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Field Age and Adsorbent Type Effects on Saffron Allelopathy Mitigation and Crop Performance under Replanting Conditions

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ABSTRACT

Declining saffron yields after 6-7 years of continuous cultivation due to allelopathic effects present a major challenge in saffron production. This study examined how allelochemical adsorbents could mitigate these effects in established saffron fields. The research was conducted during the 2021-2022 growing season in Torbat Heydarieh, Iran, using a split-plot design arranged in randomized complete blocks with three replications. The main plot factor was field age (3 and 7 years), while the subplot factor consisted of two adsorbent materials: activated carbon (at 1% and 3%) and zeolite (at 2% and 4%), each applied using two methods (mixed with soil below corms or throughout soil around corms), plus an untreated control. Results showed distinct treatment responses between field ages. Compared to controls, the 2% zeolite treatment mixed throughout the soil produced the best results in 3-year fields, improving leaf area by 99.9%, while 1% activated carbon under corms worked best in 7-year fields, increasing leaf area by 49.1%. The most striking improvement came from using 3% activated carbon in 7-year fields, which increased flower production by 250% relative to control plots. Biochemical analysis revealed that 3% activated carbon below corms in 3-year fields enhanced crocin content by 29.8% while reducing safranal by 12.7% compared to control treatments. Corm production also improved substantially, with 1% activated carbon in 3-year fields yielding 214.6 corms/m², representing a 62.2% increase over control plots. Principal Component Analysis revealed that vegetative traits and yield parameters explained 82.8% of the variation through the first three components. Our findings demonstrate that strategic adsorbent application effectively counteracts allelopathic effects, offering a practical solution for maintaining saffron productivity in aging fields. This approach could help extend field longevity and support small-scale farmers facing replanting challenges.

Keywords: Activated Carbon, Crocin, Replanting, Zeolite

INTRODUCTION

Medicinal and aromatic plants are known to synthesize a wide variety of bioactive compounds, commonly referred to as secondary metabolites, which possess significant health-promoting properties that extend beyond mere nutritional value [1]. The biosynthesis of these active substances is primarily governed by the genetic makeup of the plants[2]; however, environmental stressors and post-harvest operations also play a crucial role in influencing their production[3, 4]. A broad spectrum of these plants exhibits resilience to various environmental stresses, including both water scarcity and salinity.

Saffron (*Crocus sativus* L.) holds a distinguished position as the world's most expensive spice and represents a crucial agricultural commodity in Iran. According to agricultural statistics released by the Ministry of Jihad-e-Agriculture, saffron ranked as Iran's fifth-largest export product, generating 244.22 million dollars in revenue [5]. The crop's economic significance extends beyond direct sales; a comprehensive analysis of employment, per capita income of farmers, water use efficiency, income from saffron, and the economic value of saffron compared to other products in the provinces of Khorasan Razavi and Southern Khorasan concluded that in 2011, more than 600,000 people earned their livelihood from saffron production and processing [6]. Based on economic analysis criteria, saffron cultivation in the Torbat Heydarieh region demonstrates strong financial viability [7].

Saffron demonstrates resilience to arid conditions, making it a suitable option for the dry landscapes of Iran. Nevertheless, a considerable obstacle jeopardizes the long-term viability of saffron cultivation: the persistent decrease in economic yield observed after approximately six to seven years of farming. This decline particularly impacts small-scale farmers who face limited access to land, water, and fertilizers. These constraints often necessitate the replanting of saffron in the same field, leading to reduced yield and efficiency [8, 9]. The severity of this issue has made saffron production increasingly dependent on the expansion of cultivated areas rather than improvements in existing fields [10].

The replanting challenge extends beyond Iran's borders. Soil instability is a fundamental unsolved issue, noting that fields may become unsuitable for saffron cultivation for 30 to 50 years after one cultivation cycle. Similar difficulties have been reported in other saffron-producing countries, including Spain [11]. Traditional management approaches to this problem vary by region. In Iran, farmers typically either replace the topsoil with 30-40 centimeters of virgin soil or practice crop rotation with cereals and legumes [12]. In Spain, farmers observe a 10-year interval for irrigated fields and a 20-year interval for rainfed fields before replanting saffron. Kashmir's farmers employ a different strategy, leaving fields fallow for one year followed by wheat or mustard cultivation before reintroducing saffron.

Research has increasingly pointed to allelopathy as a primary factor in this yield decline. Multiple studies have confirmed the release of allelochemicals from saffron [13-16] suggesting a mechanism similar to that observed in asparagus cultivation. This allelopathic effect is well-documented in traditional farming practices; farmers in Qaenat, for instance, maintain that successful saffron cultivation requires a fallow period of at least twice the duration of the previous saffron cultivation cycle. The challenges in replanting have been extensively documented, with some fields requiring decades before the successful reestablishment of saffron cultivation [12].

Some studies have shown that the use of absorbent materials, including activated carbon and zeolite, can play a role in reducing the allelopathic effect. Zeolites have an open three-dimensional structure that allows them to store water and improve nutrient availability for plants [17, 18]. In addition, zeolites have a high cation exchange capacity (CEC) [19, 20]. Natural zeolite is recommended for the removal or stabilization of heavy metals and pollutants due to its high ion exchange capacity and porous structure [21]. Activated carbon has also received special attention in a few studies on plant nutrition [22]. The inherent properties of activated carbon, such as high specific surface area, high porosity, and nutrient accessibility, which make it a valuable and versatile soil amendment, enable the management of agricultural waste, improvement of soil physical-chemical properties, reduction of air, soil and groundwater pollution, and increased plant growth [23]. The use of activated carbon can improve the arrangement of soil particles as well as the physical and chemical properties of the soil, including reducing soil bulk density [24], increasing soil porosity to help plant growth and development[25], and encouraging nutrient uptake by the crop to increase crop yield [26].

While the allelopathic nature of saffron's yield decline is increasingly well-understood, effective and economically viable solutions remain elusive, particularly for small-scale farmers who cannot afford extensive fallow periods or soil replacement. Therefore, this study aimed to investigate the impact of allelochemical adsorbents on the qualitative and quantitative characteristics of saffron under field conditions. By examining the effects of activated carbon and zeolite adsorbents at various concentrations and application methods in both seven-year-old and three-year-old saffron fields under replanting conditions, this research seeks to develop practical solutions for sustaining saffron productivity in established fields.

MATERIAL AND METHODS

Study Site and Experimental Design

This research was conducted in 2021-2022 in an agricultural field 30 kilometers from Torbat Heydarieh, Iran (59.42° longitude, 35.31° latitude, 1382 meters above sea level). The experimental design investigated the interactive effects of field age and adsorbent treatments on soil allelopathic effects, growth quality, and saffron yield. The study employed a split-plot design based on a randomized complete block design with three replications. Field age served as the main factor at two levels (3 and 7 years), while adsorbent type constituted the sub-factor, comprising various concentrations and application methods of zeolite and activated carbon.

Before establishing the experiment, soil samples from the top 30 cm were analyzed for both fields. The 3-year field had a clay loam texture (32.68% sand, 39.72% silt, 27.6% clay) with pH 8.46, electrical conductivity (EC) of 1.82 dS/m, 0.907% organic carbon, 20.5% calcium carbonate, 0.14% total nitrogen, 25 ppm available phosphorus, and 160 ppm available potassium. The 7-year field had a loam texture (27.60% sand, 46.72% silt, 25.68% clay) with pH 8.33, EC of 2.86 dS/m, 0.838% organic carbon, 31.25% calcium carbonate, 0.1% total nitrogen, 10.5 ppm available phosphorus, and 224 ppm available potassium.

The experimental treatments included activated carbon at 1% and 3% and zeolite at 2% and 4%. Each adsorbent was applied using two distinct methods: mixing with the soil below the corm and mixing with the entire soil around the corm (Table 1). A control treatment without adsorbents was maintained for both field ages.

Table 1 The experimental treatments in this research

Code	Treatment	Code	Treatment
C	control treatment	Z2A	2% zeolite mixing with the entire soil around the corm
AC1A	1% activated carbon mixing with the entire soil around the corm	Z2U	2% zeolite mixing with the soil below the corm
AC1U	1% activated carbon mixing with the soil below the corm	Z4A	4% zeolite mixing with the entire soil around the corm
AC3A	3% activated carbon mixing with the entire soil around the corm	Z4U	4% zeolite mixing with the soil below the corm
AC3U	3% activated carbon mixing with the soil below the corm		

Field Preparation and Treatment Application

Before establishing the experiment, existing corms were carefully collected from the field. To eliminate potential interference from residual corm material, thorough soil cleaning was performed. The experiment was arranged in a split-plot design with three replications, where main plots (field age: 3 and 7 years) measured 16 × 12 meters, and subplots measured 2 × 1 meters. Buffer zones of 50 centimeters were maintained between subplots to prevent treatment cross-contamination. Each subplot contained 4 planting rows spaced 20 centimeters apart, with 5-centimeter spacing between corms within rows.

Selected corms for planting were sorted to ensure weights exceeding 12 grams, based on research demonstrating that corms weighing less than 5.7 grams do not flower in their first year [27].

The treatment application involved removing the top 30 centimeters of soil from each subplot. For uniform distribution, the soil was mixed with the specified percentages of activated carbon or zeolite using a mechanical mixer for 15 minutes per batch. Mixing homogeneity was verified by visual inspection and systematic sampling from different points in the mixture. For treatments applied below the corm, a 10-centimeter layer of treated soil was placed under each planting row before positioning the corms. For treatments involving the entire soil profile, treated soil was placed both below and above the corms to a total depth of 30 centimeters.

Corm planting occurred in June, with placement at 20-centimeter depth and 5-centimeter spacing within rows. Row spacing was maintained at 20 centimeters.

Irrigation followed traditional regional practices with five annual applications: Basarab (October), Zajab (post-harvest), late February, Zarab (early May), and late September. Each irrigation event provided approximately 500 m³/ha of water with EC < 2 dS/m. Water was applied through flood irrigation until the soil reached field capacity.

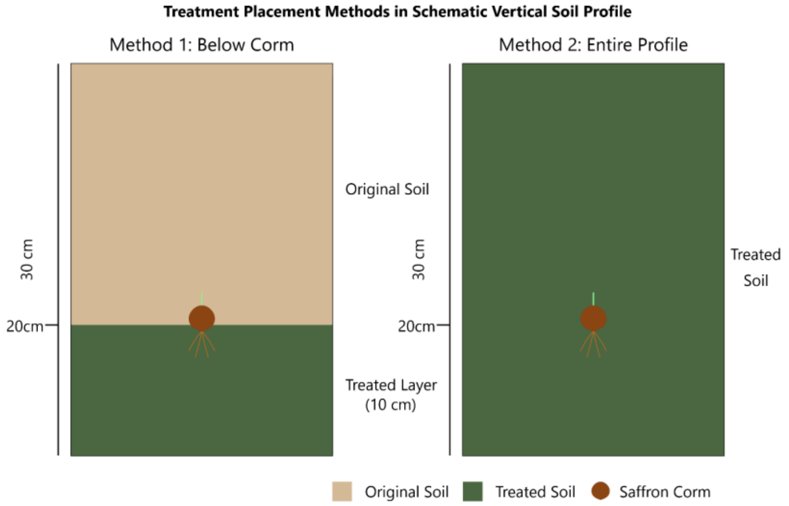


Fig. 1 schematic diagram of treatment placement in soil profile

Data Collection and Analysis

Vegetative Measurements

Leaf characteristics, including area and length, were evaluated at the end of March when leaves began yellowing. From each subplot, 10 randomly, selected plants were assessed. Leaf measurements were taken between 9:00-11:00 AM to ensure consistent moisture conditions. Leaf area was measured using a CI-202 Portable Leaf Area Meter (accuracy ±2%, resolution 1 mm^2) calibrated before each use. Leaf length was recorded as both the number and average length of leaves per corm.

Flower Yield and Quality Assessment

The flower yield assessment encompassed the number of flowers per square meter and fresh and dry weights of stigmas following the November harvest. From each subplot, three 1 m^2 areas were randomly selected for flower counting. Harvested flowers were immediately placed in sealed plastic containers and transported to the laboratory within 2 hours of collection. Three saffron stigma threads were separated at the style attachment point and dried at room temperature (22±2°C) under controlled humidity (45±5% RH) conditions.

For spectrophotometric analysis, dried stigmas were ground to pass through a 35-mesh sieve (500 micrometers). Sample preparation and analysis followed Iranian National Standard No. 2-259. The ground material (500 mg) was mixed with 900 milliliters of distilled water and stirred for one hour at 1000 rpm in darkness. After filtration through the Whatman No. 42 filter paper, absorbance measurements were recorded at 257 nm for picrocrocin, 330 nm for safranal, and 440 nm for crocin content determination. Standard solutions were analyzed every 10 samples for quality control.

Corm Yield and Quality Evaluation

After the completion of leaf measurements in late March, corm characteristics were evaluated, including total corm production per square meter, fresh and dry weights of daughter corms, and weight distribution across four categories: less than 4 grams, 4.1-8 grams, 8.1-12 grams, and greater than 12 grams. Corm dry weight determination involved drying at 60°C for 72 hours.

Statistical Analysis

Data analysis was performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). Prior to analysis, data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test. Analysis of variance was conducted using PROC MIXED, with field age as the main plot and adsorbent treatments as subplot factors. Treatment means were compared using Duncan's multiple range test at $p \leq 0.05$. Principal Component Analysis was performed using PROC PRINCOMP after standardizing variables to have zero mean and unit variance. Variables were checked for multicollinearity before analysis. Component selection was based on eigenvalues >1.0. The first three principal components were retained for interpretation based on their cumulative proportion of variance explained.

RESULTS AND DISCUSSION

Results of the Vegetative Measurements

Leaf area: Analysis of variance showed that the treatments of field age, adsorbent, and the interaction of these treatments significantly affected the leaf area of saffron at the 1% probability level (Table 2).

Comparison of the mean interaction of field age and adsorbent showed that the highest leaf area in both 3-year and 7-year field ages was obtained in the 2% zeolite treatment mixed with the entire soil, so this treatment led to an increase of 99.94% and 49.113% in leaf area, respectively, in the 3-year and 7-year fields compared to the control treatment (Table 3). The results showed that in the 3-year age, the lowest leaf area was observed in the control treatment (812.5 mm^2). The application of 2% zeolite treatment mixed with the soil below the corm, 3% activated carbon mixed with the entire soil, and 1% activated carbon mixed with the soil below the corm did not show a significant difference from the control treatment. At the age of 3 years, the 3% activated carbon treatment below the corm produced the highest leaf area (1276.4 mm^2) after the 2% zeolite treatment mixed with the entire soil. However, at the age of 7 years, this treatment not only did not have a positive effect on the leaf area of saffron but also reduced this index by 19.92% compared to the control treatment. At the age of 7 years, the 1% activated carbon treatment mixed with the soil below the corm had the highest leaf area after the 2% zeolite treatment mixed with the entire soil, which led to a 49.113% improvement in this index compared to the control. The results confirmed that in all adsorbent treatments, except for the 1% activated carbon treatment mixed with the soil below the corm, when the corms were planted in a 3-year field, the leaf area of saffron was significantly higher than in the 7-year field (Table 3).In the 7-year field, the application of 1% activated carbon with the mixing method with the soil below the corm had the highest (1398.4 mm²) and 3% activated carbon mixed with the entire soil had the lowest (540.47 mm²) effect on leaf area. In addition, in this field, the 2% zeolite treatment mixed with the entire soil around the corm had the highest (1440.9 mm²) and the 2% zeolite treatment mixed with the soil below the corm had the lowest (784.92 mm²) effect on leaf area.

Table 2 Analysis of Variance for Saffron Traits Affected by Field Age and Adsorbent Treatments

SOV	df	Leaf area (mm²)	Leaf length (mm)
Block	2	3314.3 ^{ns}	280.2 ^{ns}
Field Age (A)	1	156174.0 **	17319.0 **
Error (a)	2	337.4	89.96
Adsorbent(B)	8	324478.0 **	21043.2 **
A×B	8	147869.4 **	12141.7 **
Error (b)	32	7023.0	382.0
CV (%)	-	8.30	4.48

ns and **: non-significance, significance at the 1 percent probability level, respectively.

Table 3 Interaction Effect of Field Age and Adsorbent Type on Leaf Characteristics of Saffron

Field Age	Treatment	Leaf area (mm²)	Leaf length (mm)
3	C	812.5 e	388.9 f
	AC1A	1099.6 c	467.1 cd
	AC1U	925.7 de	421.8 ef
	AC3A	867.6 de	402.8 f
	AC3U	1276.4 b	509.5 b
	Z2A	1584.3 a	567.6 a
	Z2U	816.7 e	390.0 f
	Z4A	1019.7 cd	449.3 de
	Z4U	1169.4 bc	485.5 bc
7	C	674.92 de	334.45 e
	AC1A	1098.5 b	467.22 b
	AC1U	1398.4 a	535.00 a
	AC3A	861.10 c	401.79 cd
	AC3U	540.47 e	278.37 f
	Z2A	1440.9 a	538.88 a
	Z2U	784.92 cd	374.99 d
	Z4A	892.92 c	413.10 c
	Z4U	903.18 c	416.69 c

For each trait, based on slicing method in each of levels of the planting method, the averages that have at least one common letter, do not have a significant difference based on Duncan's test at the 5% probability level

Overall, the results showed that the use of zeolite at a concentration of 2% and mixed with the entire soil around the corm, especially in younger fields, led to a significant increase in saffron leaf area. However, activated carbon had different effects depending on the concentration and method of application, so in some cases, we observed an increase in leaf area and in others, a decrease. Various studies have proven the positive effects of zeolite on vegetative growth. Taheri et al showed that zeolite can improve all agronomic parameters and cause significant vegetative growth in Friggitello pepper plants [28]. In addition, zeolite, with its high ion exchange capacity, moisture, and nutrient retention capacity, can be used as a water and nutrient efficiency enhancer in the soil and act as a fertilizer [29].

Results of Flower Yield and Quality Assessment

Flower Number

Analysis of variance results showed that the treatments of field age, adsorbent, and the interaction of these treatments had a significant effect on the leaf area of saffron at the 1% probability level (Table 4).

Table 4 ANOVA for Saffron Traits Affected by Field Age and AdsorbentTreatments

SOV	df	Number of flowers	Fresh stigma weight (g)	Dry stigma weight (g)	Picrocrocin	Safranal	Crocin
Block	2	1.50 ns	0.28 ns	0.00ns	0.12 ns	0.001 ns	0.08 ns
Field Age (A)	1	69912.0 **	6360.4 **	1.29 **	1570.0 **	147.0 **	4801.3 **
Error (a)	2	25.68	2.78	0.00	0.01	0.02	0.72
Adsorbent(B)	8	5546.5 **	608.0 **	0.12 **	162.8 **	18.61 **	1806.9 **
A×B	8	6517.4 **	734.6 **	0.14 **	131.8 **	27.39 **	2478.0 **
Error (b)	32	1.69	0.16	0.00	0.05	0.07	0.41
CV (%)	-	4.39	6.31	4.31	2.26	1.66	1.26

ns and **: non-significance, significance at the 1 percent probability level, respectively

Comparison of the mean interaction of field age and adsorbent in the 3-year field showed that the highest number of flowers harvested was obtained in the 1% activated carbon treatment mixed with the entire soil around the corm (28.66 flowers per m2) which was significantly higher (138.8%) than control (12.00 flowers per m2). In this field, the 4% zeolite treatment mixed with the soil below the corm had the least effect on the number of flowers (7.00 flowers per square meter). This treatment not only did not increase flower production but also reduced the number of flowers by 41.6% compared to the control. In this field, all treatments except for the 4% zeolite treatment mixed with the soil below the corm (7.00 flowers per square meter) and 3% activated carbon mixed with the soil below the corm (12.00 flowers per m2) had a positive effect on the number of flowers. In the 7-year field, the number of flowers harvested per square meter was highest in the 3% activated carbon treatment mixed with the soil below the corm (186.67 flowers per m2). This treatment increased the number of flowers per square meter by 250% compared to the control treatment. In this field, the lowest number of flowers (6.66 flowers per m^2) was harvested from the 1% activated carbon treatment mixed with the soil below the corm, which was significantly different from the control and 87.5% less than it. In this field, except for the 1% activated carbon treatment mixed with the soil below the corm and 2% zeolite mixed with the entire soil, which had 6.66 and 19.66 flowers per square meter, respectively, and reduced the number of flowers compared to the control, the other treatments increased the number of flowers harvested per square meter compared to the control (Table 5).

Analysis of the results showed that the use of absorbents in combination with soil had different effects on flower production in the two fields of different ages (3 years and 7 years). The main mechanism of action of activated carbon and zeolite in flower production can be related to how they affect the physical, chemical, and biological properties of the soil. Soil amendment using activated carbon has recently become a popular amendment technique. Zeolites are beneficial in agriculture due to their high porosity, cation exchange capacity, and selectivity for ammonium and potassium cations. They can be used both as nutrient carriers and as a medium for nutrient release[25].

Fresh Weight of Stigma

Analysis of variance results showed that the effect of field age, adsorbent, and the interaction of these factors on the stigma fresh weight was significant at the 1% probability level (Table 4).

The analysis of the comparative results regarding the mean interaction between field age and adsorbent revealed that in the three-year field, the application of 3% activated carbon combined with the surrounding soil of the corm yielded the highest fresh weight of stigma, measuring 9.97 g/m2. Similarly, the treatment involving 1% activated carbon mixed with the entire soil around the corm also demonstrated a significant outcome, with a fresh weight of 9.96 g/m^2. These treatments increased the fresh weight of the stigma by 52.21% and 52.06%, respectively, compared to the control (6.55 g/m2). In this field, the lowest fresh weight of stigma was obtained in the 4% zeolite treatment mixed with the soil below the corm. In this treatment, the fresh weight of the stigma was 63.2% less than the control. In the 7-year field, the highest fresh weight of stigma was also obtained in the 3% activated carbon treatment mixed with the soil below the corm (58.73 g/m2). This treatment led to a significant increase in saffron production compared to other treatments, and its amount was 513.9% higher than the control treatment (9.42 g/m2). After that, the 4% zeolite treatment mixed with the soil below the corm had the highest fresh weight of stigma, with a fresh weight of 54.34 grams per square meter. This amount was 476.8% higher than the control treatment. However, its difference from the 3% activated carbon treatment mixed with the soil below the corm was significant. In this field, the lowest fresh weight of stigma was obtained in the 1% activated carbon treatment mixed with the soil below the corm (2.52 g/m^2) (Table 5).

Table 5 Interaction Effect of Field Age and Adsorbent Type on Quantitative and Qualitative Characteristics of Saffron

Field Age	Treatment	Number of flowers (No/m^2)	Fresh stigma weight (g)	Dry stigma weight (g)
3	C	12.00 e	6.55 c	0.094 c
	AC1A	28.66 a	9.96 a	0.142 a
	AC1U	20.66 c	6.22 d	0.088 d
	AC3A	24.66 b	9.97 a	0.142 a
	AC3U	12.00 e	4.65 e	0.066 e
	Z2A	20.00 c	7.52 b	0.107 b
	Z2U	15.66 d	4.76 e	0.068 e
	Z4A	24.00 b	7.84 b	0.112 b
	Z4U	7.00 f	2.41 f	0.034 f
7	C	53.33 f	9.42 g	0.13 g
	AC1A	105.67 d	34.64 d	0.49 d
	AC1U	6.66 h	2.52 i	0.03 i
	AC3A	55.00 f	17.67 f	0.25 f
	AC3U	186.67 a	58.73 a	0.83 a
	Z2A	19.66 g	6.47 h	0.09 h
	Z2U	84.00 e	26.60 e	0.38 e
	Z4A	138.00 c	44.83 c	0.64 c
	Z4U	163.33 b	54.34 b	0.77 b

For each trait, based on slicing method in each of levels of the planting method, the averages that have at least one common letter, do not have a significant difference based on Duncan's test at the 5% probability level

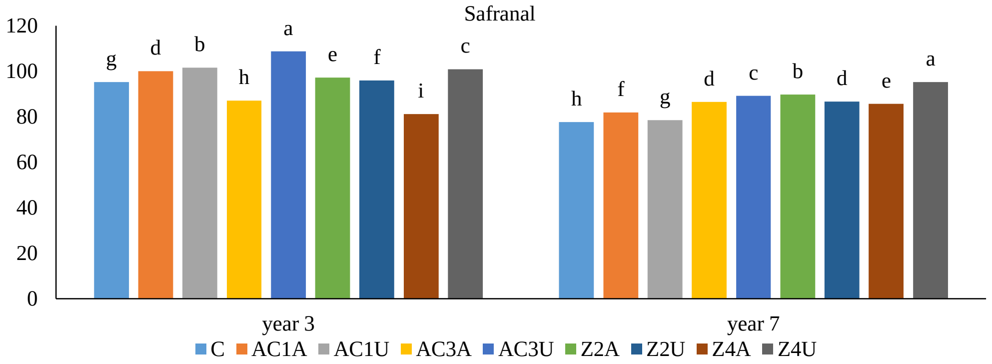
Crocin, Picrocrocin, and Safranal

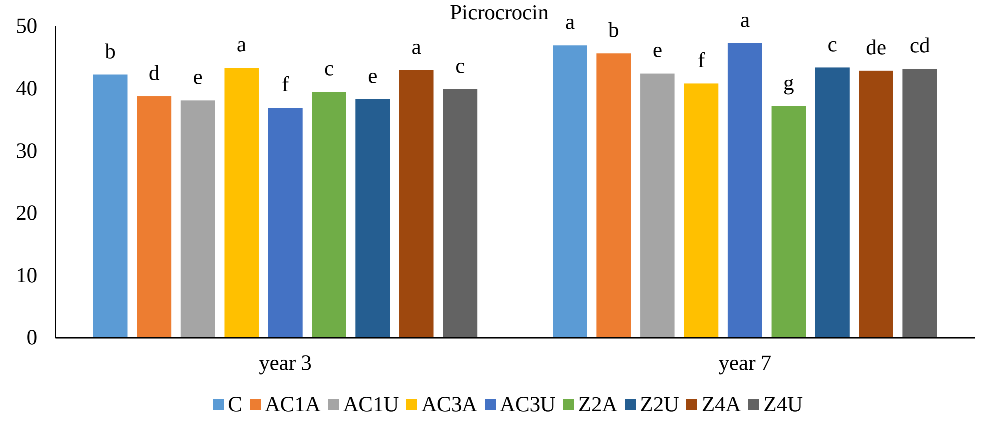
Analysis of variance results showed that the treatments of field age, adsorbent, and the interaction of these treatments had a significant effect on the amount of saffron active ingredients at the 1% probability level (Table 4).

Examination of the results of the comparison of the mean interaction of field age and adsorbent showed that in the 3-year field, the highest amount of crocin and picrocrocin (0.300 and 108.7, respectively) was obtained in the 3% activated carbon treatment mixed with the soil below the corm. This treatment also had the lowest amount of safranal (36.90). The application of this treatment increased crocin by 29.8% and picrocrocin by 1.14% compared to the control treatment (231.8 and 95.25, respectively). Also, the amount of safranal in this treatment was 12.66% lower than the control (42.25). On the other hand, the data showed that the lowest amount of crocin and picrocrocin was obtained in the 4% zeolite treatment mixed with the entire soil (197.8 and 81.19, respectively). This treatment not only had a positive effect on saffron active ingredients but also reduced these substances compared to the control, so that crocin was 14.66% and picrocrocin was 14.76% less than the control treatment. Comparison of the mean interaction of field age and adsorbent in the 7-year field showed that the 4% zeolite treatment mixed with the soil below the corm had the greatest effect on the amount of crocin (258.19) and picrocrocin (95.31). This treatment increased the amount of crocin by 28.29% and the amount of picrocrocin by 22.64% compared to the control treatment. In this field, the lowest amount of crocin (201.25) and picrocrocin (77.71) was obtained in the control treatment (Fig. 2). Also, this treatment had the highest amount of safranal (46.95). Correlation analysis between different traits showed that the amount of crocin was significantly correlated with picrocrocin in saffron stigma with a correlation coefficient of 0.888 at the 95% level. The amount of picrocrocin (with a correlation coefficient of -0.568) and crocin (with a correlation coefficient of -0.656) had a negative correlation with the amount of safranal in the stigma (Table 8).

This study showed that the use of absorbent materials (such as activated carbon and zeolite) in interaction with the age of the saffron field affects the number of active compounds in saffron (crocin, picrocrocin, and safranal). These materials may affect the production and amount of these compounds through various mechanisms. Various studies have shown that activated carbon and zeolite may have effects on improving soil and its physical properties. These materials have a high cation exchange capacity (CEC) and can absorb and release various cations, including nutrients such as potassium and ammonium. This can improve the plant's access to these nutrients [30]. These materials can also help to improve soil aeration, absorb pollutants [31, 32], better absorption and retention of nutrients[33] and water [34], root growth [22], and ultimately contribute to optimal plant growth and consequently increase active ingredients.

According to correlation analysis, there are significant relationships between the amounts of crocin, picrocrocin, and safranal. Specifically, the amount of crocin and picrocrocin are positively correlated with each other and negatively correlated with safranal. This suggests that changes in the amount of crocin and picrocrocin may affect the amount of safranal, and different absorbent materials can affect these relationships (Table 8).





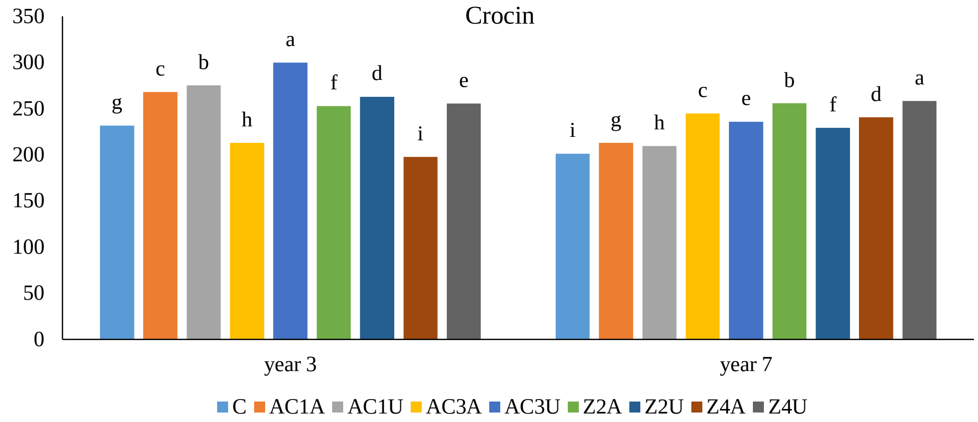


Fig. 2 Content of secondary metabolites in saffron stigmas from 3-year and 7-year fields. Values represent means ± SE (n=3). Different letters indicate significant differences at $p \leq 0.05$ according to Duncan's multiple range test.

Corm Yield

Total Number of Corms Produced Per Square Meter

Results from the analysis of variance showed that the treatments of field age, adsorbent, and the interaction of these treatments had a significant effect on the total number of corms produced per square meter at the 1% probability level (Table 6).

Results of the mean comparison of the interaction of field age and adsorbent showed that in both 3-year and 7-year fields, the highest number of corms per square meter was obtained in the 1% activated carbon treatment mixed with the entire soil. In the 3-year field, affected by this treatment, 214.6 and 230.33 corms were harvested per m2 in the 3-year and 7-year fields, respectively, which were 62.2% and 51.22% higher than the control treatment. The results showed that in the 3-year field, the lowest number of corms was obtained in the 4% zeolite treatment mixed with the soil below the corm (113.6 corms/m^2). The number of corms from this treatment was 14.13% less than the control treatment (132.3 corms/m^2), while in the 7-year field, the lowest number of corms (121.33 corms/m^2) was obtained in the 2% zeolite treatment mixed with the entire soil around the corm, which showed a 35.46% decrease compared to the control treatment (188 corms/m^2) (Table 7).

Fresh and Dry Weight of Corms

Based on the results of the analysis of variance (Table 6), the treatments of field age, adsorbent, and the interaction of these factors had a significant effect on the fresh and dry weight of produced corms at the 1% probability level.

Examination of the results of the mean comparison of the interaction of field age and adsorbent in the 3-year field showed that the 2% zeolite treatment mixed with the entire soil around the corm had the greatest effect on the fresh weight of saffron corms (10.44 grams) so that this treatment increased the corm weight by 147.98% compared to corms from the control treatment (4.21 grams). In addition, corms produced in this treatment had the highest dry weight (4.09 grams). In this field, the lowest fresh weight (3.84 grams) and dry weight (1.34 grams) of corms were obtained in the 3% activated carbon treatment mixed with the entire soil (Table 7). This study also showed that in the 7-year field, the 1% activated carbon treatment mixed with the soil below the corm (8.12 grams) increased the fresh weight of corms by 75% compared to corms produced in the control treatment (4.64 grams). The dry weight of corms produced (2.64 grams) in this treatment was also 47.48% higher than the corms produced in the control treatment (1.79 grams). On the other hand, the 3% activated carbon treatment mixed with the soil below the corm not only did not have a positive effect on the fresh weight of corms (2.53 grams) but also reduced the weight of corms by 4.45% compared to corms produced in the control treatment (Table 7).

Weight grouping of produced corms: Results of the analysis of variance showed that the treatments of field age, adsorbent, and the interaction of these treatments had a significant effect on the four weight groups of produced corms at the 1% probability level (Table 6).

Table 6 Analysis of Variance for Saffron Traits Affected by Field Age and Adsorbent Treatments

SOV	df	Fresh corm weight (g)	Dry corm weight (g)	Number of corms (per m^2)	density of daughter corms per square meter <4g	4.1-8g	8.1-12g	>12g
Block	2	0.68 **	0.11 **	9.55 ^{ns}	91.12 ^{ns}	5.24 ^{ns}	35.38 ^{ns}	9.38 ^{ns}
Field Age (A)	1	7.34 **	1.76 **	4356.0 **	4988.1 **	801.1 **	468.1 **	127.57 **
Error (a)	2	0.01	0.003	6.74	3.72	4.57	0.388	3.57
Adsorbent(B)	8	15.00 **	1.81 **	5082.7 **	3954.0 **	1643.7 **	689.7 **	641.37 **
A×B	8	5.38 **	0.93 **	2505.8 **	2570.0 **	501.6 **	337.9 **	349.19 **
Error (b)	32	0.01	0.001	9.43	4.75	10.78	6.76	4.46
CV (%)	-	7.85	5.87	8.91	2.97	6.91	9.69	16.31

ns and **: non-significance, significance at the 1 percent probability level, respectively

Table 7 The Interaction Effect of Field Age and Adsorbent Type on Quantitative and Qualitative Characteristics of Saffron

Field Age	treatment	Mean (g)		Number of corms per square meter	density of daughter corms per square meter			
		Corm Fresh weight	Corm Dry weight	(No/m^2)	<4g	4.1-8g	8.1-12g	>12g
3	C	4.21 f	1.64 e	132.3 e	78.33 c	42.00 c	10.00 e	2 e
	AC1A	6.49 c	2.65 b	214.6 a	80.33 c	54.33 b	52.33 a	27.66 b

	AC1U	6.38 c	2.48 c	131.0 e	47.33 e	31.33 de	40.66 b	12 d
	AC3A	3.84 g	1.34 f	170.0 c	90.66 a	66.00 a	13.33 e	0 e
	AC3U	5.75 e	2.41 d	126.0 f	59.66 d	26.33 ef	21.33 d	19 c
	Z2A	10.44 a	4.09 a	121.0 g	12.66 g	34.33 d	33.66 c	40 a
	Z2U	4.34 f	1.65 e	155.0 d	78.33 c	54.33 b	22.33 d	0 e
	Z4A	5.99 d	2.9 d	202.0 b	84.66 b	59.33 b	40.33 b	18 c
	Z4U	6.98 b	2.37 d	113.6 h	42.33 f	25.00 f	34.00 c	12 d
7	C	4.64 f	1.79 f	188.00 c	99.00 b	59.00 b	29.00 b	1.00 ef
	AC1A	6.04 c	2.38 c	230.33 a	89.00 c	83.66 a	34.33 a	23.33 b
	AC1U	8.12 a	2.64 a	138.00 e	41.66 f	24.66 d	37.66 a	34.00 a
	AC3A	4.95 de	1.88 e	159.33 d	73.33 d	53.66 b	28.00 b	4.33 de
	AC3U	2.53 h	0.88 g	192.33 bc	168.00 a	24.33 d	0.00 e	0.00 f
	Z2A	7.41 b	2.48 b	121.33 f	37.00 g	35.66 c	29.66 b	19.00 c
	Z2U	5.05 d	2.07 d	140.00 e	72.00 d	40.66 c	11.66 d	15.67 c
	Z4A	4.80 ef	1.87 e	163.00 d	66.00 e	81.00 a	16.00 c	0.00 f
	Z4U	4.25 g	1.77 f	195.00 b	101.33 b	59.66 b	28.66 b	5.33 d

For each trait, based on slicing method in each of levels of the planting method, the averages that have at least one common letter, do not have a significant difference based on Duncan's test at the 5% probability level

Results of the comparison of the mean interaction of field age and adsorbent in the 3-year field showed that the highest number of corms produced in groups 1 (less than 4 grams) and 2 (4-8 grams) was obtained in the 3% activated carbon treatment mixed with the entire soil, with 90.66 and 66 corms per square meter, respectively. In the 3rd weight group (8-12 grams), the highest number of corms was obtained in the 1% activated carbon treatment mixed with the entire soil (52.33 corms per square meter), and in the 4th weight group (more than 12 grams), the 2% zeolite treatment mixed with the entire soil (40 corms per square meter) had the highest number.

In the 7-year field, in the 1st weight group, the corms obtained from the 3% activated carbon treatment mixed with the soil below the corm had the highest number (168 corms per square meter). In the 2nd weight group, the 1% activated carbon treatment mixed with the entire soil and the 4% zeolite treatment mixed with the entire soil had the highest number with 88.66 and 81 corms per square meter, respectively, with a significant difference compared to the control treatment. In the 3rd weight group, the 1% activated carbon treatment mixed with the soil below the corm and the 1% activated carbon treatment mixed with the entire soil around the corm had the highest number with 37.66 and 34.33 8-12 gram corms per square meter, respectively, with a significant difference compared to the control. In this weight group, the 3% activated carbon treatment mixed with the soil below the corm had the lowest amount (zero corms per square meter). In the 4th weight group, the 1% activated carbon treatment mixed with the soil below the corm had the highest number of corms greater than 12 grams, and the 3% activated carbon treatment mixed with the soil below the corm and the 4% zeolite treatment mixed with the entire soil did not produce any corms.

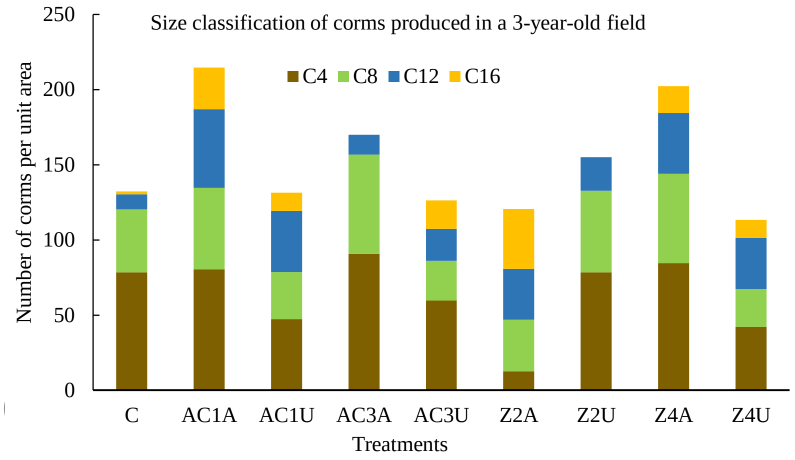


Fig. 3 Distribution of daughter corm weights in 3-year field. C4: <4g, C8: 4.1-8g, C12: 8.1-12g, C16: >12g.

Analysis of the results showed that activated carbon and zeolite, depending on the amount used and how they are mixed with the soil, have different effects on corm production, its fresh and dry weight, and the absorption of nutrients (nitrogen, phosphorus, and potassium). Various studies have shown that activated carbon has a high specific surface area and significant absorption capacity, which causes the absorption and retention of nutrients in the soil. Therefore, this feature can prevent the leaching of nutrients and improve the access of saffron corms to these elements [35, 36]. On the other hand, activated carbon improves soil structure, increases aeration and moisture retention capacity. Zeolite, due to its porous structure and high cation exchange capacity (CEC) [19, 22, 38], can store nutrients such as potassium, ammonium and other cations and gradually provide them to the plant [18]. This feature can ensure more stable absorption of nitrogen and potassium for the saffron corm. Also, various studies have shown that zeolite and activated carbon can help expand root growth by improving macro and micro pores in the soil [20, 37-39].

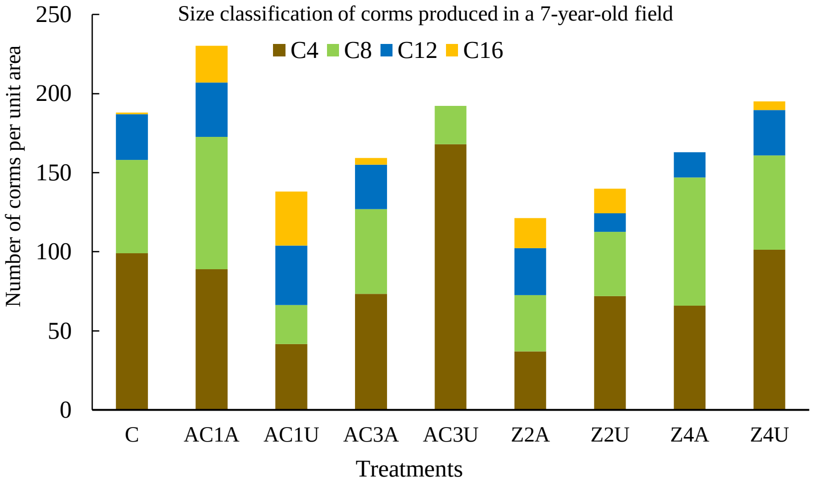


Fig. 4 Distribution of daughter corm weights in 7-year field. C4: <4g, C8: 4.1-8g, C12: 8.1-12g, C16: >12g.

Table 8 Correlation between different traits measured in this study

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15
T1	1.00														
T2	0.98 **	1.00													
T3	-0.49 **	-0.53 **	1.00												
T4	-0.45 **	-0.48 **	0.99 **	1.00											
T5	-0.45 **	-0.48 **	0.99 **	1.00 **	1.00										
T6	0.22 ns	0.24 ns	-0.25 ns	-0.21 ns	-0.21 ns	1.00									
T7	-0.56 **	-0.61 **	0.63 **	0.58 **	0.58 **	-0.71 **	1.00								
T8	0.24 ns	0.26 *	-0.12 ns	-0.09 ns	-0.09 ns	0.91 **	-0.77 **	1.00							
T9	0.88 **	0.86 **	-0.52 **	-0.51 **	-0.51 **	0.14 ns	-0.48 **	0.14 ns	1.00						
T10	0.82 **	0.82 **	-0.46 **	-0.45 **	-0.45 **	0.22 ns	-0.49 **	0.21 ns	0.96 **	1.00					
T11	-0.39 **	-0.37 **	0.51 **	0.50 **	0.50 **	-0.42 **	0.58 **	-0.41 **	-0.40 **	-0.31 *	1.00				
T12	-0.77 **	-0.81 **	0.67 **	0.65 **	0.65 **	-0.26 *	0.69 **	-0.28 *	-0.84 **	-0.80 **	0.67 **	1.00			
T13	-0.27 *	-0.20 ns	0.32 *	0.32 *	0.32 *	-0.43 **	0.36 **	-0.38 **	-0.30 *	-0.21 ns	0.67 **	0.24 ns	1.00		
T14	0.51 **	0.57 **	-0.42 **	-0.43 **	-0.43 **	0.08 ns	-0.38 **	0.10 ns	0.64 **	0.65 **	0.15 ns	-0.48 **	0.04 ns	1.00	
T15	0.81 **	0.78 **	-0.36 **	-0.34 **	-0.34 **	0.08 ns	-0.32 **	0.07 ns	0.87 **	0.87 **	-0.13 ns	-0.59 **	-0.28 *	0.58 **	1.00

ns, * and **: non-significance, significance at the 5 and 1 percent probability level, respectively

T1: Leaf area, T2: Leaf length, T3: Number of flowers, T4: Fresh stigma weight, T5: Dry stigma weight, T6: Picrocrocin content in stigma, T7: Safranal content in stigma, T8: Crocin content in stigma, T9: Fresh corm weight, T10: Dry corm weight, T11: Number of corms produced per square meter, T12: Number of corms produced less than 4 grams, T13: Number of corms produced between 4 and 8 grams, T14: Number of corms produced between 8 and 12 grams, T15: Number of corms produced more than 12 grams.

Principal Component Analysis (PCA)

PCA results (Fig. 5) showed that the first three components collectively accounted for 82.8% of the total data variation. The first component, explaining 53.3% of the variation, showed the highest positive correlation with morphological traits such as leaf area (0.305), leaf length (0.309), fresh corm weight (0.313), and dry corm weight (0.301). This indicates the significance of vegetative characteristics in saffron yield. The negative correlation of this component with the number of flowers (-0.278) and stigma weight (-0.269) suggests that under re-cultivation conditions, increased vegetative growth does not necessarily lead to increased flower yield.

Analysis of the results showed that the application of adsorbents had different effects on saffron performance in fields of different ages. In the three-year-old field, the zeolite 2% treatment mixed with the entire soil around the corm (Z2A) resulted in a 94.99% increase in leaf area compared to the control, while in the seven-year-old field, activated carbon 1% mixed with the soil under the corm (AC1U) showed the best result with a 107.2% increase in leaf area. This difference could be due to the different accumulation of allelochemicals in the soil of fields with different ages, which is consistent with the findings of Rashed Mohassel and colleagues [40]. Crocin content, as the most important compound affecting saffron quality, was significantly affected by different treatments. In the three-year-old field, the activated carbon 3% treatment mixed with the soil under the corm showed a crocin content of 300.0, a 29.8% increase compared to the control. This result is in line with the findings of Kheirabadi et al [41] regarding the positive effect of activated carbon on reducing allelopathic effects. The positive and significant correlation (r=0.91) between crocin and picrocrocin levels indicates that improving growth conditions by reducing allelopathic effects can lead to a simultaneous increase in these secondary metabolites.

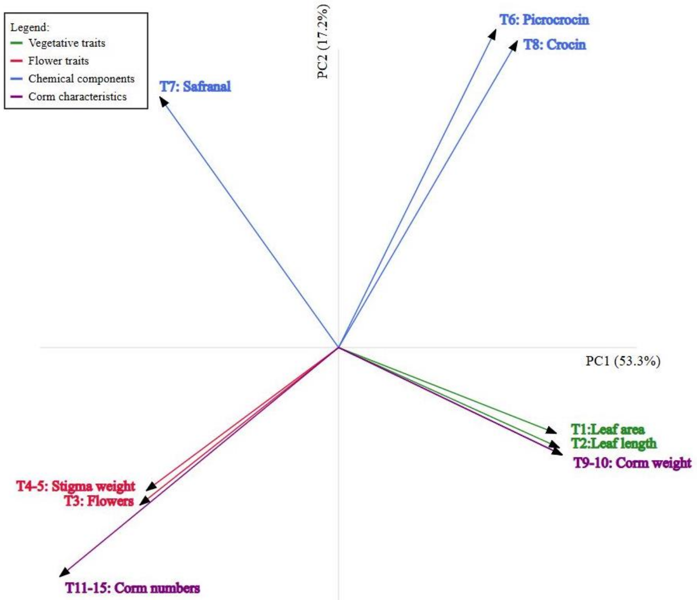


Fig. 5 Principal component analysis (PCA) biplot showing relationships between measured variables. PC1 and PC2 explain 53.3% and 17.2% of total variance, respectively. Variables: LA - leaf area, FL - number of flowers, SW - stigma weight, CR - crocin content, PC - picrocrocin content, SF - safranal content, CW - corm weight.

The number and weight of daughter corms, as important indicators of saffron propagation and establishment, showed a different pattern in response to treatments. In the three-year-old field, the AC1A treatment produced 214.6 corms per square meter, a 62.2% increase compared to the control. This treatment also resulted in the production of the highest number of corms in the 8.1-12 grams' weight group (52.33 per square meter), which is economically important. This result is consistent with the findings of Motoki and colleagues [42] regarding the positive effect of activated carbon on reducing allelopathic effects in asparagus.

The second PCA component, explaining 17.2% of the variation, showed a strong negative correlation with safranal (-0.444) and picrocrocin (-0.428) levels. This could indicate an interaction between the effective compounds of saffron. The significant negative correlation between safranal and crocin (r = -0.77) and picrocrocin (r = -0.71) also supports this hypothesis.

In summary, the results of this study show that the use of adsorbents can be an effective strategy for reducing allelopathic effects in saffron re-cultivation. However, the selection of the type and concentration of the adsorbent should be based on the field age and soil conditions. These findings can be used in the development of sustainable management methods for saffron fields and increasing their economic lifespan.

Study Limitations and Future Directions

While this study demonstrates the potential of adsorbents for alleviating replanting issues in saffron cultivation, several limitations should be addressed in future research: 1. Time constraints limited observation to one growing season 2. Site-specific soil conditions may influence treatment efficacy 3. Economic analysis based on current market conditions may fluctuate Future research should focus on: - the Long-term effects of repeated adsorbent applications - the Impact on soil microbial communities - Optimization of application methods for different soil types - Cost-reduction strategies for small-scale farmers - Multi-location trials across different climatic zones - Integration with other sustainable farming practices

CONCLUSION

The differential responses between 3-year and 7-year fields likely reflect changes in soil physical structure and allelochemical accumulation over time. In younger fields, zeolite's high cation exchange capacity may primarily benefit nutrient retention and water-holding capacity. In contrast, activated carbon's superior performance in older fields suggests its role in adsorbing accumulated allelochemicals becomes more critical over time. Soil structural analysis showed improved porosity and reduced bulk density with both amendments, but activated carbon had a more pronounced effect on microbial activity, potentially explaining its greater impact on flower production in older fields.

The investigation into the application of allelochemical adsorbents on saffron cultivation revealed critical insights into mitigating yield decline in established fields. The research demonstrated that activated carbon and zeolite treatments can significantly ameliorate allelopathic effects, with treatment efficacy varying substantially between three and seven-year-old fields. In three-year-old fields, the 2% zeolite treatment mixed with the entire soil around the corm enhanced leaf area by 94.99%, while in seven-year-old fields, 1% activated carbon mixed beneath the corm increased leaf area by 107.2%. These findings underscore the complex interactions between adsorbent type, concentration, and field age in managing soil allelopathic conditions.

Notably, the study revealed nuanced impacts on saffron's critical quality parameters. Crocin content, a primary determinant of saffron quality, experienced a 29.8% increase in the three-year-old field using 3% activated carbon treatment mixed under the corm. The research also demonstrated significant improvements in daughter corm production, with the 1% activated carbon treatment in three-year-old fields generating 214.6 corms per square meter—a 62.2% increase compared to control treatments. These results suggest that strategic adsorbent application can effectively mitigate allelopathic constraints and enhance saffron productivity, offering promising strategies for sustainable agricultural management in regions with prolonged saffron cultivation histories.

The findings from this study have important practical implications for saffron farmers facing replanting challenges. The effectiveness of different adsorbent treatments varies substantially with field age, suggesting farmers should carefully consider their field history when selecting management strategies. In younger fields of around three years, zeolite application at 2% concentration mixed throughout the soil profile offers the most promising results, particularly for vegetative growth and corm development. However, for older fields approaching seven years of cultivation, activated carbon at 1% concentration applied beneath the corms proves more effective at mitigating yield decline.

The implementation of these findings requires consideration of local conditions and resources. Farmers should assess their soil characteristics, as the effectiveness of adsorbents may vary with soil texture and organic matter content. The availability and cost of materials in different regions will influence the economic viability of these treatments. While our results demonstrate significant yield improvements, farmers may benefit from initially testing these approaches on smaller areas to optimize application methods for their specific conditions.

The timing and method of adsorbent application are crucial factors that can be integrated into existing cultivation practices. The pre-planting application allows for thorough incorporation into the soil, though this requires additional labor during the busy planting season. Farmers should consider their available equipment and labor resources when planning implementation strategies. The significant yield improvements observed, particularly in older fields, suggest that the initial investment in materials and application costs can be offset by increased production, making these treatments particularly relevant for small-scale farmers seeking sustainable solutions to replanting challenges.

These management strategies can be particularly valuable for farmers who cannot afford extended fallow periods or complete soil replacement. The ability to maintain productive saffron fields for longer periods through strategic adsorbent use offers a practical alternative to traditional management approaches, potentially reducing the pressure to expand cultivation into new areas. This approach aligns with sustainable intensification goals while providing immediate practical benefits to farmers facing yield decline in aging saffron fields.

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Authorship Contribution Statement

M. A: Writing – original draft, Investigation.
M. Az: Supervising, Writing – review & editing. Validation, Project administration, Methodology.
H.K.: Writing – review & editing. Analysis. Methodology.

Declaration of Competing Interest

Here, we declare that the authors do not have any competing interests.

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