ^{b}	2.06± 0.032 ^{b}	538± 75 ^{a}	1.65± 0.253 ^{b}	2.94± 0.141 ^{b}
+AM	41.2± 6.74 ^{a}	1.54± 0.258 ^{a}	172± 29.4 ^{a}	2.12± 0.035 ^{a}	551± 73 ^{a}	2.19± 0.352 ^{a}	3.64± 0.211 ^{a}
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.01	ns	< 0.0001	< 0.0001
Years							
2015-16	2.23± 0.037 ^{c}	0.066± 0.002 ^{c}	6.71± 0.144 ^{c}	1.85± 0.011 ^{c}	201± 4.25 ^{b}	0.52± 0.028 ^{b}	2.79± 0.160 ^{b}
2016-17	20.5± 1.63 ^{b}	0.73± 0.061 ^{b}	81± 6.63 ^{b}	2.11± 0.015 ^{b}	888± 24.6 ^{a}	3.33± 0.150 ^{a}	3.80± 0.165 ^{a}
2017-18	69.5± 5.70 ^{a}	2.62± 0.218 ^{a}	293± 26.0 ^{a}	2.30± 0.019 ^{a}	-	-	-
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

For each experimental factor, means ± standard errors with the same letter are not significantly different at $P<0.05$ according to LSD test.

COM: compost; COM+B: compost + biochar; CF: chemical fertilizers; +AM: inoculated and –AM: non-inoculated with mycorrhizal fungus

Table 4

Comparison of picrocrocin, safranal, and crocin contents obtained in different fertilizer types. Values are average of 2016-17 and 2017-18.

Fertilizer types	Picrocrocin [a] $E_{257}^{1\%}$	Safranal [a] $E_{330}^{1\%}$	Crocin [a] $E_{440}^{1\%}$
Control	68.2± 1.45 [b]	39.1± 0.936 [b]	218± 0.563 [a]
Compost	74.9± 1.21 [b]	45.1± 1.08 [a]	218± 0.634 [a]
Compost+Biochar	68.9± 1.51 [b]	43.7± 0.841 [a]	218± 0.515 [a]
Chemical fertilizers	93.1± 1.86 [a]	44.6± 0.865 [a]	219± 0.814 [a]
P-value	<0.0001	<0.05	ns

Means ± standard errors (n = 12) with the same letter are not significantly different at P<0.05 according to LSD test.

[a] The values were obtained based on ISO 3632 procedure

667 **Table 5**

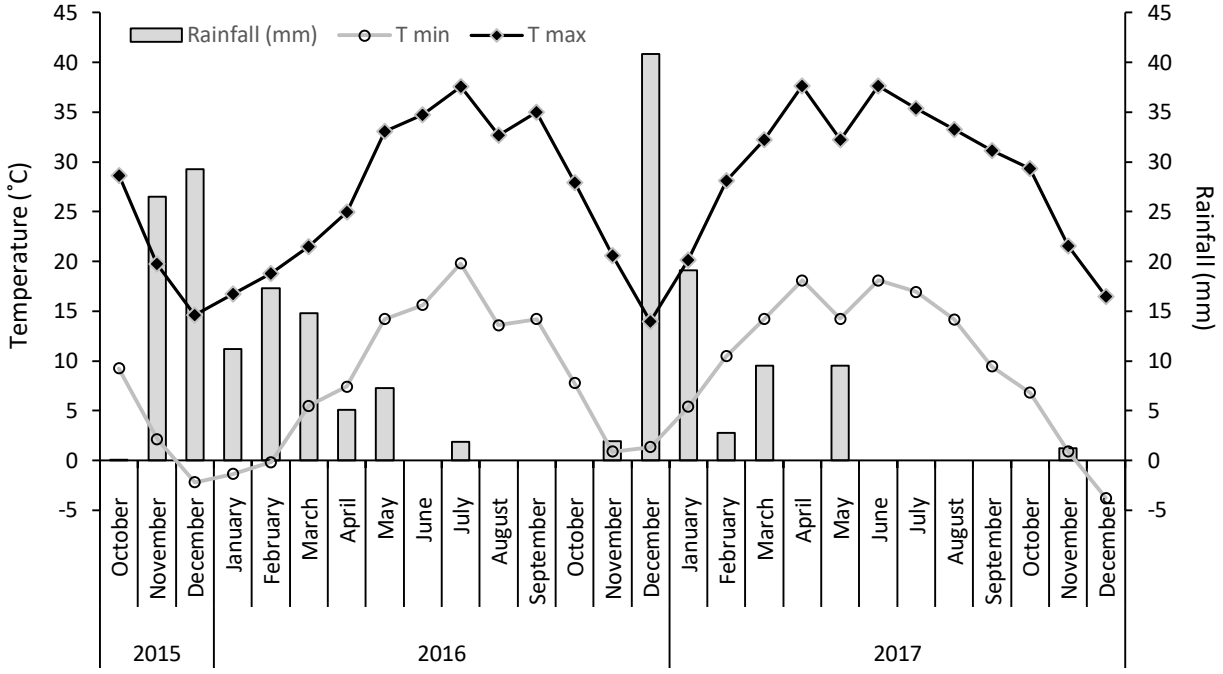
668 Comparison of total phenolic content (TPC), total flavonoid content (TFC), and DPPD radical scavenging activity of
669 saffron tepals and stigmata obtained in different experimental factors.

Experimental factors		TPC (mg GAE/g DW)		TFC (mg QE/g DW)		DPPH (IC_{50}) (µg/mL) [a]	
		Tepals	Stigmata	Tepals	Stigmata	Tepals	Stigmata
Fertilizer types (*n* = 12)							
Control		3.20± 0.249 [c]	5.51± 0.184 [a]	0.86± 0.062 [b]	3.36± 0.045 [b]	252± 4.97 [a]	266± 5.62 [a]
COM		4.75± 0.343 [a]	5.45± 0.209 [a]	0.86± 0.060 [b]	3.48± 0.191 [b]	247± 6.92 [a]	266± 5.41 [a]
COM+B		4.44± 0.165 [ab]	4.86± 0.214 [b]	0.69± 0.076 [c]	4.07± 0.177 [a]	248± 6.51 [a]	263± 4.94 [a]
CF		3.96± 0.320 [b]	4.26± 0.137 [c]	1.04± 0.080 [a]	3.51± 0.210 [b]	247± 7.53 [a]	264± 2.74 [a]
P-value		<.01	<.01	<.01	<.01	ns	ns
AM-inoculation (*n* = 24)							
-AM		4.16± 0.240 [a]	5.26± 0.154 [a]	0.84± 0.065 [a]	3.53± 0.147 [a]	249± 4.22 [a]	247± 2.91 [a]
+AM		4.02± 0.213 [a]	4.79± 0.167 [b]	0.89± 0.041 [a]	3.68± 0.109 [a]	248± 4.85 [a]	250± 3.30 [a]
P-value		ns	<.0001	ns	ns	ns	ns
Years (*n* = 24)							
2016-17		4.49± 0.263 [a]	5.24± 0.121 [a]	0.83± 0.066 [a]	4.02± 0.084 [a]	228± 1.45 [b]	237± 1.65 [b]
2017-18		3.69± 0.143 [b]	4.80± 0.194 [b]	0.90± 0.039 [a]	3.18± 0.106 [b]	269± 1.39 [a]	260± 2.39 [a]
P-value		<.0001	<.01	ns	<.0001	<.0001	<.0001
Year × Fertilizer types (*n* = 6)							
2016-17	Control	2.53± 0.099 [e]	5.47± 0.200	0.77± 0.073 [b]	3.44± 0.030 [d]	236± 2.07 [b]	238± 2.69
	COM	5.75± 0.256 [a]	5.91± 0.107	0.79± 0.027 [b]	4.00± 0.059 [bc]	226± 3.10 [c]	239± 2.03
	COM+B	4.86± 0.073 [b]	5.00± 0.139	0.48± 0.067 [c]	4.51± 0.087 [a]	227± 0.92 [c]	235± 3.78
	CF	4.83± 0.241 [b]	4.58± 0.042	1.28± 0.064 [a]	4.15± 0.038 [ab]	223± 2.22 [c]	237± 4.82
2017-18	Control	3.88± 0.285 [c]	5.55± 0.329	0.95± 0.091 [b]	3.27± 0.071 [de]	268± 1.47 [a]	259± 4.28
	COM	3.75± 0.227 [c]	5.00± 0.315	0.94± 0.114 [b]	2.96± 0.221 [ef]	269± 3.91 [a]	259± 5.07
	COM+B	4.03± 0.216 [c]	4.73± 0.419	0.90± 0.056 [b]	3.63± 0.230 [cd]	269± 2.44 [a]	259± 6.57
	CF	3.10± 0.307 [d]	3.94± 0.196	0.81± 0.035 [b]	2.88± 0.177 [f]	271± 3.36 [a]	263± 3.86
P-value		<.0001	ns	<.0001	<.01	<.05	ns

670 Means ± standard errors with the same letter are not significantly different at $P<0.05$ according to LSD test.

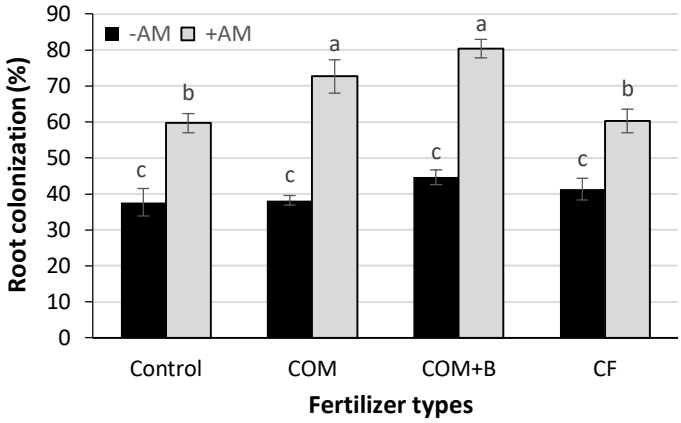
671 COM: compost; COM+B: compost + biochar; CF: chemical fertilizers; +AM: inoculated and –AM: non-inoculated

672 with mycorrhizal fungus; IC_{50}: 50% inhibitory concentration; [a] Ascorbic acid IC_{50}: 119.7 µg/ mL.



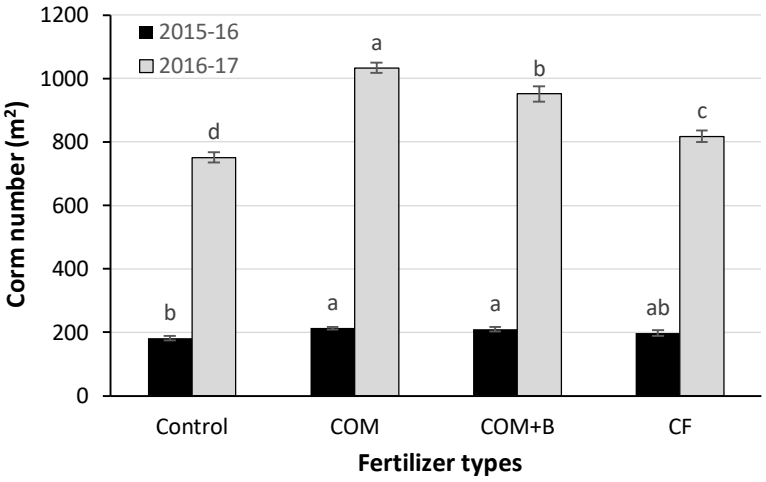
673

674 **Fig. 1.** Regional meteorological records of the study site during the three growing seasons at Kerman, Iran



675

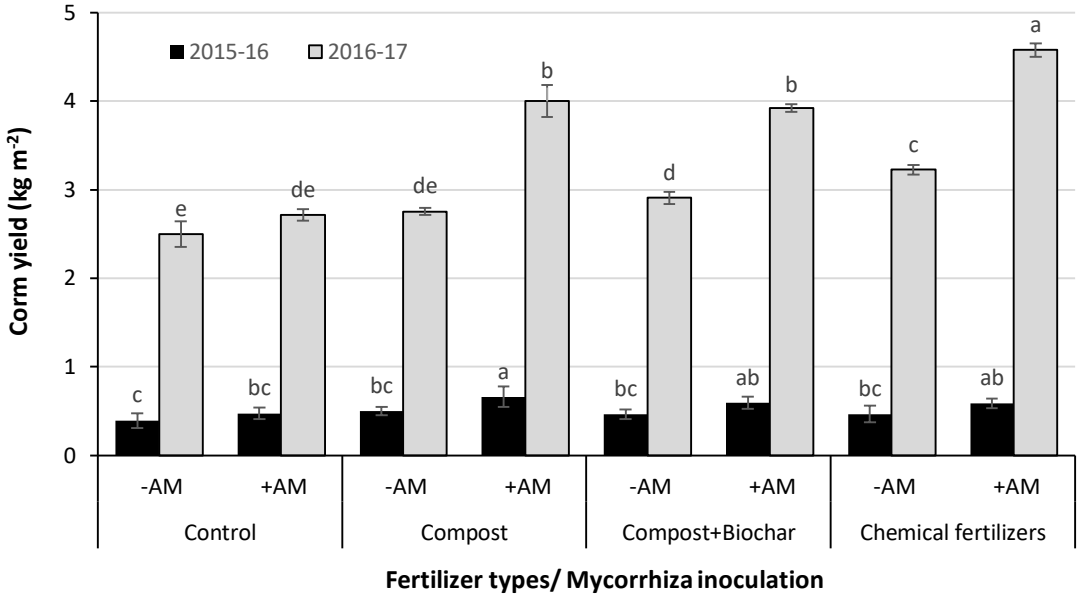
676 **Fig. 2.** Root mycorrhizal colonization (percentage) of inoculated (+AM) and non-inoculated (-AM) planting bed
677 with mycorrhizal fungus in non-amended (control) and amended soil with compost (COM), compost + biochar
678 (COM+B), and chemical fertilizers (CF). Values are mean ± SE, (n = 3).



679

680 **Fig. 3.** Interaction effect of fertilizer type × year on corm number. Values are mean ± SE, (*n* = 6).

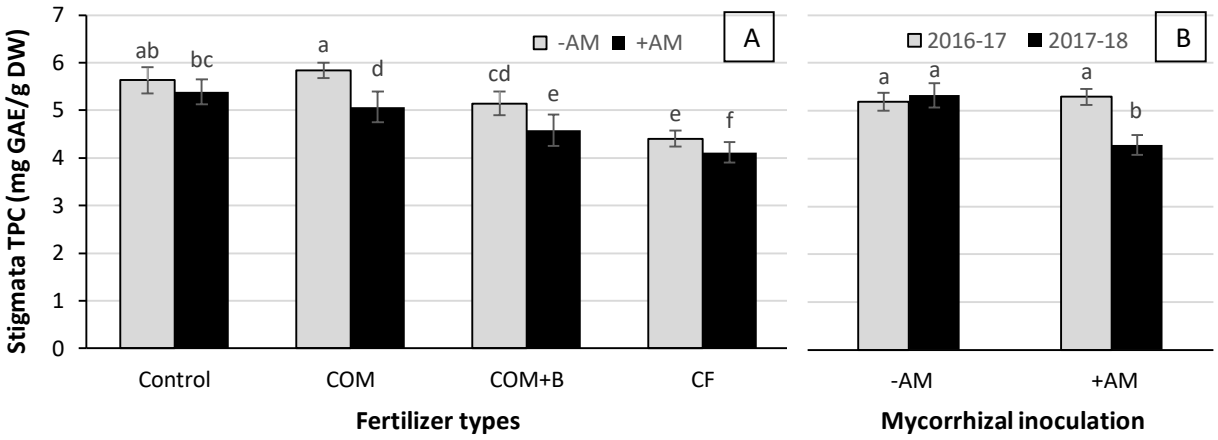
681 COM: compost; COM+B: compost + biochar; CF: chemical fertilizers



682

683 **Fig. 4.** Interaction effects of fertilizer type × mycorrhizal fungus inoculation × year on corm yield. Values are mean

684 ± SE, (*n* = 3). +AM: inoculated and –AM: non-inoculated with mycorrhizal fungus



685 **Fig. 5.** Interaction effects of fertilizer type × mycorrhizal fungus inoculation (A) and mycorrhizal fungus inoculation
686 × year (B) on total phenolic content in saffron stigmata. Values are mean ± SE, (n = 6 and n = 12, respectively).
687 COM: compost; COM+B: compost + biochar; CF: chemical fertilizers; +AM: inoculated and –AM: non-inoculated
688 with mycorrhizal fungus

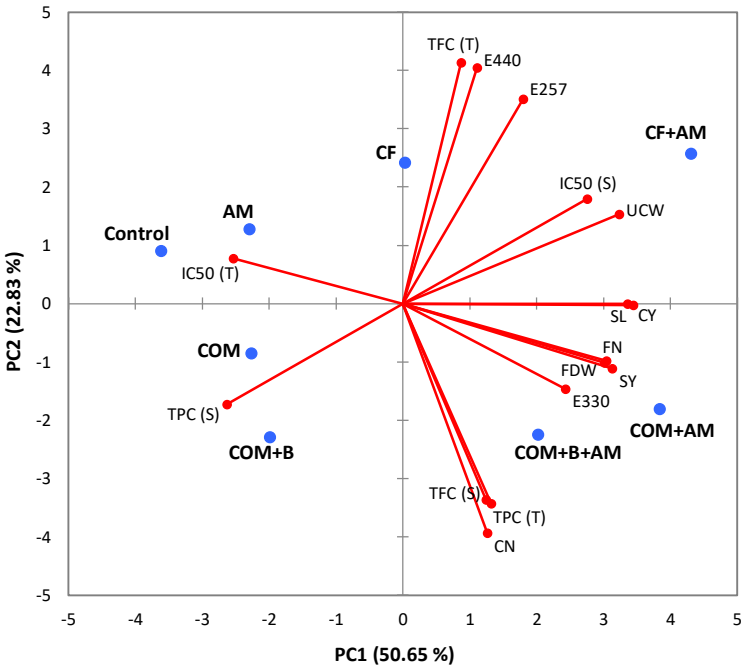


Fig. 6. PCA scatter plot of the first two principal components based on the measured traits for the nutritional treatments. COM: compost; COM+B: compost + biochar; CF: chemical fertilizers; +AM: inoculated with mycorrhizal fungus; FN: flower number: FDW: flower dry weight: SY: stigmata yield; SL: stigma length; CN: corm number; CY: corm yield; UCW: unitary corm weight; E257: picrocrocin; E330: safranal; E440: crocin; TPC: total phenolic content; TFC: total flavonoid content; IC_{50}: DPPD radical scavenging activity based on 50% inhibitory concentration; T: tepals; S: stigmata.