Investigating the effect of corm weight, Arbuscular mycorrhizal fungi and Azotobacter on the growth and yield of saffron (*Crocus sativus* L.)

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Abstract

Introduction: Saffron (*Crocus sativus* L.) is the most expensive agricultural product and one of the most valuable medicinal and spice plants that have many uses in the food and medicine industry. Iran is the largest producer and exporter of saffron in the world, and more than 90% of the world's annual saffron production is produced in Iran. A balanced supply of nutrients based on proper fertilizer management is one of the most effective factors in the sustainability of saffron production, especially in dry and semi-arid areas, so that up to 80% of the formation and yield changes of the saffron flower are determined under the influence of the variables governing the soil, especially the amount of organic matter. Soil fertility management is a proper strategy for increasing soil organic matter, strengthening microbial communities, enhancing agricultural input efficiency and eventually improving plant quantitative and qualitative yields. To attain this goal, the fertilization management program should be designed to increase nutrient use efficiency. Optimal nutrient delivery, controlled-release fertilizers, integrated fertilization management, the use of organic nutrient resources, and beneficial symbiotic

microorganisms with plant roots are all examples of methods that helps achieving nutrient use efficiency. Chemical fertilizers are the most often used fertilizers in saffron fields, and their excess can jeopardize farmed soil and water quality, and reduce the yield of saffron. As a result, biological fertilizers have attracted more interest for being more safe, low-cost, and have fewer negative environmental effects. Therefore, the present study was conducted to investigate the effect of the weight corm, mycorrhizal fungi, and *Azotobacter* biofertilizer on the growth and yield of saffron, so that by selecting the best amount of biofertilizers and the appropriate corm weight of the saffron corm.

Materials and Methods: In order to investigate the effect of corm weight, mycorrhizal fungi and *Azotobacter* biofertilizer on growth and yield of saffron, an experiment was conducted in the 2022-2023 year as factorial based in randomized complete block design at the Birjand Agricultural Faculty. The experimental factors included three levels of corm weight (0.1 to 4, 4.1 to 8 and 8.1 to 12 g), two levels of bacterial biofertilizer (application and non-application of Azotobaror-1 fertilizer containing Pantoea agglomerans strain O4 bacteria) and two levels of mycorrhizal biofertilizer (containing arbuscular mycorrhizal fungi strains Glomus mosseae, Glomus intraradices and Glomus etunicatum and a control without mycorrhiza). Bacterial treatments were applied in the first year of planting by inoculating saffron corms by preparing a suspension of the above bacteria mixed with distilled water in two times. The first time was done before planting by dipping the corms and the second time was done with irrigation 40 days after planting and also in the first irrigation of the second year according to the instructions of Zistfanavar Sabz Company. Mycorrhiza treatments were done at a rate of 10 grams for each corm by adding it to the planting hole at the time of planting according to the instructions of Danesh bonyan Zist Sepidan Hayat Paya Company.

Results and Discussion: The results of the interaction effects of corm weight, mycorrhiza fungus, and *Azotobacter* bacteria on saffron vegetative traits in the first year of the experiment showed that the highest number of leaves per plant (9.33), average leaf dry weight (0.16 g), leaf length (37.00 cm), total weight of corm with scales (23.68 g), number of corms (5), average weight of main cormlet (21.15 g), and average weight of secondary cormlets (3.42 g) were obtained from corms weighing 8.1-12 g and the use of mycorrhiza fungus and *Azotobacter* bacteria. In the second year of the experiment, the highest number of flowers per square meter (83.96), flower yield (66.37 $g.m^{-2}$), fresh stigma yield (32.2 $g.m^{-2}$), and dry stigma yield (58.0 $g.m^{-2}$) were obtained from 8.1-12 g corm and the use of mycorrhiza fungi and *Azotobacter* bacteria.

Conclusion: Overall, the results of this study indicated that growth and yield of saffron were improved with the simultaneous use of higher weight corm (8.1-12 g) and the use of mycorrhiza fungi and *Azotobacter* bacteria. Therefore, the use of biofertilizers and the development of mycorrhizal hyphae in agricultural soil, can improve saffron cultivation areas in sustainable agriculture and reduce the losses caused by the

excessive use of chemical fertilizers.

Keywords: Daughter corm, Vegetative growth, Bio-fertilizer, Medicinal plant

Introduction

Saffron ranks among the most valuable medicinal plants, holding a distinguished position in both the pharmaceutical and spice industries. Currently, Iran accounts for nearly 90% of global saffron production and over 84% of its cultivated area worldwide. Saffron is a herbaceous, perennial, triploid plant with vibrant purple flowers, propagated exclusively through corms (Habib et al., 2020). Mother corm size is a key factor in selecting high-quality saffron corms for planting (Koocheki et al., 2018). In a study by Khavari et al. (2016), they concluded that saffron corm weight can significantly influence yield. Using corms weighing 9 to 11 grams as opposed to 6 to 8 grams leads to increased production of flowers, stigma, and style in saffron. The study by Koocheki et al. (2018) revealed that heavier mother corms consistently enhanced all aspects of saffron growth and flowering. Specifically, corms with greater average weight (benefiting from their richer nutrient reserves and stronger capacity to develop both vegetative and flowering buds) confirmed significantly increased flower production.

Due to the ecological harm caused by chemical fertilizers, interest in sustainable alternatives like biofertilizers and microbial inoculants has grown in agroecosystems (Hasan, 2021). Mycorrhizal fungi, forming one of the most widespread symbiotic relationships in nature, decisively influence the growth and development of vascular plants. This mutualistic association involves a complex network of interconnected processes that both mitigate drought stress through direct water absorption and transport via fungal hyphae to the host plant, while simultaneously enhancing nutrient uptake and improving net photosynthetic rates in the host (Badr et al., 2020). Six major types of mycorrhizae have been identified: *ectomycorrhizae* (EM), *arbuscular* (AM), *arbutoid*, *monotropoid*, *orchid*, and *ericoid mycorrhizae*. Recently, agricultural applications of arbuscular mycorrhizal fungi (AMF)-based products have gained significant traction in modern farming practices. These biofertilizers optimize crop yields while improving resistance to environmental stressors and plant pathogens (Caser et al., 2019).

Arbuscular mycorrhizal fungi form symbiotic relationships with over 80% of plant species in both natural and agricultural ecosystems. These fungi significantly enhance host plant growth, nutrient

uptake, disease resistance, and overall plant development (Gianinazzi et al., 2002). *Azotobacter*, a genus of free-living aerobic soil bacteria, plays a vital role in the nitrogen cycle by fixing atmospheric nitrogen, which is otherwise unavailable to plants, and converting it into bioavailable ammonium ions in the soil. Numerous studies have indicated that *Azotobacter* contributes not only to nitrogen fixation but also influences various other plant-growth characteristics, including the production of growth hormones, antifungal compounds, siderophores, and phosphate-solubilization. Alizadeh et al. (2021) have shown that even in medicinal plants, applications of nitrogen-fixing biofertilizers can amplify plant growth and development.

Biofertilizers containing *Azotobacter* and *Azospirillum* facilitate nitrogen fixation and also produce phytohormones, enhancing plant growth, nutrient uptake, and photosynthesis (Mahfouz & Sharaf-Eldin, 2015). These free-living bacteria fix atmospheric nitrogen and release it as ammonium in the rhizosphere (Sadrabadi Haghighi & Ghavi, 2015). In some cases, it has been observed that even with sufficient levels of nitrogen-based biofertilizers, inoculating plants with *Azotobacter* further enhances plant growth and development (Hokmalipour, 2017). Based on the current findings and those of Feli et al. (2018), it can be concluded that biofertilizers improve conditions for beneficial microbial activity in the soil. By synergistically optimizing nutrient availability for saffron and amplifying synergistic effects, they also intensify the percentage of key qualitative traits.

Several researchers have reported that the combined application of plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi produces synergistic effects (Xavier et al., 2012). In the present study, treatments involving mycorrhizal fungi, growth-promoting bacteria, and their combined use all resulted in enhanced vegetative growth of the plants (Sanayei et al., 2020). Therefore, the present study was conducted to investigate the effects of corm weight, mycorrhizal fungi, and *Azotobacter* biofertilizer on the growth and yield of saffron. By determining the optimal application rates of biofertilizers and the ideal corm size, this research aims to reduce reliance on chemical fertilizers, promoting sustainable saffron cultivation while enhancing yields of this high-value medicinal crop.

Materials and Methods

In this research, a factorial experiment based on randomized complete block design (RCBD) with three replications and twelve treatments was conducted in two consecutive growing seasons

(2022–2023), at the Research and Educational Farm of the Faculty of Agriculture, University of Birjand. The experimental treatments included (1) corm weight in three levels (average weights: 0.1–4, 4.1–8, and 8.1–12 g), (2) Bacterial biofertilizer in two levels (application vs. nonapplication of *Azotobarvar*-1, containing *Pantoea agglomerans* strain O4) and (3) Mycorrhizal biofertilizer (containing arbuscular mycorrhizal fungi *Glomus mosseae*, *Glomus intraradices*, and *Glomus etunicatum* at a concentration of 10^7–10^8 CFU/g) versus a control group (no mycorrhiza). During the first Year, was treated saffron corms with bacteria in two stages: (1) soaking in sterile distilled water suspension before planting, and (2) reapplication through irrigation at 40 days post-planting. The treatment was repeated during the first irrigation of the second growing season, following the protocol of Green Biotech Company. Mycorrhizal treatments were applied at a rate of 10 g per corm by incorporating the inoculum into the planting hole during transplantation, following established protocols (Vafaee et al., 2020) and manufacturer recommendations (Zist Sepeidan Hayat Paya Knowledge-Based Company). Before trial initiation and preparation procedures, composite soil samples were randomly collected from the 0-30 cm depth for baseline soil analysis (Table 1).

Table 1. Physical and chemical properties of soil in the study area

Soil Texture	Silt (%)	Clay (%)	Sand (%)	OC (%)	N Total (%)	P (mg/kg)	K (mg/kg)	pH
Loam	7.9	300	63.7	0.013	0.15	30	24	46

Land preparation operations, including initial plowing, disking, and leveling with a roller, were completed in September 2022. Experimental plots measuring 1 × 2 meters were then established, with 50 cm spacing between plots and 2 meters between blocks. To prevent cross-mixing of water between plots, irrigation was carried out using pipes. Also, uniform and suitable corms (divided into three weight groups: 0.1– 4 g, 4.1– 8 g, and 8.1–12 g, all intact and free of cuts) were sourced from Qayenat County for cultivation. After removing the outer scales and underlying scales, saffron corms were planted in October 2021 following the experimental layout. The corms were carefully planted by hand, with trowels used to place them in 15 cm-deep furrows. The rows were

spaced 20 cm apart, with individual corms set 10 cm apart within each row, achieving a planting density of 50 corms per square meter. Once the soil reached field capacity, and a week after the first irrigation, the surface was lightly cultivated to a depth of 5 cm using a spring-tine harrow. This shallow tillage helped promote smoother flower emergence while retaining soil moisture and enhancing aeration. Additional irrigations were carried out after the flowering period ended, with supplemental waterings in December, March, and April. Notably, no chemical fertilizers, pesticides, or herbicides were applied at any stage of the experiment. Weed control during the plant's growth period was carried out manually through hand weeding.

Leaf growth-related traits were measured during the growing season (in February), including leaf length (using a ruler), leaf count, and fresh and dry leaf weight (after ensuring no soil remained on leaf surfaces, measured with a digital scale). Furthermore, corm growth metrics were evaluated in May 2023 at the onset of leaf senescence. The assessed parameters included: Total corm weight (including scales), Mean weight of primary and secondary cormlets (determined via digital scale), cormlet diameter (measured using digital calipers), cormlets count per mother corm. early November 2023. One week later, soil crusts were broken using a four-pronged metal fork. Two weeks after the initial irrigation, saffron flowers emerged and were harvested daily over the following month. Flowering-related traits were measured, including: Flower count, Fresh flower yield, Fresh and dry stigma yield (weighed to the nearest milligram using a precision digital balance). Data were analyzed using SAS 9.4, with mean comparisons evaluated via Duncan's test ($p \leq 0.05$).

Results and Discussion

Vegetative Growth Traits of Saffron

Leaves number per Plant

Analysis of variance revealed that all main effects (corm weight, mycorrhizal fungi, and *Azotobacter* bacteria) were significant ($p \leq 0.01$). Additionally, the interaction between corm weight and mycorrhizal fungi, as well as the three-way interaction of corm weight, mycorrhizal fungi, and *Azotobacter*, were significant ($p \leq 0.05$). However, none of the other treatment interactions had a statistically significant effect on saffron leaf count (Table 2). The highest leaf

count (9.33 leaves/plant) occurred when 8.1–12 g corms were treated with both mycorrhizal fungi and *Azotobacter* bacteria. In contrast, the lowest leaf count (4.66 leaves per plant) was observed with the smallest corm weight (0-4 g) in the absence of both mycorrhizal fungi and *Azotobacter* bacteria (Table 3).

In this regard, the highest number of leaves per mother corm of saffron (18.38 leaves per corm) was obtained from the 8–12-gram corm weight treatment, while the lowest value for this trait (8.41 leaves per corm) was observed in the 4–8-gram weight group. When heavier corms are used, cell division and leaf growth in saffron occur earlier compared to smaller corms. This earlier growth allows the leaves to benefit sooner and more extensively from optimal light and environmental conditions, thereby enhancing photosynthesis and further promoting leaf growth (Golzari Jahan Abadi et al., 2017). In one study, the application of 7.5 g of mycorrhizal fungus per saffron planting site resulted in an increased number of leaves per plant (7.54 leaves/plant) compared to the control (Jami et al., 2020), which aligns with the findings of the present research. Another report indicated that the use of Nitroxin biofertilizer on saffron, a medicinal plant, produced the highest number of leaves (6.33 leaves) compared to the control (4.22 leaves). The increased number of leaves in saffron plants exhibited that the application of biofertilizers containing nitrogen-fixing bacteria can stimulate vegetative growth in leaves, while also improving soil fertility and enhancing nitrogen availability, critical for photosynthesis and green leaf area expansion (Golzad, 2008).

Table2. The ANOVA (mean of squares) of the effect of corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron leaf traits

Source of variance	df	Leaf number per plant	Fresh weight of leaf	Dry weight of leaf	Leaf length
Replication	2	0.19 ^{ns}	0.00008 ^{ns}	0.0002 ^{ns}	0.006 ^{ns}
Corm Weight (CW)	2	27.02**	0.01 **	0.001 **	60.75 **
Mycorrhizal Fungi (MF)	1	7.11**	0.03 **	0.002 **	182.25 **
Azotobacter (AB)	1	11.11 **	0.06 **	0.004 **	306.25 **
CW×MF	2	0.86 *	0.002 **	0.00002 *	10.56 **
CW ×AB	2	0.19 ^{ns}	0.003 **	0.00006 *	1.27 **
MF×AB	1	0.11 ^{ns}	0.004 **	0.0007 **	113.77 **

CW ×MF×AB	2	1.027 *	0.006 **	0.00006 *	6.298 **
Error	22	0.194	0.00006	0.0007	0.059
C.V (%)	-	6.55	1.90	3.17	0.79

ns, **, *: non-significant and significant at 5% and 1% of probability levels, respectively

Table 3. Comparison of the mean interaction effect corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron leaf traits.

Corm weight (gr)	Mycorrhizal fungi	Azotobacter	Leaf number per plant	Fresh weight of leaf (gr)	Dry weight of leaf (gr)	Leaf length (cm)
0.1-4	Fertilizer	Fertilizer	6.00 cd	0.42 cd	0.13 cde	33.33 c
0.1-4	Fertilizer	No fertilizer	5.00 de	0.41 d	0.12 e	32.50 d
0.1-4	No fertilizer	Fertilizer	5.33 de	0.43 c	0.12 de	31.33 e
0.1-4	No fertilizer	No fertilizer	4.66 e	0.30 g	0.09 g	21.16 i
4.1-8	Fertilizer	Fertilizer	8.00 b	0.43 c	0.13 bc	32.66 d
4.1-8	Fertilizer	No fertilizer	6.00 cd	0.41 d	0.13 cde	30.16 f
4.1-8	No fertilizer	Fertilizer	6.66 c	0.42 cd	0.13 cd	33.33 c
4.1-8	No fertilizer	No fertilizer	6.00 cd	0.32 f	0.10 f	22.66 h
8.1-12	Fertilizer	Fertilizer	9.33 a	0.57 a	0.16 a	37.00 a
8.1-12	Fertilizer	No fertilizer	8.66 b	0.42 cd	0.13 bc	33.50 c
8.1-12	No fertilizer	Fertilizer	8.33 b	0.45 b	0.14 b	35.50 b
8.1-12	No fertilizer	No fertilizer	6.66 c	0.35 e	0.11 f	28.16 g

In each column, means with the same letter are not different significantly at 5% probability level.

Mean fresh and dry leaf weight

As shown in the ANOVA table (Table 2), fresh and dry leaf weights were significantly affected by the experimental treatments. Mean comparison results revealed that the triple interaction of corm weight (8.1-12 g) with combined application of mycorrhizal fungi and *Azotobacter* bacteria produced the highest fresh and dry leaf weights (0.45 g and 0.14 g, respectively; Table 3).

According to the study by Koocheki & Sabet Teimouri (2014), which examined four weight categories of corms, the highest dry leaf weight was obtained from corms weighing 8 to 12 grams. The findings suggested that heavier corms likely produced larger leaves with greater mass, leading to increased fresh and dry leaf weight in the 8–12gram weight group. This weight range enhances saffron's dry leaf weight due to the corms' greater capacity for photosynthetic production. Furthermore, consistent with the findings of this study, biofertilizers positively impacted saffron leaf vegetative growth by fixing and supplying plant-available nitrogen. Researchers attribute this stimulatory effect on leaf growth to the provision of essential nitrogen from nitrogen-fixing biofertilizers and other microbial amendments (Golzari Jahan Abadi et al., 2017).

Mean leaf length

Analysis of variance (Table 2) revealed that all experimental treatments significantly influenced mean leaf length ($p \leq 0.01$). The lowest mean leaf length (21.16 cm) was observed in the treatment combining 0.1– 4 g corm weight with no application of mycorrhizal fungi or *Azotobacter* bacteria, as shown in the three-way interaction table. In contrast, the longest mean leaf length (35.5 cm) occurred with 8.1–12 g corm weight and *Azotobacter* inoculation (without mycorrhizal fungi), representing a 64% increase in leaf length (Table 3).

In this regard, studies have reported that using heavier-weight saffron corms considerably increases the average leaf length of the plants. Specifically, corms weighing 10 to 12 grams produced the longest leaves (26.56 cm), while the lowest leaf length (23.14 cm) was observed in corms weighing 4 to 6 grams. The use of heavier corms (due to their greater nutrient reserves) positively influences leaf growth, thereby enhancing saffron leaf length (Golzari Jahan Abadi et al., 2017). According to findings by Jami et al. (2020), the greatest leaf length (40.6 cm) was achieved in the treatment combining 8 tons per hectare of vermicompost and 10 grams of mycorrhizal fungi per planting site, while the lowest leaf length (25.33 cm) was observed in the absence of mycorrhizal application. Additionally, the use of Nitroxin biofertilizer alongside urea fertilizer notably influenced saffron leaf length. The maximum leaf length (25.82 cm) and minimum saffron leaf length (21.15 cm) were recorded in the control treatment (Golzad, 2008).

Total corm weight (including scales)

Table 4 results from the first year demonstrated that individual treatment effects significantly affected total corm weight (including scales) ($p \leq 0.01$). Furthermore, various treatment interactions also indicated statistically significant effects on total corm weight with scales. Combining mycorrhizal fungi and *Azotobacter* bacteria gave the highest total corm weight (with scales). Analysis of first-year three-way treatment interactions showed that the highest total corm weight (including scales) (23.68 g) was achieved using 8.1–12 g corms with the combined application of fungi and bacteria. In contrast, the lowest weight (4.23 g) occurred with 0.1–4 g corms and no application of mycorrhizal fungi or *Azotobacter* bacteria (Table 5).

A study revealed that planting heavier corms improved both daughter corm traits and saffron flower yield. Mother corms weighing 7-9 grams produced the highest corm yield per unit area (809 g/m²) (Hassanzadeh Aval et al., 2015). Therefore, using corms in the 8.1-12 gram weight range can optimize yield potential.

The application of biofertilizers such as arbuscular mycorrhizal fungi increases phosphorus availability by increasing root density and phosphatase enzyme secretion, which converts non-absorbable phosphorus into its bioavailable form. Moreover, by expanding the host plant's root system, these fungi improve soil nutrient uptake efficiency and facilitate subsequent translocation to corms, ultimately increasing total mother corm weight. Studies have demonstrated that using corms weighing over 7 grams in combination with mycorrhizal inoculation significantly boosts total saffron corm weight (Bekhradiyaninasab et al., 2020). Moreover, any nutritional factor, including biofertilizers, that enhances other yield components can improve corm characteristics

Cormlets number per mother corm

According to Table 4, both the main and interaction effects of treatments on cormlet number per mother corm were statistically significant ($p \leq 0.01$). The three-way interaction analysis revealed that corms weighing 8.1–12 g, when treated with a combined application of mycorrhizal fungi and *Azotobacter* bacteria application produced the highest number of cormlets per mother corm (5 cormlets) (Table 5).

Research has attested that mother corms produce more cormlets when larger corms (9–10 g) are planted. The use of heavier corms, due to their greater nutrient reserves, faster growth rates, and enhanced nutrient uptake, results in increased cormlet production (Koocheki et al., 2015). This

finding has been corroborated by multiple studies, with researchers consistently reporting higher cormlet numbers from larger mother corms (Sahabi et al., 2017; Golzari Jahan Abadi et al., 2017; Sharifi et al., 2021; Khavari et al., 2016).

A study by Noori et al. (2023) showed that inoculation with mycorrhizal fungi combined with planting larger corms (8.1-12 g) notably increased the number of cormlets per mother corm. Similarly, other research demonstrated that the biofertilizers mycorrhizal fungi and phosphate-solubilizing bacteria (Barvar-2) had a statistically significant effect ($p \leq 0.05$) on cormlet production. Specifically, application of *Glomus mosseae* increased cormlet numbers by 13% compared to untreated controls (Bekhradiyaninasab et al., 2020).

The application of biofertilizers such as mycorrhizal fungi in saffron corm planting sites enhances soil microbial activity. This improves nutrient uptake by converting non-available elements into bioavailable forms, thereby promoting plant growth and yield. Enhanced access to both macro- and micronutrients, total biomass increases. During nutrient remobilization from leaves back to the corms, this results in greater nutrient allocation to the corms, ultimately boosting corm productivity, including increased cormlet production per mother corm. The study demonstrated that using heavier corms (8.1-12 g) combined with mycorrhizal inoculation dramatically increased cormlet production per mother corm to 67 cormlets per square meter (Noori et al., 2023). Earlier work by Koocheki et al. (2014) similarly found that nitrogen-fixing bacterial treatments yielded the highest corm density per square meter compared to other fertilizer regimes. The study of biofertilizers' effects on saffron yield and quality in Zaveh County further illustrated that nitrogen-fixing bacteria treatments markedly increased cormlet production per mother corm ($p \leq 0.01$). Pre-planting inoculation of saffron corms with these bacteria also enhanced daughter corm formation (Poorreza & Amirshkari, 2020).

Mean weight of primary cormlet

The ANOVA results (Table 4) revealed considerable main treatment effects on primary cormlet mean weight. Both the corm weight × *Azotobacter* bacteria interaction and the three-way treatment interaction significantly affected primary cormlet weight ($p \leq 0.05$), while other treatment interactions showed no significant effects (Table 4). The highest mean weight of primary cormlet (18.27 g) was achieved using 8.1-12 g corms inoculated with *Azotobacter* bacteria. However, mean comparison analysis of three-way treatment interactions demonstrated no significant difference in

first-year primary cormlet weight between: (1) 8.1-12 g corms with combined mycorrhizal fungi and bacteria application, and (2) 8.1-12 g corms with mycorrhizal fungi alone (Table 5). Koocheki and Sabet Teimouri's (2014) study of four corm weight classes revealed that the maximum primary corm weight (15 g) was obtained from mother corms weighing 8-12 g. Subsequent research on 8-10 g corms by Salehi et al. (2022) established that Bacillus inoculation increased mother corm weight to 22.48 g, while untreated controls produced the lowest weights (9.84 g).

Mean weight of secondary cormlets

Analysis of variance demonstrated substantial main treatment effects ($p \leq 0.01$) on secondary cormlet mean weight (Table 4). However, the mycorrhizal fungi × *Azotobacter* bacteria interaction suggested no significant effect, while other treatment interactions markedly impacted secondary cormlet weight (Table 4). The three-way treatment interaction considerably affected the mean weight of secondary cormlets ($p \leq 0.05$) (Table 4). First-year mean comparison analysis revealed that the highest (3.42 g) and lowest (0.61 g) secondary cormlet weights were obtained from 8.1-12 g corms treated with both mycorrhizal fungi and *Azotobacter* bacteria, and 0.1-4 g corms receiving neither fungal nor bacterial treatment, respectively (Table 5).

Noori et al. (2023) proved that the highest secondary cormlet weights resulted from treating 8.1–12 g corms with both mycorrhizal fungi and humic acid. Furthermore, the use of heavier corm weight classes appreciably enhanced both the density and yield of secondary cormlets per unit area, ultimately increasing saffron flower production along with fresh and dry stigma and flower weights (Hassanzadeh Aval et al., 2015). Additional trials similarly illustrated significantly higher daughter corm fresh weights when planting heavier mother corms (Sahabi et al., 2017). Subsequent studies further confirmed the positive influence of larger mother corm size on both the quantity and mean weight of secondary saffron cormlets (Mollafilabi et al., 2014). Research findings indicate that the greater weight of secondary cormlets results from the translocation of reserves from primary cormlets. Meanwhile, the mother corm serves as a photosynthetic reserve bank during initial growth stages. Earlier leaf development enhances light interception and environmental resource utilization, ultimately yielding larger corms by the end of the growing season.

Mean diameter of primary and secondary cormlets

The ANOVA results (Table 6) revealed that all main effects and treatment interactions had a notable effect on primary cormlet diameter ($p \leq 0.01$). Similarly, corm weight main effects and other interactions decisively influenced secondary cormlet diameter ($p \leq 0.01$) (Table 6). Mean comparison analysis showed that the largest diameters for both primary and secondary cormlets (37.50 mm and 21.32 mm, respectively) occurred in the first year with 8.1–12 g corms treated with both mycorrhizal fungi and *Azotobacter* bacteria (Table 7).

A study highlighted that applying 10 g of mycorrhizal fungi with 16 tons of vermicompost per hectare markedly increased average saffron corm diameter (3.2 cm) compared to non-inoculated controls. The arbuscular mycorrhizal hyphae boost root system density and absorption surface area, thereby improving root efficiency in acquiring both macro- and micronutrients. This mechanism ultimately boosts corm growth parameters, including primary and secondary cormlet diameter (Jami et al., 2020). Evaluating various concentrations of nitrogen-fixing bacteria on 8-10 g corms revealed that the maximum and minimum primary cormlet diameters (23.69 mm and 14.85 mm, respectively) were obtained from treatments with bacterial inoculation at 10^8 CFU/mL and non-inoculated controls. The study found that plant growth-promoting bacteria enhance root access to essential nutrients, thereby stimulating cell expansion and division while improving vegetative growth (Salehi et al., 2022).

Number of buds with flowering potential

The results validated significant main effects of corm weight ($p \leq 0.01$) on both flower-potential bud count and small bud formation. Additionally, all treatment interactions (except the corm weight × *Azotobacter* bacteria interaction) showed significant effects on flower-potential bud number (Table 6). Only the interaction between corm weight and *Azotobacter* application strongly influenced small bud count (Table 6). Mean comparison analysis revealed that first-year treatments using 8.1-12 g corms without mycorrhizal inoculation but with *Azotobacter* bacteria produced the highest number of buds with flowering potential (3 buds) (Table 7).

Mother corm weight substantially impacted the number of buds with flowering potential. Heavier corms were shown to produce more buds and consequently greater numbers of daughter corms

(Amirshekari et al., 2006). Subsequent research has emphasized selecting optimal corm weights to activate buds with flowering potential and optimize saffron flower production. Corms exceeding 8 grams can substantially increase flowering rates while playing a crucial role in both floral yield and corm productivity (Bayat, 2015).

Other research indicated that biofertilizer application in saffron cultivation enhanced the development of buds with flowering potential in mother corms. These meristematic buds serve as the primary sites for generating new daughter corms, thereby accelerating corm multiplication. The study further revealed that higher numbers of buds with flowering potential correlated with increased flower production per corm, ultimately boosting both flower and stigma yields in saffron (Behdani et al., 2016).

Table 4. The ANOVA (mean of squares) of the effect of corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron corm traits

Source of variance	df	Total corm weight with scale	Number of cormlets	Weight of corms	Weight of cormlets (g)
Replication	2	1.23 ^{ns}	0.11 ^{ns}	19.22 ^{ns}	0.006 ^{ns}
Corm Weight (CW)	2	584.96**	16.02 **	484.83**	4.47**
Mycorrhizal Fungi (MF)	1	129.84**	12.25 **	159.76**	3.53 **
Azotobacter (AB)	1	30.74 **	4.69 **	29.59*	3.68 **
CW×MF	2	17.98**	1.08 **	32.36 ^{ns}	0.52 **
CW ×AB	2	3.48 *	0.52 *	15.45*	0.32 *
MF×AB	1	18.16**	0.50 *	57.76 ^{ns}	0.05 ^{ns}
CW ×MF×AB	2	2.69 *	0.50 *	17.26*	0.24*
Error	22	0.62	0.17	4.08	0.08
C.V (%)	-	6.054	16.76	19.37	15.51

ns, **, *: non-significant and significant at 5% and 1% of probability levels, respectively

Table 5. Comparison of the mean interaction effect corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron corm traits

Corm weight (gr)	Mycorrhizal fungi	Azotobacter	Total Corm weight with scale (g)	Number of cormlets	Weight of Corms (g)	Weight of cormlets (g)
0.1-4	Fertilizer	Fertilizer	7.11 ^{e}	2.00 ^{ef}	5.58 ^{ef}	1.27 ^{d}
0.1-4	Fertilizer	No fertilizer	6.93 ^{e}	1.66 ^{efg}	6.59 ^{de}	1.40 ^{d}
0.1-4	No fertilizer	Fertilizer	5.28 ^{e}	1.33 ^{efg}	3.77 ^{ef}	1.29 ^{d}
0.1-4	No fertilizer	No fertilizer	4.23 ^{f}	1.00 ^{g}	2.06 ^{f}	0.61 ^{e}
4.1-8	Fertilizer	Fertilizer	14.85 ^{c}	3.00 ^{cd}	9.80 ^{cd}	2.52 ^{b}
4.1-8	Fertilizer	No fertilizer	14.83 ^{c}	2.33 ^{de}	11.08 ^{c}	1.74 ^{cd}
4.1-8	No fertilizer	Fertilizer	14.67 ^{c}	2.00 ^{ef}	13.33 ^{bc}	2.09 ^{bc}
4.1-8	No fertilizer	No fertilizer	10.00 ^{d}	1.33 ^{efg}	4.39 ^{ef}	1.40 ^{d}
8.1-12	Fertilizer	Fertilizer	23.68 ^{a}	5.00 ^{a}	21.15 ^{a}	3.42 ^{a}
8.1-12	Fertilizer	No fertilizer	22.59 ^{ab}	4.33 ^{ab}	21.02 ^{a}	2.38 ^{b}
8.1-12	No fertilizer	Fertilizer	18.56 ^{b}	3.66 ^{bc}	15.39 ^{b}	2.18 ^{bc}
8.1-12	No fertilizer	No fertilizer	14.47 ^{c}	2.00 ^{ef}	11.00 ^{c}	1.40 ^{d}

In each column, means with the same letter are not different significantly at 5% probability level.

Table 6. The ANOVA (mean of squares) of the effect corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron corm traits

Source of variance	df	Mean diameter of primary cormlets	Mean diameter of secondary cormlets	Number of buds with flowering potential
Replication	2	1.12 ^{ns}	2.15 ^{ns}	0.083 ^{ns}
Corm Weight (CW)	2	500.82**	74.99**	5.08 **
Mycorrhizal Fungi (MF)	1	276.22**	0.04 ^{ns}	0.02 ^{ns}
Azotobacter (AB)	1	40.91**	0.04 ^{ns}	0.69 ^{ns}
CW×MF	2	39.76 **	8.07 **	0.19*
CW ×AB	2	17.29 **	56.26 **	0.36 ^{ns}
MF×AB	1	70.166 **	83.570**	2.250**

CW ×MF×AB	2	3.401 **	44.700 **	1.583**
Error	22	0.382	0.685	0.053
C.V (%)	-	2.27	6.40	16.25

In each column, means with the same letter are not different significantly at 5% probability level.

Table 7. Comparison of the mean interaction effect corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron corm traits

Corm weight (gr)	Mycorrhizal fungi	Azotobacter	Mean diameter of original cormlets (mm)	Mean diameter of lateral cormlets (mm)	Number of buds with flowering potential
0.1-4	Fertilizer	Fertilizer	24.13 f	10.40 ef	1.00 c
0.1-4	Fertilizer	No fertilizer	26.90 e	11.64 e	1.00 c
0.1-4	No fertilizer	Fertilizer	17.63 h	10.74 ef	1.00 c
0.1-4	No fertilizer	No fertilizer	15.92 i	9.39 f	1.00 c
4.1-8	Fertilizer	Fertilizer	27.34 e	11.84 e	1.00 c
4.1-8	Fertilizer	No fertilizer	27.23 e	11.76 e	1.00 c
4.1-8	No fertilizer	Fertilizer	29.78 c	10.33 ef	1.33 c
4.1-8	No fertilizer	No fertilizer	21.63 g	17.92 b	1.00 c
8.1-12	Fertilizer	Fertilizer	37.50 a	21.32 a	2.00 b
8.1-12	Fertilizer	No fertilizer	36.81 a	10.81 ef	2.66 ab
8.1-12	No fertilizer	Fertilizer	33.31 b	14.15 d	3.00 a
8.1-12	No fertilizer	No fertilizer	28.40 d	15.83 c	1.00 c

In each column, means with the same letter are not different significantly at 5% probability level.

Flower-related traits

Flowers number

Second-year results demonstrated considerable main and interaction effects ($p \leq 0.05$) on flower density per square meter, with only the corm weight × *Azotobacter* bacteria interaction showing no significant impact (Table 8). Analysis of treatment interactions revealed that combining 8.1-12 g corms with both mycorrhizal fungi and *Azotobacter* bacteria yielded maximum flower density (96.83 flowers/m²), while untreated 0.1-4 g corms produced the lowest density (32 flowers/m²) - representing a 202% increase attributable to treatment application (Figure 1).

A study has shown that planting heavier corms results in greater flower production, with corms weighing 10-12 grams yielding the highest flower counts and those in the 4-6-gram range producing the lowest (Golzari Jahan Abadi et al., 2017).

Furthermore, another study indicated that mother corms in higher weight classes enhanced both the number and fresh weight of saffron flowers, as well as stigma yield (Sahabi et al., 2017). Additionally, findings from a study by Koocheki et al. (2018) revealed that increasing maternal corm weight positively influenced all vegetative and reproductive traits of saffron. Heavier corms -due to their greater reserve nutrients and higher potential for activating vegetative and reproductive buds- produced a significantly higher number of flowers. A study on the role of mycorrhizal fungi in saffron flower traits showed that the simple application of arbuscular mycorrhiza dramatically increased the number of flowers ($p \leq 0.01$) (Habibi et al., 2021). Conversely, the absence of mycorrhizal inoculation resulted in the lowest saffron flower count.

Also, the combined application of three biofertilizers (*Azotobacter*, *Bacillus subtilis*, and Pseudomonas) led to a 92% increase in saffron flower production compared to the control treatment (Alizadeh et al., 2021). Notably, the highest flower count was achieved with the application of 0.2% *Azotobacter* treatment. In this study, maximum and minimum flower densities were recorded at 53.2 and 41 flowers per square meter, respectively (Kheiry et al., 2018). In a separate trial, *Bacillus subtilis* inoculation displayed a significant effect on field flower production. Peak flower numbers during harvest were obtained using a bacterial concentration of 10^8 CFU/ml (Salehi et al., 2022). Given the direct correlation between corm weight and flowering potential, the application of biofertilizers to enhance corm weight may effectively increase saffron flower production. Moreover, combining biofertilizer with heavier mother corms significantly increased saffron flower yield.

Flower Yield

The second-year results presented in Table 8 revealed considerable effects of both individual and interactive factors on flower yield, with the exception of the interaction between corm weight and *Azotobacter* application, which illustrated no significant effect. The combined treatment of 8.1-12 g corms with mycorrhizal inoculation significantly improved flower yield. Mean comparison analysis of treatment interactions demonstrated that the maximum flower yield (37.66 g/m²) was achieved in the second year with the 8.1-12 g weight class treatment incorporating both mycorrhizal fungi and *Azotobacter* application (Figure 2).

A study suggested that fresh flower yield per hectare was substantially higher in mother corms weighing 9-10 grams compared to lighter corms (4-5 grams) (Koocheki et al., 2015). Subsequent investigations by Khavari et al. (2016) further confirmed the significant influence of saffron corm weight on crop performance, a finding supported by multiple studies (Khavari et al., 2016; Sahabi et al., 2017; Mardani Asl et al., 2018). Results of biofertilizer application methods showed that separate use of nitrogenous and phosphorus biofertilizers, along with small amounts of chemical fertilizers, could increase saffron yield (Golzad, 2008). In another study, the highest yield was obtained either from simultaneous inoculation with mycorrhizal fungi and nitrogen-fixing bacteria in low-input systems or from the control treatment in high-input systems (Jahan et al., 2008). The application of bacterial biofertilizers significantly enhanced saffron flower yield in both years of the study period. By optimizing the plant's nutrient uptake, these biofertilizers improved vegetative growth, consequently exerting a positive influence on reproductive development (Salehi et al., 2022). The mechanism likely involves rhizospheric microorganisms that fix atmospheric nitrogen. These beneficial microbes enrich nutrient availability in the root zone (rhizosphere), creating a symbiotic relationship that promotes overall plant growth and productivity.

Fresh and Dry Stigma Yield

The second-year ANOVA results (Table 8) demonstrated significant effects ($p < 0.05$) of all main treatments and their interactions on both fresh and dry stigma yields. Figures 3 and 4 illustrate that the highest yields (2.32 g/m² fresh and 0.58 g/m² dry) were achieved with 8.1-12 g mother corms treated with both mycorrhizal fungi and *Azotobacter* inoculation. In contrast, the lowest yields

(0.44 g/m² fresh and 0.04 g/m² dry) occurred when using smaller 0.1-4 g corms without either microbial treatment. The study by Koocheki and Sabet Teimouri (2014) on four corm weight classes showed that the highest saffron stigma yield (0.62 g/m²) was obtained from 8.1-12 g corms. Another report indicated that the maximum dry stigma-plus-style yield (0.25 g/m²) came from corms weighing over 15 grams (Sahabi et al., 2017). Other researchers have reported similar findings (Mardani Asl et al., 2018; Ansaryan Mahabadi et al., 2019). Corresponding results indicated that combining mycorrhizal fungi application with full irrigation significantly increased both fresh and dry saffron stigma yields compared to the control group (Habibi et al., 2021). The bacterial biofertilizer Bacillus appreciably increased fresh and dry saffron stigma weights in the study, with reported dry stigma weights ranging from 6.14 to 7.13 g/m² (Salehi et al., 2022). Research on nitrogen biofertilizers also indicated that Nitroxin biofertilizer increased dry stigma and style yield by 83% compared to the control (Golzad, 2008).

Planting heavier corms from higher weight classes leads to better stigma yields, as these corms contain greater nutrient reserves and establish roots more quickly in soil. This gives the plant more opportunity to utilize light, environmental factors, and nutrients, ultimately increasing overall performance, including stigma production. Saffron leaves serve as the photosynthetic source for corm development. Fewer leaves reduce photosynthesis, decreasing food production and nutrient transfer to the corms, which ultimately lowers saffron stigma yield (Khazaei et al., 2013).

Table 8. The ANOVA (mean of squares) of the effect of corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron flower traits in two years

Source of variance	df	Number of flower per m^2	Yield of flower	Fresh yield of stigma	Dry yield of stigma
Replication	2	67.84 ^{ns}	1.92 ^{ns}	0.02 ^{ns}	0.001 ^{ns}
Corm Weight (CW)	2	1129.04 **	156.21 **	1.16 **	0.08 **
Mycorrhizal Fungi (MF)	1	189.06 *	67.50 *	1.63 **	0.14 **
Azotobacter (AB)	1	269.50 *	78.34 *	1.41 **	0.11 **
CW×MF	2	665.43 **	112.23 **	0.40 **	0.03 **
CW ×AB	2	63.46 ^{ns}	11.77 ^{ns}	0.18 **	0.01 **

MF×AB	1	1566.84 **	160.16**	0.13 **	0.01**
CW ×MF×AB	2	1076.29**	151.53 **	0.27 **	0.02 **
Error	22	41.59	7.70	0.01	0.006
C.V (%)	--	12.18	13.61	12.02	11.69

ns, **, *: non-significant and significant at 5% and 1% of probability levels, respectively

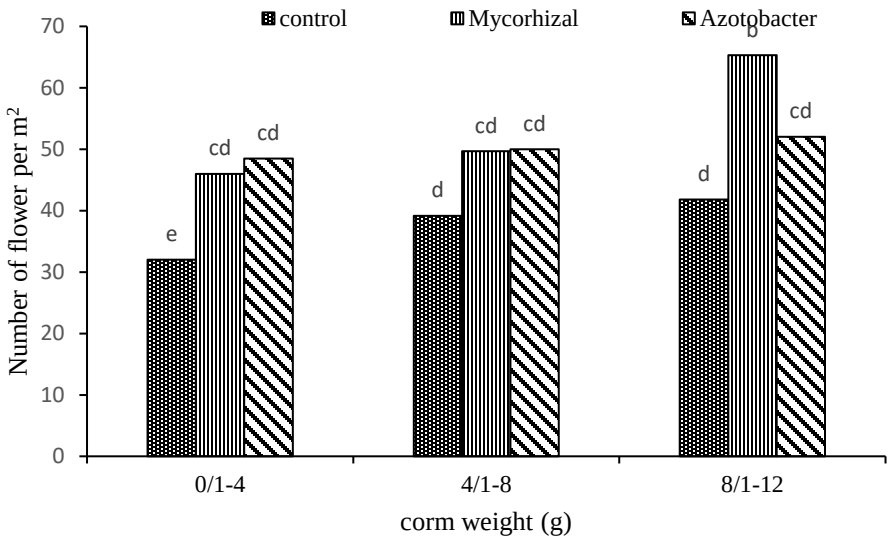

Figure 1. Interaction effect of corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron flower number in two years

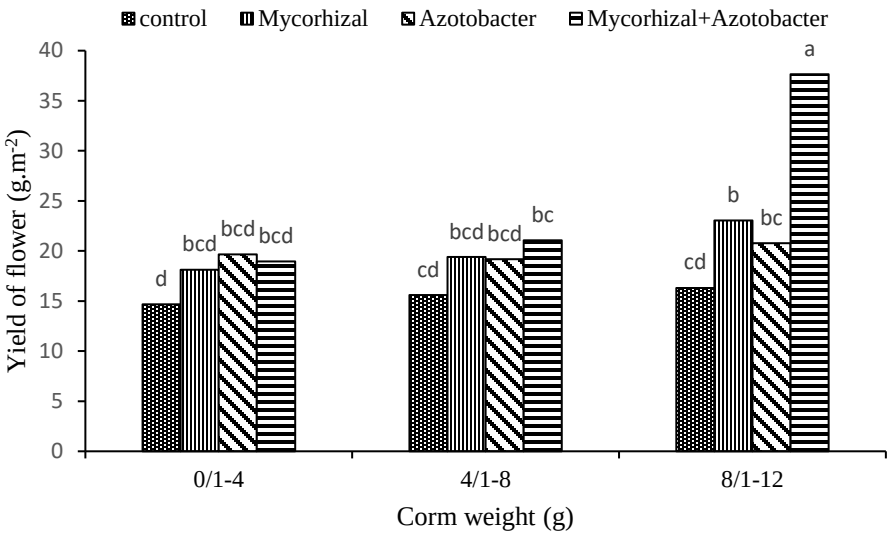

Figure 2. Interaction effect of corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron flower yield in two years

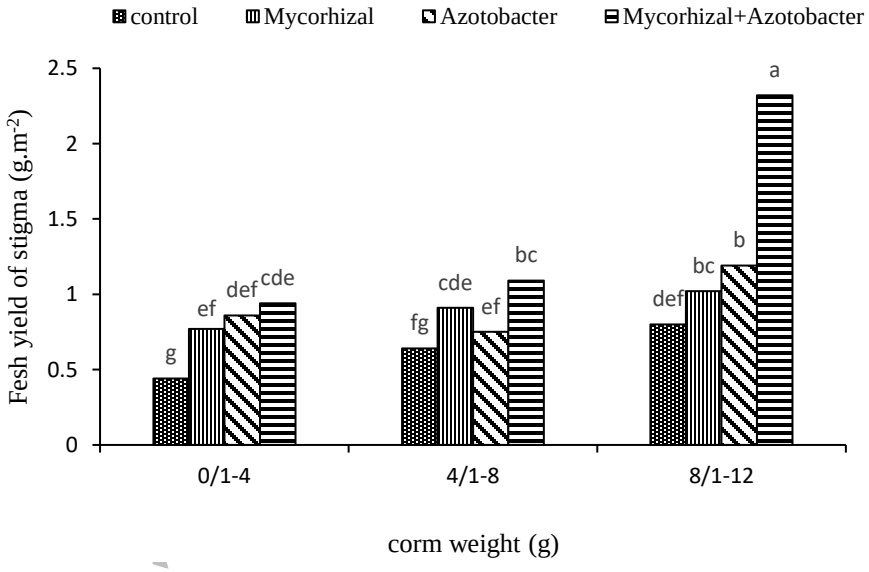

Figure 3. Interaction effect of corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron fresh yield of stigma in two years

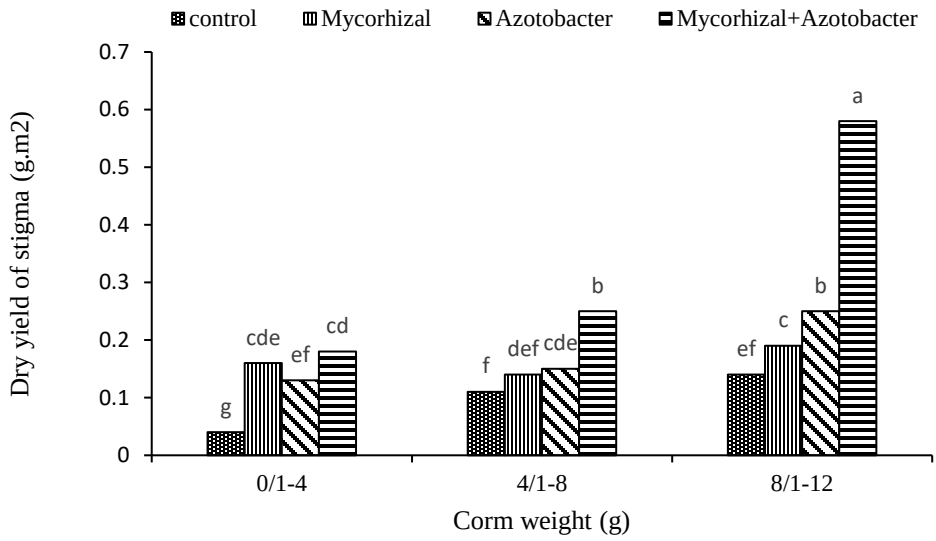

Figure 4. Interaction effect of corm weight, mycorrhizal fungi and Azotobacter bacteria on saffron fresh yield of stigma in two years

Conclusion

The combined application of *Azotobacter* bacteria and mycorrhizal fungi with corms weighing 8.1-12g significantly improved saffron growth and yield of saffron. These findings demonstrate that incorporating biofertilizers and fostering mycorrhizal hyphae in agricultural soils, particularly in saffron planting beds, enhance cultivation efficiency while reducing the damaging effects of chemical fertilizer overuse.

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