**Original Research Article**

**The Effect of Organic Acid Treatment on Some Chemical Indicators of Several Varieties of Wild and Cultivated Saffron (Crocus sp.)**

**Abstract:**

The research carried out in Banias-Syria during the agricultural season 2023/2024 and in the scientific research laboratory affiliated to the Department of Field Crops at the Faculty of Agricultural Engineering, Tishreen University to study the effect of treatment with organic acids (humic and fulvic) at concentrations (0, 25, and 50 mg/L) on some chemical indicators of cultivated and wild saffron. Saffron corms (Iranian, Spanish, Abu Reiha, yellow wild) were planted according to a randomized complete block design (RCBD) with three replications. Some chemical indicators were measured: chlorophyll and carotenoid content (μg/gFw), content of sugars in leaves and corms (%), content of active components crocin, picrocrocin, and safranal (μg/g). The results showed the positive effect of organic acid treatment where the concentration (50 mg/L) outperformed the control and the treatment with concentration (25 mg/L) in all studied traits and characteristics and for all varieties. The plants of the Spanish variety treated with concentration (50 mg/L) excelled in the traits (carotenoid content (μg/gFw), content of sugars in corms (%), crocin content (μg/g)), reaching (157.608 μg/g - 4.742% - 186.006 μg/g) respectively. Meanwhile, the plants of the Iranian variety treated with concentration (50 mg/L) excelled in the following characteristics (chlorophyll content (μg/gFw), picrocrocin (μg/g), and safranal (μg/g)), reaching (1262.312 μg/g - 63.218 μg/g - 63.676 μg/g) respectively. The yellow wild variety gave the highest content of sugars in leaves reaching (5.042%). Therefore, we recommend using the concentration (50 mg/L) for treating saffron with organic acids through irrigation during the growth season.

Keywords: **saffron-Organic acids - Chlorophyll - Crocin – Safranal -Picrocrocin.**

**Introduction:**

Saffron, known as red gold or the golden spice, belongs to the Iridaceae family [1] and the genus Crocus sp. which is distributed in Asia, Europe, and North Africa [2], comprising about 80 species mainly distributed in Southeast Asia and the Mediterranean basin [1]. Saffron is considered one of the most expensive agricultural and medicinal plants, enjoying a unique position among industrial and export products, and important for securing numerous job opportunities [3]. Saffron spice is obtained from the stigmas of its flowers, and saffron contains more than 100 biologically active compounds, the most important being crocin, picrocrocin, safranal, and crocetin [4], and it is considered the most expensive agricultural and pharmaceutical product in the world.

Documented in "The Modern Flora of Syria and Lebanon" [5], there are 11 species of the genus Crocus in Syria, of which only three species are in the coastal region: Crocus kotschyanus K. Koch, Crocus graveolens Boiss. & Reut, and Crocus vitellinus Wahlenb.

Many studies have shown that the physical, chemical, and biological properties of soil are key indicators and can serve as criteria in determining and designing crop production plans, thus helping in selecting fertilizers to improve crop production by enhancing soil properties [6]. Some studies confirm that 16% to 80% of variations in flower production of this plant depend on soil characteristics [7,8], so soil conservation is key to obtaining high-quality production.

Organic acids are characterized by improving soil physical properties, increasing soil porosity and aeration, providing oxygen necessary for root respiration and microbial activity, in addition to increasing soil water retention capacity [9]. It was reported [10] that foliar spraying of humates enhances the penetration of nutrient ions into leaves, stimulates the formation of some physiologically active metabolites, and activates the roots' ability to absorb nutrients, especially N and K, which increases the number of chloroplasts per cell in addition to improving photosynthetic efficiency and increasing sugar content [11]. The chemical composition of saffron is complex, containing primary compounds in large proportions such as carbohydrates, proteins, and amino acids, and lower proportions of vitamins, fats, and minerals, and secondary compounds such as flavonoids and carotenoids [12]. Saffron stigmas contain biologically active elements such as flavonoids, vitamins, pigments, and volatile aromatic oils [13]. Chemical analyses of saffron stigmas have shown that it contains more than 150 volatile and non-volatile compounds, but no more than 50 compounds have been identified to date [14]. Crocin (C₄₄H₆₄O₂₄) is a carotenoid rare in nature with water solubility and high effectiveness as a dye in medicines and foods, and picrocrocin (C₁₆H₂₆O₇) which gives saffron its flavor and bitter taste [15], while safranal (C₁₀H₁₄O) is responsible for the aroma and its presence is low in fresh stigmas [16]. The proportion of the presence of these three compounds in the stigmas constitutes the main standard for determining saffron quality [17,18,19], and the concentration of these compounds varies significantly according to many factors, the most important being country of origin, stigma drying method, method of extracting these compounds, in addition to the method of analysis and estimation [20,21,22]. A study was conducted on the effect of humic acid on the quantitative and qualitative properties of saffron at four levels (0, 10, 20, and 30 kg/ha) with three replications using the randomized complete block method, where the treatment gave a positive and clear effect on the percentage (picrocrocin, safranal, and crocin) [23].

Another study [24] showed the effect of fulvic acid, proving its positive effect on saffron stigma components (picrocrocin, crocin), as well as on antioxidant compounds (anthocyanins and total phenols). Given that saffron is a crop with great benefits and high economic return, cultivated in few places in the world, and considering the possibility of cultivating saffron in Syria and the lack of clear knowledge regarding optimal fertilization for saffron to improve its productivity and quality, comprehensive research is required to reach a reliable long-term strategy to provide the best knowledge and understanding of fertilization requirements and the effect of organic acids on many growth, productivity, and quality characteristics. Therefore, this research aimed to study the effect of organic acid treatment on some biochemical characteristics of several varieties of wild and cultivated saffron, in addition to determining the optimal concentration of these acids that gives the best biochemical traits which are mainly responsible for saffron quality.

**Materials and Methods:**

The experiment was implemented in Banias, Syria, at an altitude of 35-60 meters above sea level, during the period from mid-September to the end of April 2024, and the Mediterranean climate prevails in the region, characterized by a hot and humid summer, and a rainy winter. The annual average temperature ranges between (16-24°C), and the average annual precipitation in the region is about (800-1000 mm); most of which falls in winter and spring. The climatic data in Table (1) indicate that the climatic conditions accompanying the crop growth period (from September to May) were within the appropriate limits for plant growth and production during the cultivation season.

**Table (1) Average temperatures and amount of rainfall during the research season (2023-2024)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Min. Temp. (°C)** | **Max. Temp. (°C)** | **Rainfall (mm/month)** | **Month** |
| **21.6** | **28.6** | **6.7** | **Septembre** |
| **17.1** | **23.9** | **154.7** | **Octobre** |
| **16.3** | **22.5** | **150.0** | **Novembre** |
| **11.1** | **15.3** | **157.0** | **Décembre** |
| **7.2** | **11.3** | **360.0** | **January** |
| **9.5** | **12.7** | **290.0** | **February** |
| **12.9** | **15.7** | **270.0** | **March** |
| **15.0** | **21.0** | **320.0** | **April** |
| **19.5** | **27.1** | **80.8** | **May** |

 **(Meteorological Station in Al-Sinn, 2023-2024)**

Using two introduced saffron strains (Iranian, Spanish) characterized by high productivity, corms free from mechanical damage and diseases were selected, and the size of corms used for planting was standardized to a diameter of (3.5-4) cm. Additionally, two types of wild saffron common in the Syrian environment: Abu Reiha saffron and yellow saffron. Planting was carried out in mid-September in a mixture of peat soil, agricultural soil, and organic fertilizer at a ratio of 1:1:1 with a little sand to increase permeability. The corms were planted manually in bags with diameters of (40\*40\*40 cm) filled with the soil mixture at a depth of about 4 cm and a distance of 10 cm between each corm. A total of 180 corms were planted in 60 bags at a rate of three corms per bag. The experiment was irrigated immediately after planting with water only using surface irrigation, and then irrigation was continued at a rate of one irrigation per week, taking into account the spacing of irrigations during rainfall during the growth period. Irrigation was stopped when the plant entered the dormancy stage in mid-May. Weeding was done manually whenever needed without resorting to herbicides.

**Studied Treatments:**

* **Saffron types**: (Iranian, Spanish, Yellow wild, and Abu Reiha)
* **Organic acids**: Treatment was done by irrigation with acids (humic and fulvic) in one preparation at concentrations (0-25-50 mg/L) at a rate of twice during the growing season according to dates (23/11 and 5/12 2023) during plant growth stages.
* **O0**: Irrigating plants with fresh water only.
* **O25**: Irrigating plants with humic and fulvic acids at concentration (25 mg/L).
* **O50**: Irrigating plants with humic and fulvic acids at concentration (50 mg/L).

The experiment was factorial using a randomized complete block design (RCBD), with three replications per treatment, thus the number of treatments was (12) and the number of experimental units was (36) experimental units.

**Studied Traitsand Characteristics:**
Five plants from each experimental unit were labeled two weeks after the last organic acid treatment (for measuring chlorophyll, carotenoids, and sugars), while the content of (crocin, picrocrocin, and safranal) was measured when reaching maturity and proper drying of the stigmas.

**1- Leaf** **content of chlorophyll and carotenoids (microgram/gram fresh weight):**
Known weight samples of saffron leaves from plants of each treatment were crushed in pure acetone, then the optical absorption of the extract was measured using a spectrophotometer at wavelengths 470, 645, and 662 nanometers, then from the equations according to the researcher's method [25]:

1. Chl a (µg mL⁻¹) = 11.24 DO662 - 2.04 DO645
2. Chl b (µg mL⁻¹) = 20.13 DO645 - 4.19 DO662
3. Chl (µg mL⁻¹) = Chl a + Chl b
4. Car (µg mL⁻¹) = (1000 DO470 - 1.90 Chl a - 63.14 Chl b) / 214

Then the total pigment content was estimated relative to the fresh weight of leaves (microgram/gram fresh weight).

**2- content of sugars in leaves and corms:**

 The content of soluble sugars in leaves and corms was analyzed according to the method of [26] with some modifications. Where 100 mg of fresh saffron leaves and corms were crushed in 4 ml of 80% ethanol, then the tubes were placed in a hot water bath at 80°C for 10 minutes until the alcoholic extract dried, then 5% phenol and concentrated sulfuric acid (96%, density = 1.86) were added to the mixture producing a yellow-brown color. Optical absorption was measured at wavelength 490 nm using a spectrophotometer, then the percentage of sugars in the samples was estimated based on a standard curve of pure glucose.

**3- Analysis of active components:**

 Saffron stigma samples were analyzed in the graduate studies laboratory in the Department of Field Crops to determine the concentration of the main active components in saffron: crocin, picrocrocin, and safranal, using a spectrophotometer according to the method of [27] at the following wavelengths: 440 nm to determine crocin concentration responsible for color, then at wavelength 330 nm to measure safranal concentration responsible for aroma, then at wavelength 257 nm to measure picrocrocin concentration responsible for taste.

Saffron stigmas designated for analysis were dried in the designated drying area, then the samples were ground, then 100 mg of dry ground saffron was taken and placed in tubes containing 200 ml of distilled water. The tubes were placed in a water bath at 60°C with shaking every 5 minutes for 20 minutes. Then the tubes were stored in darkness for 24 hours, and 1 ml of the previous solution was taken and measured with a spectrophotometer by taking three readings for each sample, taking into account the presence of a control sample containing only distilled water [17].

The previous readings were subjected to the following equation:
E¹% = (D × 10000) / (m × (100 - H))
D: Device reading at the specific wavelength for each active substance.
m: Weight of stigma sample used (g).
H: Moisture percentage in the stigma sample.

To determine quality, the result of applying the previous equation will be compared with the standard numbers set by the International Organization for Quality Classification (ISO)¹.

Table 2 shows the quality standards of saffron plant [17].

|  |  |  |
| --- | --- | --- |
|  **Test method** | **Concentration in dried stigmas (μg/g)** | **characteristic** |
| **ISO3632-2****Clause13** | **Picrocrocin****(257nm)** | **70** | **Category I** |
| **55** | **Category II** |
| **40** | **Category III** |
| **30** | **Category IV** |
| **Safranal****(330 nm)** | **20** | **Min** |
| **50** | **Max** |
| **Crocin****(440 nm)** | **190** | **Category I** |
| **150** | **Category II** |
| **110** | **Category III** |
| **80** | **Category IV** |

**4-** **Flower weight (g):** Formed flowers were weighed periodically from each corm and each experimental plot, and flowers were harvested daily in the early morning before full flowering.

**Analysis of results:** The results were statistically analyzed using the Genstat-12 statistical analysis program and the least significant difference (LSD) was calculated at a 5% significance level by comparing the means**.**

**Results and Discussion:**

**1-Effect of organic acid treatment (humic and fulvic)on chlorophyll** **content** **(μg/gFw):**

The results of Figure (1) showed significant differences (P<0.05) between the studied treatments in terms of chlorophyll percentage (μg/gFw), where a significant increase (P<0.05) in chlorophyll content (μg/gFw)was observed when treated with concentration (50 mg/L) in saffron varieties (Iranian, Spanish, Abu Reiha, yellow wild) compared to other treatments, reaching (1262.312 - 1237.22 - 1171.397 - 1251.452 μg/gFw) respectively. The chlorophyll content (μg/gFw) in control plants was (1136.479 - 988.695 - 1018.526 - 1070.684 μg/gFw).

A significant superiority (P<0.05) of Iranian variety plants over saffron variety plants (Spanish, Abu Reiha, yellow wild) was observed in the average total chlorophyll content of the plant, reaching (1200.348 - 1102.166 - 1164.793 - 1113. μg/gFw) respectively.

Plants treated with concentration (50 mg/L) also showed significant superiority (P<0.05) over plants treated with (25 mg/L) and control plants in the average total chlorophyll content of the plant, reaching (1230.595 - 1151.612 - 1053.596 μg/gFw) respectively.

The increase in total chlorophyll pigment content in saffron leaves when treated with organic acids may be due to the role of these acids in improving nutrient absorption, such as activating the absorption of nitrogen N and nitrates NO3 and their metabolism within plant cells, which improves protein synthesis efficiency and thus increases chlorophyll content in leaves [28].

**Figure (1) Effect of organic acid treatment on chlorophyll content (μg/gFw) in Spanish, Iranian, Abu Reiha, and yellow wild saffron varieties. All data indicate averages of 5 replicates,** n = 5, **and different letters indicate significant differences between means for each trait (ANOVA-Tukey test, P<0.05).**

In addition to the role of spraying with organic acids, especially humic acid, in increasing plant cell permeability, improving oxygen absorption, activating the photosynthesis process, and raising the efficiency of iron and phosphorus absorption and increasing root elongation, as a role somewhat similar to the effect of plant hormones [29,30,31]. In this context, [32] indicated that spraying with humic acid HA stimulates and activates H⁺-ATP pumps and supports secondary ion transporters in the cell membrane, leading to increased absorption of plant nutrients.

**2- Effect of organic acid treatment (humic and fulvic) on carotenoid** **content (μg/gFw):**

The results of Figure (2) showed significant differences (P<0.05) between the studied treatments in terms of carotenoid content (μg/gFw), where a significant increase (P<0.05) in carotenoid content (μg/gFw) was observed when treated with concentration (50 mg/L) in saffron varieties (Iranian, Spanish, Abu Reiha, yellow wild) compared to other treatments, reaching (78.839 - 157.608 - 125.780 - 92.231 μg/gFw) respectively. The carotenoid content (μg/gFw) in control plants was (78.569 - 106.991 - 76.807 - 76.775 μg/gFw).

A significant superiority (P<0.05) of Spanish variety plants over saffron variety plants (Iranian, Abu Reiha, yellow wild) was observed in the average total carotenoid content of the plant, reaching (77.919 - 126.707 - 95.606 - 84.561 μg/gFw) respectively. Plants treated with concentration (50 mg/L) also showed significant superiority (P<0.05) over plants treated with (25 mg/L) and control plants in the average total carotenoid content of the plant, reaching (113.614 - 85.285 - 89.696 μg/gFw) respectively. The positive effect of organic acid treatment on carotenoid content in saffron leaves is explained by the role of these acids in stimulating the physiological and biochemical processes occurring in the plant, as these acids participate in building proteins, manufacturing carbohydrates, and activating the photosynthesis process by contributing to increased synthesis of photosynthetic pigments such as chlorophyll and carotenoids [33,34].

**Figure (2) Effect of organic acid treatment on carotenoid content (μg/gFw) in Spanish, Iranian, Abu Reiha, and yellow wild saffron varieties. All data indicate averages of 5 replicates,** n = 5, **and different letters indicate significant differences between means for each trait (ANOVA-Tukey test, P<0.05).**

**3- Effect of organic acid treatment (humic and fulvic) on sugar content in corms(%):**
The results of Figure (3) indicate significant differences (P<0.05) between the studied treatments in terms of sugar content in corms (%), where a significant increase (P<0.05) in sugar content in corms (%) was observed when treated with concentration (50 mg/L) in saffron varieties (Iranian, Spanish, Abu Reiha, yellow wild) compared to other treatments, reaching (4.168% - 4.742% - 3.979% - 4.415%) respectively. The sugar content in corms (%) in control plants was (3.998% - 4.447% - 3.843% - 4.189%).

**Figure (3) Effect of organic acid treatment on sugar content in corms (%) in Spanish, Iranian, Abu Reiha, and yellow wild saffron varieties. All data indicate averages of 5 replicates,** n = 5, **and different letters indicate significant differences between means for each trait (ANOVA-Tukey test, P<0.05).**

A significant superiority (P<0.05) of Spanish variety plants over saffron variety plants (Iranian, Abu Reiha, yellow wild) was observed in the average sugar content in corms (%) of the plant, reaching (4.567% - 4.080% - 4.316% - 3.927%). Plants treated with concentration (50 mg/L) also showed significant superiority (P<0.05) over plants treated with (25 mg/L) and control plants in the average sugar content in corms (%), reaching (4.326% - 4.223% - 4.119%) respectively.

**4- Effect of organic acid treatment (humic and fulvic) on sugar content in leaves(%):**
The results of Figure (4) showed significant differences (P<0.05) between the studied treatments in terms of sugar content in leaves (%), where a significant increase (P<0.05) in sugar content in leaves (%) was observed when treated with concentration (50 mg/L) in saffron varieties (Iranian, Spanish, Abu Reiha, yellow wild) compared to other treatments, reaching (4.825% - 4.921% - 4.689% - 5.042%) respectively. The sugar content in leaves (%) in control plants was (3.610% - 4.305% - 3.962% - 3.603%).

A significant superiority (P<0.05) of Spanish variety plants over saffron variety plants (Iranian, Abu Reiha, yellow wild) was observed in the average sugar content in leaves (%) of the plant, reaching (4.694% - 4.273% - 4.266% - 4.264%).

Plants treated with concentration (50 mg/L) also showed significant superiority (P<0.05) over plants treated with (25 mg/L) and control plants in the average sugar content in leaves (%), reaching (4.869% - 4.385% - 3.87%) respectively.

**Figure (4) Effect of organic acid treatment on sugar content in leaves (%) in Spanish, Iranian, Abu Reiha, and yellow wild saffron varieties. All data indicate averages of 5 replicates,** n = 5, **and different letters indicate significant differences between means for each trait (ANOVA-Tukey test, P<0.05).**

The increase in soluble sugar content (%) in corms and leaves can be attributed to the positive effect of organic acid treatment on the soluble sugar content in saffron corms and leaves because plant cells can easily absorb these compounds to contribute to activating all physiological, biochemical, and metabolic processes occurring in cells, in addition to providing ready-made components for building and synthesizing proteins, synthesizing carbohydrates and plant sugars [35]. It was indicated [36] that the direct absorption of humic substances by the plant significantly and directly affects photosynthesis, which leads to increased accumulation of stored materials and their gathering in the bulbs in the case of saffron. Additionally, the presence of acids in the soil affects the development of the root system and improves the nutritional status of the plant through the availability of essential elements, in addition to improving the enzymatic system in the plant [37].

**5- Effect of organic acid treatment (humic and fulvic) on active components of saffron stigmas (μg/g):**

**5-1- Effect of organic acid treatment (humic and fulvic) on crocin percentage (μg/g):**
The results of Figure (5) indicated significant differences (P<0.05) between the studied treatments in terms of crocin percentage (μg/g), where a significant increase (P<0.05) in crocin percentage (μg/g) was observed when treated with concentration (50 mg/L) in saffron varieties (Iranian, Spanish, Abu Reiha, yellow wild) compared to other treatments, reaching (171.81 - 186.006 - 151.861 - 144.986 μg/g) respectively. The crocin percentage (μg/g) in control plants was (126.47 - 161.133 - 97.648 - 93.030 μg/g).

A significant superiority (P<0.05) of Spanish variety plants over saffron variety plants (Iranian, Abu Reiha, yellow wild) was observed in the average crocin percentage (μg/g) of the plant, reaching (166.279 - 153.755 - 128.410 - 122.985 μg/g).

Plants treated with concentration (50 mg/L) also showed significant superiority (P<0.05) over plants treated with (25 mg/L) and control plants in the average crocin percentage (μg/g), reaching (163.665 - 149.837 - 119.570 μg/g) respectively.

**Figure (5) Effect of organic acid treatment on crocin percentage (μg/g) in Spanish, Iranian, Abu Reiha, and yellow wild saffron varieties. All data indicate averages of 5 replicates,** n = 5, **and different letters indicate significant differences between means for each trait (ANOVA-Tukey test, P<0.05).**

**5-2- Effect of organic acid treatment (humic and fulvic) on picrocrocin percentage (μg/g):**

The results of Figure (6) showed significant differences (P<0.05) between the studied treatments in terms of picrocrocin percentage (μg/g), where a significant increase (P<0.05) in picrocrocin percentage (μg/g) was observed when treated with concentration (50 mg/L) in saffron varieties (Iranian, Spanish, Abu Reiha, yellow wild) compared to other treatments, reaching (63.218 - 61.454 - 51.944 - 49.184 μg/g) respectively. The picrocrocin percentage (μg/g) in control plants was (56.114 - 31.684 - 34.895 - 51.62 μg/g).

A significant superiority (P<0.05) of Iranian variety plants over saffron variety plants (Spanish, Abu Reiha, yellow wild) was observed in the average picrocrocin percentage (μg/g) of the plant, reaching (59.923 - 56.332 - 43.129 - 39.001 μg/g). Plants treated with concentration (50 mg/L) also showed significant superiority (P<0.05) over plants treated with (25 mg/L) and control plants in the average picrocrocin percentage (μg/g), reaching (56.45 - 48.759 - 43.579 μg/g) respectively.

**Figure (6) Effect of organic acid treatment on picrocrocin percentage (μg/g) in Spanish, Iranian, Abu Reiha, and yellow wild saffron varieties. All data indicate averages of 5 replicates,** n = 5, **and different letters indicate significant differences between means for each trait (ANOVA-Tukey test, P<0.05).**

**5-3- Effect of organic acid treatment (humic and fulvic) on safranal percentage (μg/g):**
The results of Figure (7) showed significant differences (P<0.05) between the studied treatments in terms of safranal percentage (μg/g), where a significant increase (P<0.05) in safranal percentage (μg/g) was observed when treated with concentration (50 mg/L) in saffron varieties (Iranian, Spanish, Abu Reiha, yellow wild) compared to other treatments, reaching (63.676 - 58.508 - 47.595 - 51.144 μg/g) respectively. The safranal percentage (μg/g) in control plants was (56.708 - 50.484 - 31.764 - 28.226 μg/g).

A significant superiority (P<0.05) of Iranian variety plants over saffron variety plants (Spanish, Abu Reiha, yellow wild) was observed in the average safranal percentage (μg/g) of the plant, reaching (59.810 - 54.394 - 41.494 - 37.662 μg/g).

Plants treated with concentration (50 mg/L) also showed significant superiority (P<0.05) over plants treated with (25 mg/L) and control plants in the average safranal percentage (μg/g), reaching (55.230 - 47.99 - 41.795 μg/g) respectively.

**Figure (7) Effect of organic acid treatment on safranal percentage (μg/g) in Spanish, Iranian, Abu Reiha, and yellow wild saffron varieties. All data indicate averages of 5 replicates,** n = 5, **and different letters indicate significant differences between means for each trait (ANOVA-Tukey test, P<0.05).**

The positive effect of organic acid treatment on the content of crocin, picrocrocin, and safranal can be explained in light of the increase in plant carotenoid and antioxidant content resulting from treatment with organic acids, and this agrees with study [39]. And to its role as an enzyme activator that stimulates the oxidation of carotenoids to carotenoid derivatives and their association with sugars [32].

The increase in safranal concentration with the use of organic acids may be due to the addition of both acids stimulating the synthesis of the enzyme β-glucosidase [40], which is responsible for converting picrocrocin to safranal during the drying process [41]. Furthermore, saffron quality differs due to the formation of specific secondary metabolites instead of others, in addition to some factors that must be considered during saffron production. The first is flower picking. This step is usually performed during the early hours of the day while the flowers are still closed and light is not suitable for the crocin inside the stigmas. Light may indeed cause loss of these compounds. The second aspect to consider is the drying process. Additionally, the variation in quality standards (crocin, picrocrocin, safranal) can be attributed to the genetic material used, environmental conditions, as well as cultivation, harvesting, and post-harvest (drying and storage), as indicated by [41,42,43]. It seems that any factor that enhances plant carbohydrate concentration will have a direct effect on increasing volatile compounds in saffron. [44] mentioned that the content of picrocrocin, safranal, and crocin in saffron was higher by 73%, 77%, and 83% respectively, due to the combined application of organic fertilizer and biofertilizers compared to chemical fertilizers. Other researchers showed that biofertilizers improved saffron essential oil more than chemical fertilizers. For example, [45] mentioned that the application of biofertilizers increased picrocrocin and safranal in saffron stigmas more than chemical fertilizers.

**6- Effect of organic acid treatment (humic and fulvic) on Flower weight (g):**

The results of (Figure 8) showed significant differences (P<0.05) between the studied treatments in terms of flower weight per plant (g), as a significant increase (P<0.05) in flower weight (g) was observed when treated with a concentration (50 mg/L) for saffron varieties (Iranian - Spanish - Abu Riha - Safrawi) compared to the rest of the treatments, and reached (0.515 - 0.601 - 0.1026 - 0.0902 g), respectively. The flower weight of the control plants reached (0.43 - 0.0439 - 0.1005 - 0.0885 g). The Spanish variety plants were significantly (P<0.05) superior to the saffron variety plants (Abu Riha-Safrawi) in the average flower weight per plant, which reached (0.5-0.10-0.08 g), respectively, but it did not outperform the Iranian variety, which reached (0.46 g). The plants treated with the concentration (50 mg/L) were significantly (P<0.05) superior to the plants treated (25 mg/L) and the control plants in the average number of flowers per plant, which reached (0.32-0.27-0.26 g), respectively.

**Figure (8) Effect of organic acid treatment on** **Flower weight (g) in Spanish, Iranian, Abu Reiha, and yellow wild saffron varieties. All data indicate averages of 5 replicates,** n = 5, **and different letters indicate significant differences between means for each trait (ANOVA-Tukey test, P<0.05).**

The increase in flower weight (g) can be explained by the fact that these organic acids contain substances that contribute to increasing the photosynthesis process and the accumulation of carbohydrates in the plant. Balanced and timely feeding of these fertilizers is a basic condition for obtaining optimal and high-quality saffron production [46]. The content of organic acids has a positive effect on the quantity of saffron flowers and improving its productivity and quality [47]. In this context, [48] showed that organic acids play an effective role in increasing flower production and stimulating production. These results were consistent with the results of [47]), which showed an increase in the number of flowers in plants treated with organic acids compared to untreated plants. The genetic factor represented by the variety and its interaction with the environmental conditions accompanying growth is considered one of the most important factors affecting the growth, development and production characteristics of saffron, which in turn has a positive and significant impact on the number and weight of flowers in the plant.

**Conclusions and Recommendations:**

Treatment with organic acids at concentration 50 mg/L led to a significant increase in most of the studied traits such as chlorophyll and carotenoid content (μg/gFw), sugar content in leaves and corms (%), content of active components crocin, picrocrocin, and safranal (μg/g). The varieties (Iranian, Spanish, Abu Reiha, yellow wild) differed in their response to organic acid treatment. The Spanish variety recorded the highest value for carotenoid content (μg/gFw), sugar content in leaves and corms (%), crocin content (μg/g), while the Iranian variety showed the highest content of chlorophyll (μg/gFw), picrocrocin (μg/g), and safranal (μg/g). In light of these results, it is recommended to use the concentration 50 mg/L of organic acids (humic and fulvic) for their stimulating physiological role in increasing plant growth and development.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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