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Interactive effects of bacterial biofertilizers and soil properties on yield and quality of saffron (*Crocus sativus L.*): A Meta-Analysis

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ABSTRACT

This meta-analysis systematically reviewed the effects of bacterial biofertilizers on quantitative and qualitative traits of saffron (Crocus sativus L.) using data from 32 articles retrieved from Web of Science (50%), Scopus (53%), ScienceDirect (16%), and Magiran (47%). Statistical analyses included multivariate regression, correlation matrix analysis, and principal component analysis (PCA). These methods identified relationships between soil properties, management practices, and saffron yield and quality. Bacterial species richness and soil organic matter percentage were the strongest positive predictors of saffron traits, together explaining 59–81% of the variation in yield and secondary metabolite content. Fresh flower yield increased by 43% per additional bacterial species and by 51% per 1% increase in organic matter. Dry stigma yield increased by 27% per additional bacterial species and by 163% per 1% increase in organic matter. Conversely, each unit increase in EC and chemical fertilizer reduced fresh flower yield by 2.4% and 0.4%, and dry stigma yield by 18.2% and 0.3%, respectively. PCA revealed that biological soil fertility parameters (organic matter, biofertilizer complexity, and treatment duration) clustered separately from chemical inputs, indicating that saffron yield and quality are driven primarily by biological rather than chemical nutrient intensity. Soil texture analysis showed that loam-clay-sandy soils maximized fresh flower yield (124% increase), whereas clay loam soils optimized crocin and picrocrocin production (18–38% increases). These findings demonstrate that sustainable saffron production requires integrated bio-organic management strategies prioritizing bacterial richness , organic matter enrichment, and soil pH optimization over synthetic chemical fertilizers.

Keywords: Organic Matter, Electrical conductivity, Stigma, Crocin, Safranal, Picrocrocin

Abbreviations: Cl^-: chloride ion, dS/m: deciSiemens per meter, EC: soil electrical conductivity, INM: integrated nutrient management, Na^+: sodium ion, NPK: nitrogen, phosphorus, potassium, ns: not statistically significant, PC1: principal component 1, PC2: principal component 2, PCA: principal component analysis, PGPR: plant growth promoting rhizobacteria, R^2: coefficient of determination, r: correlation coefficient, β: standardized coefficient, SAS: Statistical Analysis System

INTRODUCTION

Saffron (*Crocus sativus* L) is a herbaceous, perennial, tuberous plant of the Iridaceae family without aerial stems [1]. Saffron is regarded as one of the most expensive spices worldwide because of its distinctive medicinal and nutritional properties, which are largely attributed to the secondary metabolites crocin, picrocrocin, and safranal [2]. The excessive application of chemical fertilizers in recent decades has led to serious environmental contamination, particularly of soil and water resources. These contaminants consequently enter the human food chain and pose risks to public health [3]. Biofertilizers, especially microbial inoculants, are therefore increasingly promoted as effective alternatives to chemical fertilizers, as they regulate and enhance nutrient uptake while supporting sustainable and safe agricultural production [4]. Microbial and bacterial biofertilizers can improve crop performance through multiple mechanisms. These include nitrogen fixation, phosphorus and potassium solubilization, phytohormone secretion, improvement of soil structure, suppression of soil-borne diseases, and enhancement of plant tolerance to environmental stresses [4, 5]. As highlighted in a comprehensive review of plant microbiomes, beneficial microorganisms can enhance crop quality and yield through both direct mechanisms (such as nitrogen fixation, phosphorus solubilization, and phytohormone production) and indirect mechanisms (such as inducing systemic resistance and pathogen suppression) [6]. The beneficial effects of nitrogen-fixing and phosphate-solubilizing bacterial inoculants on the physiological performance and stress tolerance of medicinal plants have been well documented [7].

With rising global demand for saffron, the optimization of its cultivation has become a key research priority [8]. Among the proposed strategies, the integration of beneficial microorganisms with organic and mineral fertilizers is emerging as a promising approach to increase both yield and quality. The positive influence of plant growth-promoting rhizobacteria (PGPR) on the physiological traits and productivity of medicinal plants, particularly under drought stress, further underscores their potential for saffron cultivation systems [9, 10]. Bacterial biofertilizers contribute to agricultural sustainability by improving soil health and promoting plant growth, thereby reducing dependence on synthetic inputs. These inputs can enhance the physicochemical properties of soil, enrich the soil microbial community, and help maintain microecological balance, which collectively improves nutrient acquisition, boosts crop yield, and strengthens plant resistance to diseases [11]. The application of co-inoculated PGPRs in combination with organic amendments has been shown to increase nutrient uptake and achieve higher levels of yield and quality in medicinal plants, supporting the shift toward sustainable agricultural practices [12].

In recent years, studies have shown that microbial and bacterial inputs can affect key agronomic parameters - including flower number, fresh flower yield and stigma production - as well as the biosynthesis of important saffron compounds such as crocin, picrocrocin and safranal [8]. For example, inoculation of saffron with Bacillus subtilis has resulted in increased quality and quantity of saffron flowers, possibly due to bacterial siderophore production [13]. Other studies have shown that certain bacterial strains can increase fresh and dry stigma weight, stigma length, leaf number, corm weight, and dry stigma yield in saffron [14-16]. A study showed that PGPR can also be used as a biofungicide for corm rot diseases in saffron [17].

Although individual studies have reported positive effects of bacterial biofertilizers on saffron yield and quality, a comprehensive quantitative synthesis of these findings is lacking. To date, no meta-analysis has systematically evaluated how soil properties (e.g., organic matter, pH, EC) and management practices (e.g., manure application, chemical fertilizer use) interact with bacterial richness to influence saffron performance. Furthermore, the relative contribution of bacterial species richness versus organic matter content to yield improvement remains unclear. To address these gaps, we conducted a meta-analysis of 32 studies to (i) quantify the effects of bacterial biofertilizers on fresh flower yield, dry stigma yield, safranal, crocin, and picrocrocin; (ii) identify the most influential soil and management predictors through multivariate regression and correlation analyses; and (iii) assess the underlying patterns using principal component analysis (PCA). Our study provides the first quantitative estimates of percentage changes per unit increase in key predictors (bacterial species number, organic matter, EC, pH) and reveals that biological soil fertility parameters outweigh chemical inputs in determining saffron yield and quality. This novel insight supports a paradigm shift toward integrated bio-organic management strategies for sustainable saffron production.

MATERIALS AND METHODS

This study conducted a systematic review and meta-analysis to assess the effects of bacterial biofertilizers on the quantitative and qualitative traits of saffron. Relevant studies were identified through a systematic search of the Web of Science (50%), Scopus (53%), ScienceDirect (16%), and Magiran (47%) databases covering the period from 2009 to 2024. Following the screening and eligibility assessment, data from 32 articles were extracted for the final meta-analysis. Studies were included if they met the following minimum requirements: (1) study design – controlled field or greenhouse experiments that compared at least one bacterial biofertilizer treatment with a concurrent non-bacterial control under identical management; (2) sample size – studies had to report at least three replicates per treatment and provide a measure of variance (standard deviation, standard error, or 95% confidence interval); studies with single-plot observations (no true replication) or without any variance measure were excluded; (3) reported data – studies had to provide means and variance measures for at least one quantitative outcome of interest (fresh flower yield, dry stigma yield, safranal, crocin, or picrocrocin); data presented exclusively in figures without numerical values were excluded unless raw data were obtained from the authors. Additionally, studies lacking complete numerical data or with unclear methodology were excluded.

From each eligible article, information was extracted on bacterial genus and species, number of species used, soil properties (texture, pH, EC, percentage of organic matter), number of years since treatment, amount of animal/chemical/biofertilizers, microbial inoculant concentration, and saffron yield and quality parameters. Bacterial richness was defined as the number of distinct bacterial species co-inoculated in the biofertilizer formulation (species richness), as all included studies reported this count (range 1–6) and none used diversity indices such as Shannon or Simpson.

For all quantitative and qualitative traits, we calculated the percentage change of the bacterial biofertilizer treatment relative to the non-bacterial control using the formula:

$$Change\ percentage = \frac{Treatment\ value - control}{Control} \times 100 \quad \text{(Eq: 1)}$$

This normalized data across studies regardless of original units (e.g., kg/ha, %, absorbance, mg/g).

Statistical analyses included the following:

(1) correlation analysis (Pearson's coefficient) to assess relationships between independent variables (e.g., soil organic matter percentage, bacterial species) and saffron traits; (2) multiple regression models (linear, logarithmic, polynomial) to predict trait changes, with model selection based on R^2; and (3) principal component analysis (PCA) to reduce dimensionality, identify treatment-trait relationships, and visualize patterns through 2D plots. In the regression models, “unit increase” refers to a one-unit change on the original measurement scale: one additional bacterial species for bacterial richness , a 1% absolute increase for soil organic matter, and a 1 dS/m increase for EC. All correlation and regression analyses were performed using R software (version 4.2.1). Principal component analysis (PCA) was also conducted in R using the ‘FactoMineR’ package. SAS (version 9.4) was used for additional regression diagnostics, and Minitab (version 19) was used for initial data exploration. Python (version 3.9) was used for data extraction and management but not for the final statistical models.

RESULTS

Fresh Flower Yield

Based on the results of multivariate regression analysis (Table 1), the statistical model obtained with $R^2 = 0.68$ explained 68% of the variation in fresh saffron flower yield. The indices of the number of bacterial species in the bacterial biofertilizer formulation, the percentage of organic matter, the number of years after treatment, and the use of animal manure had a positive relationship with fresh flower yield, while the relationship of EC, pH, and chemical fertilizer consumption with this index was inverse.

Table 1 Relationship between fresh saffron flower yield and various soil parameters and planting management

Variable	Parameter estimate	t value	Standardized estimate
Intercept	832.37	-	-
Number of bacterial species in bacterial biofertilizer formulation	43.01	2.81 *	0.70
EC (dS/m)	– 2.36	4.68 **	– 0.04
pH	– 112.17	2.28 *	– 0.28
Organic matter (percentage)	51.25	3.91 **	0.60
Number of years after treatment	12.70	1.82 ns	0.13
Manure consumption (tons/hectare)	0.23	4.97 **	0.35
Fertilizer consumption/hectare	– 0.43	2.31 *	– 0.33
Model $R^2 = 0.68$			

ns, * and **: non-significant and significant at $p < 0.05$ and $p < 0.01$, respectively

Among the variables studied, several factors showed a significant effect on fresh saffron flower yield. These included the number of bacterial species in the biofertilizer formulation, the percentage of organic matter, pH, EC, and the use of animal and chemical fertilizers. Specifically, the number of bacterial species (β = 0.70) and the percentage of organic matter (β = 0.60) had the greatest positive effect on yield. With each unit increase, fresh saffron flower yield increased significantly by 43% and 51%, respectively. Manure application (β = 0.35) also showed a positive and significant effect on yield. With each unit increase, flower yield improved by 0.23%. In contrast, pH (β = –0.28), soil EC (β = –0.04), and chemical fertilizer application (β = –0.33) had a negative and significant effect on fresh flower yield. An increase in any of these variables caused a decrease in yield. Each unit increase in EC and chemical fertilizer application decreased flower yield by 2.36% and 0.42%, respectively. For pH, the regression coefficient indicated a strong negative effect (β = –0.28, $p < 0.05$), but because the model used absolute yield values, the percentage decrease is not directly interpretable from the coefficient. Qualitatively, higher pH consistently reduced yield across studies. The variable of number of years after treatment did not have a significant effect on yield (ns) and was therefore considered statistically insignificant in the final model.

Correlation matrix analysis also showed (Fig. 1) that the highest positive correlation with fresh flower yield was related to the number of bacterial species in the bacterial biofertilizer formulation (*r* = 0.42) and the percentage of organic matter (*r* = 0.24), such that increased microbial activity and improved soil organic status were associated with increased yield. A strong positive relationship was also observed between organic matter percentage and bacterial species number (*r* = 0.69). In contrast, soil pH showed a relatively strong negative correlation with yield (*r* = –0.34). Animal manure consumption (*r* = 0.11) and the number of years after treatment (*r* = 0.17) had weak positive correlations with yield, while chemical fertilizer consumption (*r* = –0.19) showed a negative relationship. Regarding soil type and texture (Fig. 2), the bacteria in biofertilizers had the greatest effect on increasing saffron flower yield in sandy loam soil. In this soil texture, bacterial biofertilizers increased fresh flower yield by 124% on average.

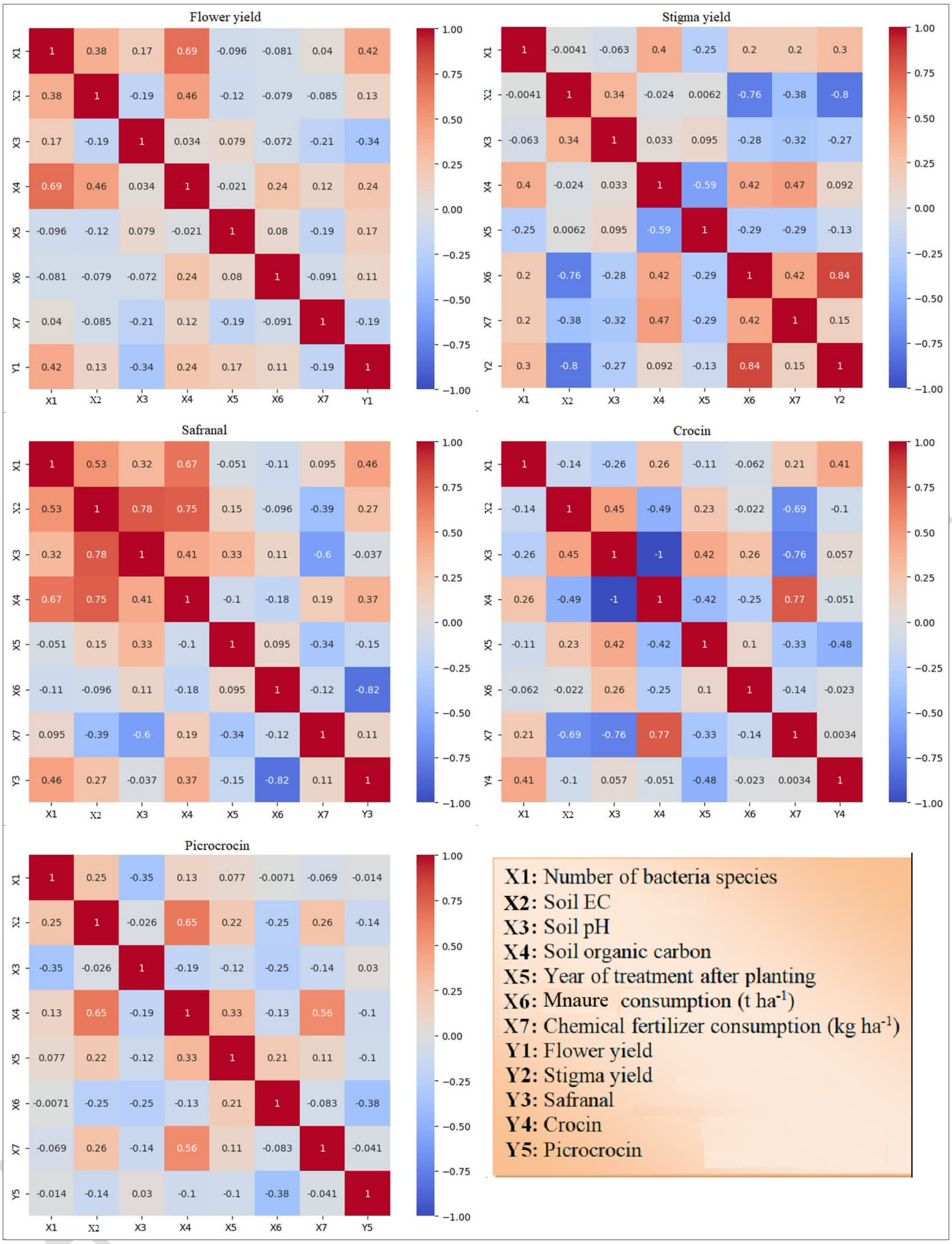

Fig. 1 Correlation of some quantitative and qualitative characteristics of saffron with soil parameters and planting management when using bacterial biofertilizers.

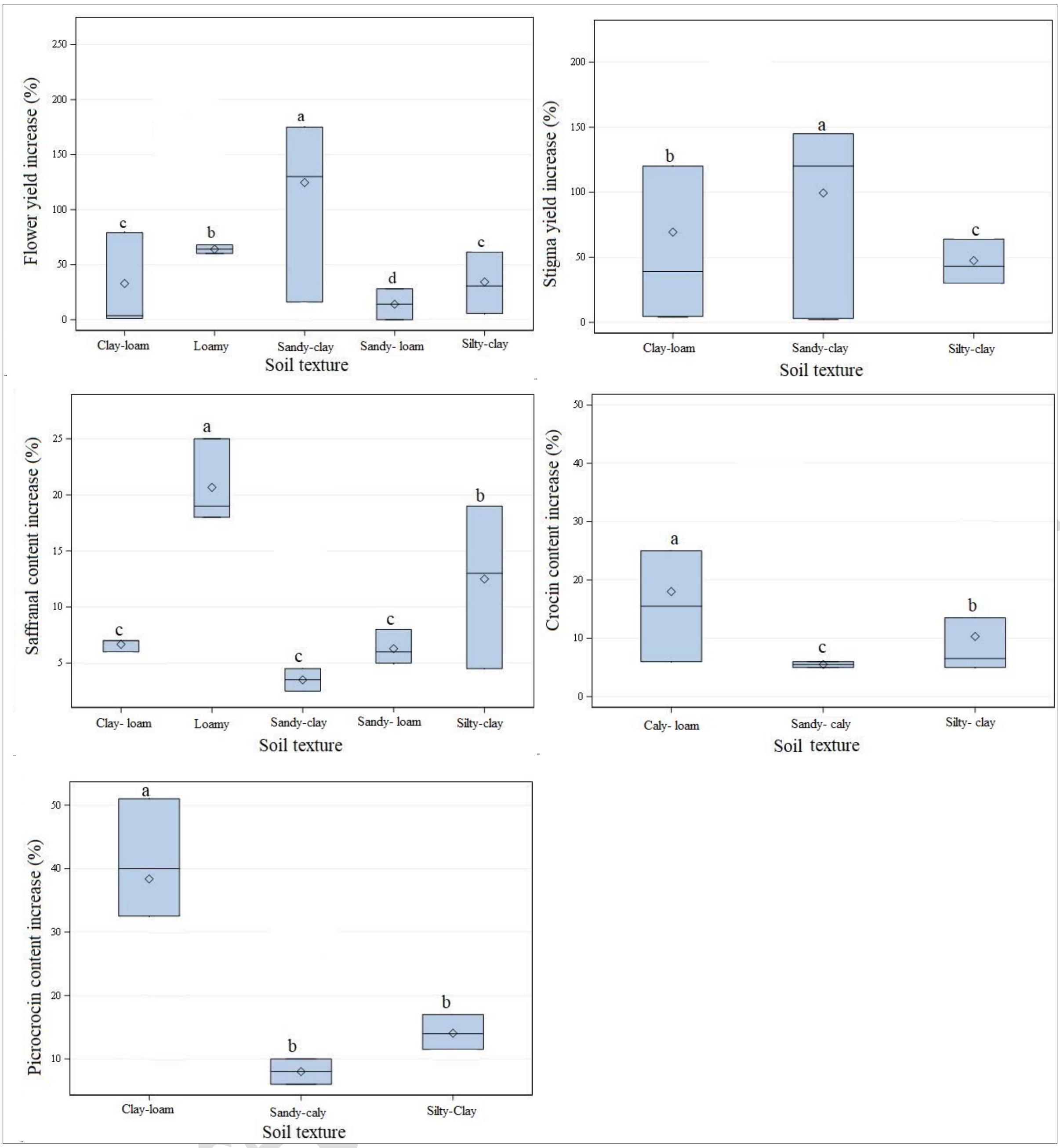

Fig. 2 Effect of soil texture on the efficiency of bacteria in bacterial biofertilizers on quantitative and qualitative traits of saffron

Dry stigma yield

Based on the results of multivariate regression analysis (Table 2), the statistical model with $R^2 = 0.72$ explained 72% of the variation in saffron dry stigma yield. Number of bacterial species in the bacterial biofertilizer formulation, organic matter, and manure consumption had a positive relationship with dry stigma yield, whereas EC, pH, years after treatment, and chemical fertilizer consumption had an inverse relationship.

Table 2 Relationship between dry stigma yield and various soil parameters and planting management

Variable	Parameter estimate	t value	Standardized estimate
Intercept	– 270.85	-	-
Number of bacterial species in bacterial biofertilizer formulation	27.87	2.37 *	0.50
EC (dS/m)	– 18.19	3.07 **	– 0.35
pH	– 43.92	0.49 ns	– 0.09
Organic matter (percentage)	163.51	2.23 *	0.78
Number of years after treatment	– 3.21	0.19 ns	– 0.03
Manure consumption (tons/hectare)	6.79	3.50 **	0.86
Fertilizer consumption/hectare	– 0.35	2.71 *	– 0.13
Model $R^2 = 0.72$			

ns, * and **: non-significant and significant at $p < 0.05$ and $p < 0.01$, respectively

Among the variables studied, the number of bacterial species in the bacterial biofertilizer formulation, EC, percentage of organic matter, chemical fertilizer consumption, and manure consumption showed a significant effect on saffron dry stigma yield. Specifically, manure consumption (β = 0.86) and organic matter percentage (β = 0.78) had the most positive effect on dry stigma yield. With each unit increase, dry stigma yield increased significantly by 6.79% and 163%, respectively. The number of bacterial species in the bacterial biofertilizer formulation (β = 0.50) also showed a positive and significant effect on dry stigma yield. With each additional species, dry stigma yield improved by 27%. In contrast, soil EC (β = –0.35) and chemical fertilizer use (β = –0.13) had a negative and significant effect on dry stigma yield. An increase in either variable caused a decrease in yield. Each unit increase in EC and chemical fertilizer use decreased dry stigma yield by 18.18% and 0.34%, respectively. The variables of number of years after post-planting treatment and pH did not have a significant effect on dry stigma yield (ns) and were therefore considered statistically insignificant in the final model.

Correlation matrix analysis also showed (Fig. 1) that the highest positive correlations with dry stigma yield were for manure consumption (*r* = 0.84) and bacterial species number (*r* = 0.30). Thus, increased microbial activity and improved soil organic status were associated with increased yield. Relatively strong correlations were also observed. These included the correlation between organic matter percentage and chemical fertilizer consumption (*r* = 0.47), between organic matter percentage and manure consumption (*r* = 0.42), and between organic matter percentage and bacterial species number (*r* = 0.47). These findings indicate the importance of organic matter as the axis of soil biological activity and fertility, especially when combined with animal and chemical fertilizers. The lowest correlation with dry stigma yield was for EC (*r* = –0.80). Regarding soil type and texture, the bacteria in biofertilizers had the greatest effect on increasing dry stigma yield in sandy loam soil (Fig. 2). In this soil texture, bacterial biofertilizers increased dry stigma yield by 99.42% compared to no bacterial application.

Safranal

Based on multivariate regression analysis (Table 3), the statistical model with $R^2 = 0.81$ explained 81% of the variation in saffron safranal content. The indices of the number of bacterial species in the bacterial biofertilizer formulation, the percentage of organic matter, and EC had a positive relationship with the amount of safranal. While, the relationship between pH and manure consumption with this index was inverse.

Table 3 Relationship between Safranal and various soil parameters and planting management

Variable	Parameter estimate	t value	Standardized estimate
Intercept	104.46	-	-
Number of bacterial species in bacterial biofertilizer formulation	6.55	2.75 **	0.41
EC (dS/m)	2.75	2.06 **	0.20
pH	– 13.28	3.18 **	– 0.46
Organic matter (percentage)	7.02	2.48 *	0.39
Number of years after treatment	0.66	0.36 ns	0.05
Manure consumption (tons/hectare)	– 0.21	4.34 **	– 0.57
Fertilizer consumption/hectare	0.01	0.10 ns	0.02
Model $R^2 = 0.81$			

ns, * and **: non-significant and significant at $p < 0.05$ and $p < 0.01$, respectively

Among the variables studied, the number of bacterial species in the bacterial biofertilizer formulation, EC, pH, and manure consumption showed a significant effect on the amount of safranal. Specifically, the number of bacterial species (β = 0.41) and organic matter percentage (β = 0.39) had the greatest positive effect on safranal content. With each unit increase, safranal increased significantly by 6.5% and 7%, respectively. EC (β = 0.20) also showed a positive and significant effect on safranal content. With each unit increase, safranal increased by 2.74%. In contrast, manure use (β = –0.57) and pH (β = –0.46) had a negative and significant effect on safranal content. An increase in either variable caused a decrease in safranal. Each unit increase in manure use and pH decreased safranal content by 0.2% and 13.28%, respectively. The variables of number of years after post-planting treatment and chemical fertilizer use did not have a significant effect on safranal content (ns) and were therefore considered statistically insignificant in the final model.

Correlation matrix analysis also showed (Fig. 1) that the highest positive correlations with safranal content were for bacterial species number (*r* = 0.46) and organic matter percentage (*r* = 0.37). Strong positive correlations were also observed: between pH and EC (*r* = 0.78), between organic matter percentage and EC (*r* = 0.75), and between organic matter percentage and bacterial species number (*r* = 0.67). In contrast, livestock manure use showed a strong negative correlation with safranal content (*r* = –0.82). EC (*r* = 0.27) and chemical fertilizer use (*r* = 0.11) had relatively weak correlations with safranal content, while the number of years after treatment had a weak negative correlation (*r* = –0.15). In general, these results indicate that soils with higher organic matter (not manure added in the same year) and higher microbial activity provide a more favorable environment for microorganisms and increased saffron safranal. In contrast, manure use drives saffron toward quantitative and vegetative properties. It acts in the opposite direction for quality traits such as safranal. Bacteria in loamy soil had the greatest effect on increasing safranal compared to no bacterial application. In this soil texture, bacterial biofertilizers increased safranal by 20.66%.

Crocin

Based on the results of multivariate regression analysis (Table 4), the statistical model obtained with $R^2 = 0.59$ explained 59% of the variation in saffron crocin content. The indices of bacterial species number in the biofertilizer formulation, organic matter percentage, and animal manure use had a positive relationship with crocin content, while the number of years after treatment had an inverse relationship.

Table 4 Relationship between Crocin and various soil parameters and planting management

Variable	Parameter estimate	t value	Standardized estimate
Intercept	31.12	-	-
Number of bacterial species in bacterial biofertilizer formulation	3.66	2.83 *	0.37
EC (dS/m)	– 0.93	0.22 ns	– 0.05
pH	– 0.75	0.09 ns	– 0.02
Organic matter (percentage)	11.21	3.19 **	0.40
Number of years after treatment	– 7.34	2.34 *	– 0.50
Manure consumption (tons/hectare)	0.02	2.29 *	0.36
Fertilizer consumption/hectare	0.00	0.03 ns	0.01
Model R^2 = 0.59			

ns, * and **: non-significant and significant at $p < 0.05$ and $p < 0.01$, respectively

Among the variables studied, bacterial species number, organic matter percentage, years after treatment, and animal manure use showed a significant effect on crocin content. Specifically, organic matter percentage (β = 0.40), bacterial species number (β = 0.39), and animal manure use (β = 0.36) had the greatest positive effects. With each unit increase, crocin increased significantly by 11.2%, 3.65%, and 0.024%, respectively. In contrast, the number of years after treatment (β = –0.50) had a negative and significant effect. Each additional year decreased crocin content by 34.7%. The variables EC, pH, and chemical fertilizer use did not have a significant effect on crocin content (*p* > 0.05) and were therefore considered statistically insignificant in the final model.

Correlation matrix analysis also showed (Fig. 1) that the highest positive correlation with crocin content was for bacterial species number (*r* = 0.41). Increasing microbial richness was associated with increasing crocin content. A strong positive correlation was also found between organic matter and chemical fertilizer use (*r* = 0.77) for crocin production. In contrast, the number of years after treatment showed a relatively strong negative correlation with crocin content (*r* = –0.48). Interestingly, a very strong negative correlation was observed between organic matter percentage and soil pH (*r* = –1.00) in crocin production. In general, these results show that microbial richness , organic matter, and proper nutrition increase crocin content when bacterial biofertilizers are used. The effect of bacteria decreases over many years for crocin production. Furthermore, a strong negative correlation was observed between pH and organic matter percentage. Regarding soil type and texture, bacteria in clay loam soil had the greatest effect on increasing crocin compared to no bacterial application. In this soil texture, bacterial biofertilizers increased crocin by 18%.

Picrocrocin

Based on the results of multivariate regression analysis (Table 5), the statistical model with R^2 = 0.68 explained 68% of the variation in saffron picrocrocin content. The number of bacterial species in the biofertilizer formulation and organic matter percentage had a positive relationship with picrocrocin content, while EC and manure consumption had an inverse relationship.

Table 5 Relationship between Picrocrocin and various soil parameters and planting management

Variable	Parameter estimate	t value	Standardized estimate
Intercept	42.11	-	-
Number of bacterial species in bacterial biofertilizer formulation	0.73	2.20 *	0.24
EC (dS/m)	– 10.40	2.21 *	– 0.42
pH	0.30	1.04 ns	0.01
Organic matter (percentage)	9.47	3.24 **	0.27
Number of years after treatment	– 1.10	0.43 ns	– 0.08
Manure consumption (tons/hectare)	– 0.14	2.98 **	– 0.36
Fertilizer consumption/hectare	0.00	0.81ns	0.04
Model R^2 = 0.68			

ns, * and **: non-significant and significant at $p < 0.05$ and $p < 0.01$, respectively

Among the variables studied, bacterial species number, EC, organic matter percentage, and manure consumption showed a significant effect on picrocrocin content. Specifically, bacterial species number (β = 0.24) and organic matter percentage (β = 0.27) had the greatest positive effects. With each unit increase, picrocrocin increased significantly by 0.72% and 9.47%, respectively. In contrast, EC (β = –0.42) and manure application (β = –0.36) had a negative and significant effect. An increase in either variable caused a decrease in picrocrocin. Each unit increase in EC and manure application decreased picrocrocin by 10.4% and 0.13%, respectively. The variables pH, number of years after treatment, and fertilizer application did not have a significant effect on picrocrocin content (*p* > 0.05) and were therefore considered statistically insignificant in the final model.

Correlation matrix analysis also showed (Fig. 1) that the only positive correlation was with pH (*r* = 0.03), which was very weak and can be ignored. Strong positive relationships were also observed: between EC and organic matter percentage (*r* = 0.65) and between organic matter percentage and fertilizer application (*r* = 0.56). In contrast, manure application showed a relatively strong negative correlation with picrocrocin content (*r* = –0.38), and EC showed a weak negative correlation (*r* = –0.13). In general, these results indicate that soils with higher organic matter (not manure added in the same year) provide a favorable environment for microorganisms and increased saffron picrocrocin. Manure use drives saffron toward quantitative and vegetative properties and has the opposite effect on quality and picrocrocin production. Regarding soil type and texture, bacteria in clay loam soil had the greatest effect on increasing picrocrocin compared to no bacterial application. In this soil texture, bacterial biofertilizers increased picrocrocin by 38.37%.

PCA Results

Principal component analysis revealed that the first two components captured the dominant structure of the dataset, effectively separating the soil and management variables from the saffron yield and quality responses (Figs. 3 and 4). PC1 was strongly and positively associated with organic matter, year of treatment application, and the level of biofertilizer complexity (number of bacterial strains), while showing a negative association with soil EC. PC2 was primarily influenced by manure and chemical fertilizer inputs. The clustering of organic matter, multi-strain biofertilizer use, and treatment year in the same quadrant indicated that these variables co-varied and collectively represented a broader management–soil fertility gradient.

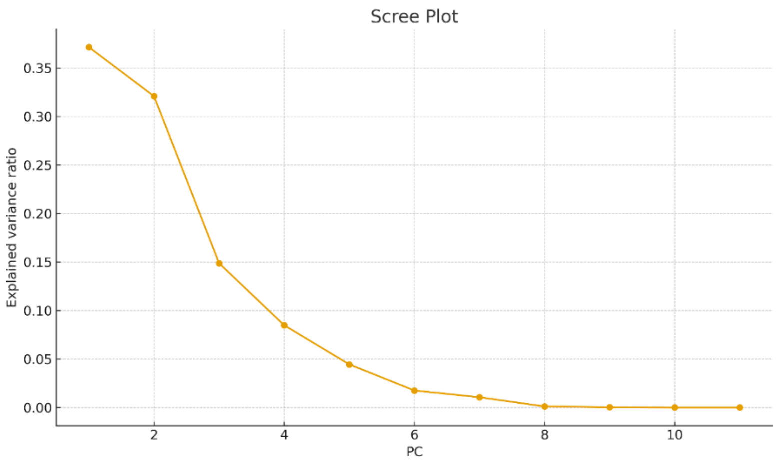

Fig. 3 Scree plot of principal component analysis based on soil properties, management variables, and saffron yield and quality traits.

The biplot demonstrated that the dependent variables were most closely aligned with the axis defined by organic matter, treatment year, and bacterial strain number. These dependent variables included changes in fresh flower yield, dry stigma yield, safranal, crocin, and picrocrocin contents. This alignment suggests that improvements in soil biological activity and fertility status were the primary drivers of enhanced saffron yield. In contrast, soil EC and chemical fertilizer inputs contributed minimally or antagonistically to quality-related traits. On PC1, a coherent cluster showed that a higher number of bacterial species and higher pH were associated with larger percentage improvements in fresh flower yield (Y1), dry stigma yield (Y2), and picrocrocin (Y5). Higher chemical fertilizer application was associated with lower observed percent-change responses in flower yield, dry stigma, and picrocrocin. PC2 received its strongest positive loadings from bacterial number, soil organic carbon, and chemical fertilizer consumption, all associated with safranal content. The number of bacterial species applied was positively associated with increases in safranal content. Overall, PCA confirmed that saffron yield and quality were more responsive to biologically driven improvements in soil conditions and multi-strain bacterial inoculation than to conventional chemical inputs. This highlights the central role of soil organic enrichment and biofertilizer richness in determining treatment efficacy.

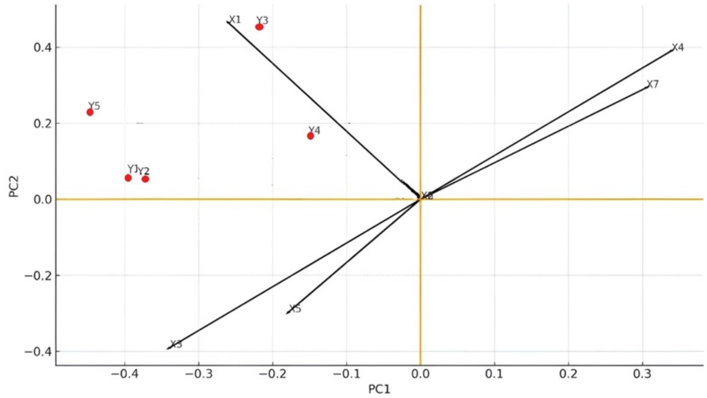

Fig. 4 PCA biplot of soil properties, management practices, and saffron yield and quality variables. X1: number of bacterial species, X2: Soil EC, X3: Soil pH, X4: Soil organic carbon, X5: year of treatment after planting, X6: manure consumption, X7: chemical fertilizer consumption, Y1: Fresh flower yield, Y2: stigma yield, Y3: Safranal, Y4: Crocin and Y5: Picrocrocin.

DISCUSSION

Regression and correlation analyses showed that two variables – the number of bacterial species in the biofertilizer formulation and the level of soil organic matter – exerted the strongest positive effects on both quantitative traits (flower and fresh stigma yield) and qualitative traits (safranal, crocin, and picrocrocin) of saffron. Notably, while animal manure application showed the greatest effect on dry stigma yield ($\beta = 0.86$), soil organic matter percentage ($\beta = 0.78$) and bacterial species richness ($\beta = 0.50$) also contributed significantly to yield improvement (Table 2). Overall, our correlation analysis indicates that soils with higher organic matter and microbial activity create a more favorable environment for saffron growth, whereas high pH and excessive chemical fertilizer use reduce yield by disrupting soil

biological and chemical balance. These results are consistent with findings that higher soil organic matter content increases soil structure, porosity, water retention, and nutrient buffering capacity, thereby increasing the efficiency of mineral and organic nutrient use [18, 19].
The strong positive correlation observed between organic matter percentage and bacterial species number (r = 0.69) suggests that these two factors interact synergistically to enhance soil biological fertility. The positive correlations between safranal content and both bacterial species number (r = 0.46) and organic matter (r = 0.37) suggest that enhanced microbial activity and soil organic status promote safranal accumulation. Soils enriched with organic matter maintain microbial activities. These activities cause nutrient mineralization and convert macro and micro elements into plant-available forms. They also promote long-term soil fertility. All of these processes are essential for maintaining saffron yield and stimulating secondary metabolism. The synergistic effect of microbial richness is due to functional complementarity between bacterial species, including nitrogen fixation by *Azotobacter* and *Azospirillum* and phosphate solubilization by *Pseudomonas* and *Bacillus* strains [20, 21]. Inoculation of different species reduces antagonistic interactions, reduces the accumulation of inhibitory metabolites, and supports a stable rhizosphere microbiome that suppresses pathogens and improves nutrient uptake efficiency [22]. This microbial synergy facilitates plant-microbe signaling and activates biosynthetic pathways related to terpenoid and carotenoid formation the biochemical basis for the synthesis of safranal, crocin, and picrocrocin [23]. In contrast, the introduction of chemical fertilizers alongside microbial inoculations has had negative effects on yield and quality traits, corroborating evidence that synthetic fertilizers reduce soil organic matter and suppress beneficial PGPR [24, 25].
Continuous use of such fertilizers leads to nutrient imbalances, reduced enzyme activity, and weakened microbial richness , resulting in reduced crocin and safranal biosynthesis. Previous work has shown that reliance on high nitrogen inputs (especially urea) or NPK diets often results in reduced flower and stigma yields compared to systems enriched with manure or compost [26, 27]. However, the observed positive correlation (*r* = 0.47) between manure and chemical fertilizer application with respect to dry stigma yield supports the concept of INM. Combining organic amendments with mineral fertilizers under controlled ratios can benefit from the rapid availability of mineral nutrients and long-term improvement of soil by organic matter. Such integrated strategies have been shown to increase flower number, stigma length, and dry weight while maintaining biological activity and soil stability [28, 29].
Regression and correlation analyses showed that manure application significantly and consistently increased saffron quantitative traits, including fresh flower yield and dry stigma yield. However, manure application had a decreasing effect on qualitative traits such as crocin, picrocrocin, and safranal. The reduction in quality parameters is largely attributed to the high availability of nutrients especially nitrogen introduced through manure, which shifts plant metabolism toward vegetative biomass accumulation at the expense of secondary metabolite synthesis. This trade-off has been well documented in various aromatic and medicinal crops [30, 31].
A similar pattern was reported by Esmailian et al. (2022), who found that bacterial biofertilizers without manure significantly increased picrocrocin content, while manure-amended treatments did not increase crocin concentration. Nevertheless, the quantitative benefits of manure are consistently supported by experimental data. For example, one study showed that combined manure and microbial inoculation treatments produced 19–27% higher dry matter yield than control treatments, indicating the synergistic effects of organic inputs and beneficial microorganisms on crop yield [32]. While manure exerts contrasting effects on yield versus quality, other soil chemical properties also play critical roles in determining saffron performance. Among these, soil pH and salinity have emerged as key factors that can either limit or enhance the efficacy of biofertilizers. Accordingly, the following sections examine the influence of soil pH and electrical conductivity.
Regarding soil pH, the regression analysis showed no positive effect, with relatively strong negative effects observed for fresh flower yield (β = –0.28) and safranal content (β = –0.46) (Table 1). The negative correlation between soil pH and fresh flower yield (r = –0.34) indicates that increased alkalinity reduces bacterial efficiency and, consequently, saffron growth and yield. High soil pH limits the richness and activity of rhizobacteria and reduces microbial-mediated nutrient mineralization and chelation processes of metals essential for plant nutrition [33]. Alkaline conditions favor microbial species that do not produce organic acids, which are critical for solubilizing micronutrients. The resulting deficiency of essential elements impairs terpenoid biosynthesis and leads to reduced safranal accumulation [34]. The very strong negative correlation between organic matter percentage and soil pH (r = –1.00) in crocin production underscores the vital importance of soil pH management. Maintaining an appropriate pH is necessary to preserve the benefits of organic matter for saffron quality traits. Soil salinity (EC) also showed a strong negative correlation with flower yield, stigma yield, and stigma color. However, qualitative responses were different: salinity increased safranal content, decreased picrocrocin, and had negligible effects on crocin. High EC induces osmotic stress and ion toxicity (especially Na^+ and Cl^-), limits water uptake, and suppresses metabolic processes, which dramatically reduces saffron yield beyond 2 dS/m [34, 35]. Furthermore, salinity stress disrupts the establishment and function of rhizobacteria and reduces the effectiveness of biofertilizers [8, 36]. Despite yield reduction, moderate salinity often stimulates the production of secondary metabolites as a protective response, although this is not uniform across compounds, confirming the complex role of stress physiology in saffron biochemistry [23, 37, 38]. These findings highlight the importance of soil organic matter enrichment and maintaining conditions that support bacterial viability, particularly under saline conditions, to achieve better stigma yield. Furthermore, the duration of biofertilizer application did not show any significant effect on fresh flower or stigma yield. However, it was negatively correlated with crocin (β = –0.50) and weakly correlated with picrocrocin (β = –0.08), while safranal was not affected (Table 5). This suggests that biofertilizer efficacy may decline over time due to changes in soil microbial composition, nutrient depletion, or reduced root–microbe signaling efficiency, potentially reducing the bioavailability of precursors for crocin synthesis [8, 39]. Such variability highlights the dynamic nature of microbial interactions, the long-term effects of which depend on soil physicochemical balance, environmental conditions, and microbial community stability [8, 23].
Bacterial biofertilizers had the greatest effect on quantitative traits (fresh flower and stigma yield) in loam-clay-sandy soil. In this soil texture, they increased fresh flower yield by 124% and dried stigma yield by 99.42% (Fig. 2). Bacterial biofertilizers exerted differential effects on secondary metabolites depending on soil texture. In loam-clay soil, these inoculants enhanced crocin and picrocrocin by 18% and 38.37%, respectively. Conversely, in loam soil, safranal production increased by 20.66% (Fig. 2). The superior performance observed

in loam-clay-sandy soil for quantitative traits (124% increase in fresh flower yield and 99.42% increase in dry stigma yield) can be attributed to optimal soil conditions. These conditions balance water retention, aeration, and nutrient availability – critical factors for PGPR survival, colonization, and activity. Sandy loam soils provide a balanced ratio of sand, silt, and clay, along with good drainage, adequate moisture retention, and sufficient oxygen. These properties increase microbial and enzymatic activities, thereby enhancing nutrient mineralization by these microbes [34, 40, 41]. Moreover, the loose matrix of this soil texture allows roots and microbes to explore a larger soil volume, thereby maximizing nutrient absorption and microbial proliferation [42-44]. Studies also show that the performance benefits of biofertilizers are maximized in sandy loam soils with moderate alkalinity, limited nutrients, or low organic matter [34, 44, 45]. The differential effects on quality traits reflect the regulation of apocarotenoid biosynthesis pathways by nutrients. Specifically, crocin and picrocrocin increased in loam-clay soil (18% and 38.37%, respectively), whereas safranal increased in loam soil (20.66%). Crocin and picrocrocin respond positively to increased nitrogen and phosphorus availability, while safranal production is favored under moderate nutrient conditions [23, 39, 46]. These findings demonstrate that the importance of adapting biofertilizer formulations to specific soil textures to optimize PGPR efficacy and saffron secondary metabolite profile, and provide a sustainable approach to improve saffron production while reducing dependence on chemical fertilizers.

The PCA results provide robust evidence that saffron yield and quality are governed by a biological soil fertility gradient rather than by mineral nutrient intensity alone. The distinct separation of organic matter, treatment year, and biofertilizer complexity (PC1) from chemical inputs (PC2) underscores a fundamental divergence in how these management systems influence saffron physiology. The strong positive association between fresh flower yield, dry stigma yield, and secondary metabolite content (crocin, picrocrocin, and safranal) with the "biological axis" (PC1) suggests that multi-strain bacterial inoculants and organic amendments synergistically enhance the soil microbiome. They likely do so by solubilizing recalcitrant nutrients and producing phytohormones that stimulate corm growth and metabolic activity [39, 47]. This aligns with findings by Esmaeilian et al. (2022), who reported that integrating organic and biofertilizers significantly outperforms chemical regimes in sustaining long-term stigma quality [16]. Conversely, the antagonistic or minimal contribution of chemical fertilizers and soil EC to quality traits evidenced by their negative or orthogonal alignment highlights the sensitivity of *Crocus sativus* to salt stress and excessive mineral salts. High electrical conductivity (EC) has been previously identified as a limiting factor that imposes osmotic stress, thereby inhibiting the biosynthetic pathways responsible for apocarotenoid accumulation [48, 49]. The observation that chemical fertilizers were associated with lower percent-change responses in yield and quality further supports the hypothesis that while synthetic inputs may provide immediate nutrients, they fail to sustain the rhizosphere health required for optimal secondary metabolite synthesis [50]. Furthermore, the clustering of treatment year with organic parameters reflects the cumulative nature of soil biological fertility; unlike chemical fertilizers which are rapidly depleted, organic amendments build soil structure and microbial richness over successive growing seasons, leading to progressive improvements in crop performance [51]. Ultimately, these results advocate for a shift toward bio-organic management strategies, prioritizing bacterial richness and organic matter enrichment to maximize both the agronomic productivity and pharmaceutical value of saffron.

CONCLUSION

This meta-analysis demonstrates that the greatest positive effects of bacterial biofertilizers on saffron yield and quality are achieved under conditions of high soil organic matter and the use of diverse microbial consortia. While the application of animal manure enhances quantitative traits such as fresh flower and dry stigma yield, it often reduces secondary metabolite concentrations due to a metabolic shift toward vegetative growth. In contrast, chemical fertilizers, likely through depletion of soil organic matter and suppression of beneficial microbial populations, negatively affect both yield and quality. Integrated approaches that combine organic amendments with minimal mineral fertilizer inputs can sustain high yields while improving stigma quality traits. Principal component analysis revealed that biological soil fertility factors including organic matter content, biofertilizer richness , and treatment duration form a distinct cluster separate from chemical inputs, indicating that saffron yield and quality are primarily governed by biological processes rather than nutrient intensity. Furthermore, soil pH and salinity were found to constrain yield and certain qualitative traits by limiting microbial activity and nutrient availability, underscoring the importance of optimizing both biological and chemical soil properties for sustainable saffron production. Regarding soil texture, loam–clay–sandy soils were most favorable for quantitative traits (fresh flower and stigma yield), loam–clay soils supported higher crocin and picrocrocin content, and loam soils were optimal for safranal accumulation.

Consent for Publication

Not applicable. This manuscript does not contain any individual person's data in any form (including individual details, images, or videos).

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. The meta-analysis was conducted using data extracted from published articles identified through systematic database searches (Web of Science, Scopus, ScienceDirect, and Magiran) covering the period from 2009 to 2024. All extracted data and statistical analysis codes are available from the corresponding author upon reasonable request.

Conflict of Interests

The authors declare no competing interests.

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Authors' Contributions

Ghaffar Khezri (Corresponding Author): Conceptualization, project administration, methodology development, formal analysis, investigation, supervision, writing-original draft, writing-review and editing. Rooholla Moradi: Conceptualization, methodology, data curation, formal analysis, software, validation, visualization, writing-review and editing. Samira Hossein Jafari: Writing-review and editing. Iman Yousefi Javan: Writing-review and editing.

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