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**NEW APPROACHES FOR SAFFRON (*CROCUS SATIVUS* L.)  
FERTILIZATION IN LEBANON**

**ABSTRACT OF A DISSERTATION**

for awarding of educational and scientific degree “Doctor”

Field of higher education: 6.1  
Professional field: Plant growing  
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The dissertation is written on 127 pages and contains 14 tables and 28 figures and 5 appendix. The list of references includes 226 titles. A total of 8 conclusions and 4 recommendations were made and 5 scientific and applied contributions. The study material is outlined in 5 sections.

The dissertation defense will be held on 11.01.2023, at 11:00 in the Academic Hall “M. Dakov” at Building A of the University of Forestry, Sofia, 10 Kliment Ohridski Blvd. at an open meeting of a scientific jury approved by Order ZPS-641/05.12.2022 of the Rector of the University of Forestry with the following members:

**Chairman:** Assoc. Prof. Dr. Vera Zamfirova Petrova

**Members:** Prof. Dr. Ivanka Georgieva Mitova  
Prof. Svetla Stoyanova Kostadinova, Phd  
Assoc. Prof. Dr. Margarita Todorova Nikolova  
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The materials on the defense (dissertation, abstract, reviews and opinions) are available to those interested on the website of the University of Forestry ([www.ltu.bg](http://www.ltu.bg)) and in the dean's office of AF – Sofia, 10 “Kliment Ohridski” blvd.

## I. INTRODUCTION

Saffron (*Crocus sativus*) is a flowering plant of the genus *Crocus* in the family Iridaceae. It is one of the most valuable agricultural and medicinal plants that for centuries has been cultivated in Iran, India, and southern Europe (Menia et al., 2018). A red spice called saffron is obtained from the dried red stigmas of *Crocus sativus* L. flowers and is used in the pharmaceutical, cosmetic, perfume, and textile dye industries. Saffron contains over 100 biologically active compounds, the most important of which are crocin, crocetin, picrocrocin, and safranal (Singletary, 2020). In Lebanon, the objective of saffron cultivation was to replace illegal crops (cannabis and poppies) in underdeveloped areas as a potential means of fostering socioeconomic development (Chalfoun, 2014). A variety of factors affect saffron cultivation, including climate, crop density, irrigation, and other agricultural methods (Al Madini et al., 2019; Rezvani-Moghaddam, 2020), but a balanced and timely supply of fertilizer is a pre-requisite for getting optimum yield and quality of saffron (Ghanbari and Khajoei-Nejad, 2021). So far, this plant was mostly developed in the Baalbeck-Hermel area, the Aakkar Plain, and South Lebanon. However, this type of cultivation might have a potential to succeed in other Lebanese growing regions if suitable agricultural practices (fertilization, irrigation, species and corm selection etc.) are adopted. To attain this goal, the fertilization management program should be designed to increase nutrient use efficiency. optimal nutrient delivery, controlled-release fertilizers, integrated fertilization management, and the use of organic nutrient resources are all examples of methods that helps achieving nutrient use efficiency (Ghanbari and Khajoei-Nejad, 2021). The use of nanofertilizers may increase the effectiveness of nutrient use, lessen nutrient waste, replenish soil fertility, boost crop output, maintain ecosystem health, and prevent significant soil issues (Chhipa, 2017). Studies have showed their positive effect on saffron flowering and production traits, like flower number, stigma length, fresh and dry stigma weights, and dry stigma yield (Amirnia et al. 2014). Super absorbent polymers and hydrogels have recently demonstrated a variety of benefits for soil amendments, including preserving water content, decreasing the consumption of soil nutrients, and limiting the detrimental effects of dehydration and moisture stress on crops (Elshafie and Camele, 2021). Therefore, the main aim of this research was to investigate the effect of climate on saffron as well as to identify the saffron grown in North Lebanon, and applying nano and organic fertilizers, and SAP to different sized corms grown in north Lebanon. The strategy of this study is to determine the optimal method to increase saffron yield and quality under Lebanese climate conditions.

## II. AIM AND TASKS

AIM of the study: To evaluate and optimize saffron cultivation under Lebanese climate conditions in order to expand this type of cultivation into a new Lebanese region. In addition, the effect of nano-fertilizers has not yet been tested on saffron especially in the Lebanese region. However, water availability plays a role in many growth effects on saffron, hence SAP (super absorbent polymer) was one of the solutions several studies reported to its beneficial and efficient effect in this field. Thus, the study aimed to optimize saffron production under Lebanese climate conditions by providing a strategy where nano and organic fertilizers are applied in combination with SAP so that less fertilizers amounts and irrigation water are needed. The study was done in three separate phases: The first phase of the study (2019) was to investigate the effect of climate conditions of North Lebanon (Douma) on saffron yield and quality in comparison to those obtained in saffron native country of production; Iran (Mahallat). The second phase of the experiment was conducted in 2020 to identify the saffron species being cultivated in Douma (North Lebanon). The third phase (2021) of research was to investigate the effects of different corm weight, nano and organic fertilizers, and SAP application on the performance of saffron cultivated in Lebanon.

Many tasks were targeted during this study:

1. Assess and compare the phenological dates, quantitative and qualitative indicators of saffron between Douma and Mahallat.
2. Conduct DNA sequencing on corm samples to identify the species cultivated in Douma
3. Assess and compare the phenological dates, quantitative and qualitative indicators of saffron of different corm weights as affected by the application of fertilizers and SAP alone or combined.

The study was achieved to give answers to the following questions:

- How did local climate conditions of North Lebanon affect saffron production and quality?
- What is the saffron species normally cultivated in Douma?
- What is the effect of the three fertilizers tested (Seaumic, LITHOvit FORTE, Super plus ZFM++) on saffron production and quality?
- What is the effect of super absorbent polymers (SAP) on saffron production and quality?
- Are fertilizers and SAP better used alone or in combination?

### III. MATERIALS AND METHODS

#### III.1. FIRST PHASE

##### III.1.1. Experimental sites

Saffron corms of *Crocus sativus* were grown in two locations characterized by different geographical and weather conditions: Douma situated in North Lebanon and Mahallat situated in Iran. Experiments in Douma and Mahallat were conducted simultaneously. In specific, 1000 corms were planted in the experimental field in Douma, and other 1000 corms were planted in the experimental field of the research center in Mahallat, Iran.

##### III.1.2. Weather and soil conditions

The two experimental sites had different weather conditions and soil properties (Table 3). Meteorological data were collected from local meteorological stations located at the regions where experimental sites were located; meteorological data were collected from the weather station of the Lebanese Agricultural Research Institute (LARI) station in Lebanon while data of Mahallat from was procured from the Ornamental Plants Research Center (OPRC), Alborz Province, Iran.

In order to carry out the physico-chemical analysis of the soil (texture and structure), soil samples were taken from the plot, at 0 to 30 cm depth, since the majority of the roots develop up to this depth and that tillage ensures nutrients are mixed up to about 15 to 30 cm. Soil samples were collected randomly following the zigzag method. Collected samples were mixed together, put in a plastic bag, and sent to the laboratory of the Lebanese Agricultural Research Institute (LARI) at Fanar station, to carry out soil analysis (Fig. 1).

**Table 1. Physico-chemical characteristics of soils from the two experimental sites.**

| Site     | Soil texture    | Sand (%) | Silt (%) | Clay (%) | OM (%) | pH  | EC (mS. cm <sup>-1</sup> ) | Ntotal (%) | P <sub>2</sub> O <sub>5</sub> av (ppm) | K <sub>2</sub> Oav (ppm) |
|----------|-----------------|----------|----------|----------|--------|-----|----------------------------|------------|--|--------------------------|
| Mahallat | Sandy Loam      | 56       | 32       | 12       | 0.51   | 7.9 | 1.3                        | 0.051      | 462.4                                  | 293                      |
| Douma    | Sandy Clay Loam | 53.7     | 24.7     | 21.5     | 5.04   | 7.6 | 0.4                        | 0.406      | 464.1                                  | 529.5                    |

Ntotal: Total nitrogen; P<sub>2</sub>O<sub>5</sub>av: Available phosphate; K<sub>2</sub>Oav: Available potassium



**Fig. 1. Steps of soil analysis at the laboratories of LARI-Fanar**

### **III.1.3. Agricultural practices**

Sowing trials in each location were of 16 m<sup>2</sup> and consisted on raised beds with rows 50 cm apart. In each row, corms were planted at 10 cm depth and were distant of 15 cm. Both fields were managed organically, as no pesticides or herbicides were used during the growing season, and weeds were controlled manually. Drip irrigation started by first September and was done weekly providing 1 liter/plant at each irrigation time. This was done similarly at both locations.

### III.1.4. Vegetative and reproductive indicators.

In each experimental site, quantitative indicators were evaluated on 30 corms, collected from the same place that was tagged at the beginning of the experiment. The number of flowers (NF) harvested was recorded at each harvest date. Moreover, leaf number and leaf length were recorded after harvest of flowers. Phenological dates as date of leaf and flower cataphylls appearance (days after plantation (DAP)), and date of first harvest (days from planting) were recorded based on daily observation.

### III.1.5. Stigma collection and preparation

Flower-picking started as soon as flowers appeared in the field, and it was done by hand on daily basis. Methods for removal of the stigma from flowers and drying conditions were kept identical to the methods used by farmers. Stigmas were brought indoors where they were separated by hand shortly after collecting in the field and the total fresh weight of collected stigmas (FWS) was recorded (Fig. 2). Stigmas were dried in a forced air oven at 30°C for 24 h, and then stigmas dry weight (DWS) was recorded.

### III.1.6. Qualitative indicators

Saffron stigma samples collected from both locations were analyzed for quality. The quality of saffron in international commercial agreements is determined according to ISO 3632 (International Organization for Standardization, 2011), which classifies it into three categories depending upon their physical and chemical characteristics (Carmona et al., 2006). The measurement of E1% of an aqueous saffron extract at 440 nm for crocin (colour strength), 257 nm for picrocrocin (bitterness) and 330 nm for Safranal (aroma) were carried out with a 1 cm pathway cell on a UV-Visible spectrophotometer. Moisture content was also determined according to ISO 3632 (International Organization for Standardization, 2011). The amount of effective compounds of saffron has been deter-

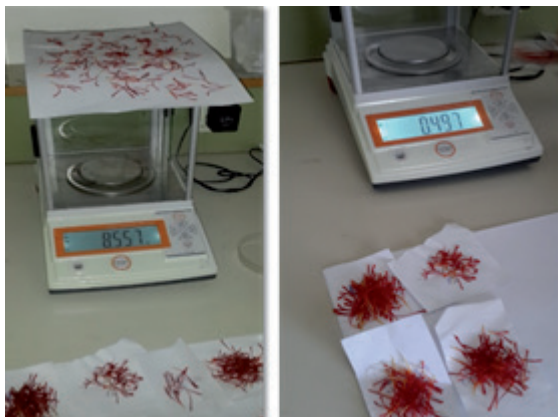


Fig. 2. Measurement of stigma weight

mined by HPLC method, which is a very accurate method for determining the amount of these compounds (Mahmoodi et al., 2021).

### **III.1.7. Statistical analysis**

All recorded data were statistically analysed using STASTICA (data analysis software system), version 12. copyright StatSoft, Inc. (2014). One-way ANOVA was adopted and the p-values equal or less than 0.05 was considered statistically significant. Correlation coefficients were established among various indicators independently of the environmental conditions.

## **III.2. SECOND PHASE**

### **III.2.1. Saffron sampling**

To identify the saffron grown in North Lebanon, plants were collected from the Douma experimental site during the flowering period of the studied species. Five saffron samples were chosen, labeled, packed, and shipped to Macrogen Inc. in Korea for DNA extraction (Fig. 3).



**Fig. 3. Samples taken for DNA test**

## **III.3. THIRD PHASE**

### **III.3.1. Field preparation and planting**

The third phase of the study was conducted in the same location used in the first phase of the study (Douma, North Lebanon) starting in August 2021 (date of corm plantation). Field was plowed 30 cm deep into the soil, and before planting, weeds were removed manually, to reduce competition for water



and nutrients with saffron. Likewise, the rows were prepared keeping 20 cm between them (Fig. 4). During the first week of August, corms were planted distant of 15 cm in each row. The saffron irrigation used a localized drip system with a distance of 20 cm between the irrigation lines and 15 cm between the emitters of the same line. The first irrigation was done at the same time as planting and the second irrigation was done 15 days after the first irrigation in order to facilitate the sprouting of the corms (Khorrarnadel et al., 2013; Zarch et al., 2020). Transmitters with a flow rate of 4 l/h were used. Each irrigation was conducted for 1 hour and 30 min.



**Fig. 4. Planting corms (left); treatment of corms with fungicide (right)**

### **III.3.2. Experimental design**

During the experiment, 2000 corms were planted; 1000 corms of 4-6 g (CW1) and 1000 corms of 6-8 g (CW2). Sixteen treatments were the subject of the study, which investigated the separate and combined effects of three fertilizers: Seaumic, Super Plus ZFM++ (ZFM++), and LITHOvit FORTE (LIForte); and SAP on saffron performance. Fertilizers and SAP were tested alone (CW1/Seaumic, CW1/ZFM++, CW1/LIForte, CW1/SAP, and CW2/Seaumic, CW2/ZFM++, CW2/LIForte, CW2/SAP) or combined (CW1/Seaumic/SAP, CW1/ZFM++/SAP, CW1/LIForte/SAP, and CW2/Seaumic/SAP, CW2/ZFM++/SAP, CW2/LIForte/SAP), compared to two controls; non-treated corms of weight 1 (CW1) and weight 2 (CW2).

Effectively, the experimental design was a randomized complete block design (Fig. 5), with five blocks, and sixteen treatments per block, with 25 replicates per treatment. Therefore, each treatment consisted of 125 corms distributed into five blocks.

| Block 1 | Block 2 | Block 3 | Block 4 | Block 5 |
|---------|---------|---------|---------|---------|
| T1      | T11     | T3      | T12     | T1      |
| T8      | T3      | T1      | T15     | T14     |
| T4      | T6      | T15     | T8      | T13     |
| T12     | T14     | T12     | T13     | T4      |
| T2      | T10     | T13     | T5      | T15     |
| T9      | T5      | T9      | T2      | T12     |
| T13     | T4      | T2      | T9      | T2      |
| T16     | T16     | T8      | T1      | T8      |
| T14     | T15     | T4      | T7      | T7      |
| T7      | T2      | T16     | T4      | T11     |
| T15     | T12     | T5      | T3      | T10     |
| T3      | T7      | T11     | T14     | T6      |
| T5      | T1      | T14     | T16     | T9      |
| T11     | T8      | T7      | T10     | T5      |
| T10     | T9      | T6      | T11     | T16     |
| T6      | T13     | T10     | T6      | T3      |

**Fig. 5. Experimental design of phase 3 experiment; T1: CW1, T2: CW1/ SAP, T3: CW1/ Seamic, T4: CW1/ ZFM++, T5: CW1/ LIForte, T6: CW1/Seamic/SAP, T7: CW1/ZFM++/SAP, T8: CW1/LIForte/SAP, T9: CW2, T10: CW2/SAP, T11: CW2/ Seamic, T12: CW2/ZFM++, T13: CW2/LIForte.**

### III.3.3. Application of fertilizers

All products were applied as fertigation; LITHOvit FORTE (1 kg/ha as a 0.5% suspension; 0.5 kg g of LITHOvit per 100 l of water), Seamic (2 l/ha) and Super Plus ZFM++ (3 l/ha).

### **III.3.4. Application of SAP**

For evaluation of the impact of SAP rates on flowering indicators and underground traits of saffron, it was applied at the time of saffron planting. The used SAP was made of potassium polyacrylate based polymer. It was applied at a rate of 40 kg/ha. SAP was used below and beside of mother corms (Fallahi et al., 2017a).

### **III.3.5. Data collection**

#### **III.3.6. Leaf and flower appearance**

The date of appearance of the first leaf and flowers and the date of the first harvest were recorded following daily observation of the site. These dates were expressed in terms of days after planting (DAP).

#### **III.3.7. Harvesting and conservation of flowers**

Harvesting is carried out in order to extract the pistils with three stigmas from each saffron flower using tweezers. Drying is an essential step because it determines the organoleptic quality of the spice and its shelf life. The Spanish drying method was adopted which consists in drying the stigmas on a hot plate at a constant temperature of 65° C for a period of 45 minutes, in order to reach a dry matter content.

#### **III.3.8. Saffron production**

The number of leaves produced by each saffron corm and their lengths were measured and recorded. Before harvesting, the number of flowers produced by each corm were counted and then calculated by unit of area (m<sup>2</sup>). Similarly, after harvesting, followed by drying of the stigmas, the dry weight of the stigmas produced by each flower, and therefore the dry weight produced by each corm, were measured and recorded. All these indicators were assessed on the representative samples of each type of saffron selected at the site.

#### **III.3.9. Replacement corms**

Corm lifting from soil was carried out after leaf withering in mid-June. Corm measured indices included the number of replacement corms per m<sup>2</sup>, and total weight of replacement corms (g/m<sup>2</sup>). Eventually, replacement corms developed on initial corms were counted and weighed (using Shimadzu TX4202L electronic balance). Counting and weighing of replacement corms was done on all initial corms of every treatment, and the results were expressed per square meters.

### **III.3.10. Saffron quality**

To test the physico-chemical quality of saffron, three samples of each treatment of saffron were prepared. Each sample to test will be 0.5 g. The quality tests concerning the characteristic components of saffron, such as crocin, picrocrocin, and safranal was carried out at a laboratory by spectrophotometric analysis. The contents of crocin, picrocrocin and safranal was determined according to the international standard ISO-3632.

### **III.3.11. Statistical analysis**

One-way ANOVA was adopted and the p-values equal or less than 0.05 was considered statistically significant. Pearson correlations were established between different tested indicators. The significance of correlation was evaluated considering 95 and 99% confidence levels. Statistical analysis was applied using SPSS® program.

## **IV. RESULTS AND DISCUSSION**

### **IV.1. RESULTS OF PHASE I: CLIMATE CONDITIONS EFFECT ON SAFFRON**

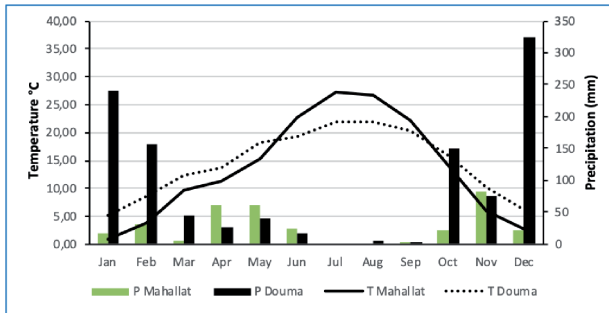
#### **IV.1.1 Results of soil analysis**

In Mahallat the percentages of sand, silt and clay were 56, 32 and 12%, respectively, and then the soil was sandy loam (Table 3). In Douma the soil was sandy clay loam with 53.7% sand, 24.7% silt and 21.5% clay. Both soils had almost similar pH with 7.9 and 7.6 in Mahallat and Douma respectively. On the other hand, the soil analysis detected a higher EC in Mahallat's soil ( $1.3 \text{ mS.cm}^{-1}$ ) compared to Douma's soil ( $0.4 \text{ mS.cm}^{-1}$ ).

Regarding nutrient content, the available phosphorus was similar in both soils with 462.4 ppm. The total nitrogen and the available potassium as well as the organic matter were lower in Mahallat's soil. As shown in the table 3 the total N was 0.051%, the available K was 293.0 ppm and the organic matter was 0.51% in Mahallat. In Douma, soil test recorded 0.406% total N, 529.5 ppm available K and 5.04% organic matter.

#### **IV.1.2. Results of climate analysis**

The mean temperature during the trial period from May to October was higher in Mahallat-Iran ( $21.4^{\circ}\text{C}$ ) compared to Douma-Lebanon ( $19.5^{\circ}\text{C}$ ), while rainfall during the same time period was higher in Douma-Lebanon (218.2 mm) compared to Mahallat-Iran (106.9 mm) (Fig. 6).



**Fig. 6. Meteorological trend in the experimental sites (Mahallat and Douma) during Phase I. (P) for mean monthly precipitations and (T) for mean monthly temperature.**

The precipitation pattern between corm planting and flower harvest was quite similar in the two sites, up until October, when Douma recorded 150 mm of precipitation compared to 25 mm for Mahallat, or five times as much precipitation. From mid-summer until beginning of flowering, the temperature in Douma and Mahallat dropped 7°C and 15°C respectively, indicating the rapid change and higher fluctuation in temperature in Mahallat compare to that in Douma.

#### **IV.1.3. Effect of both location on saffron**

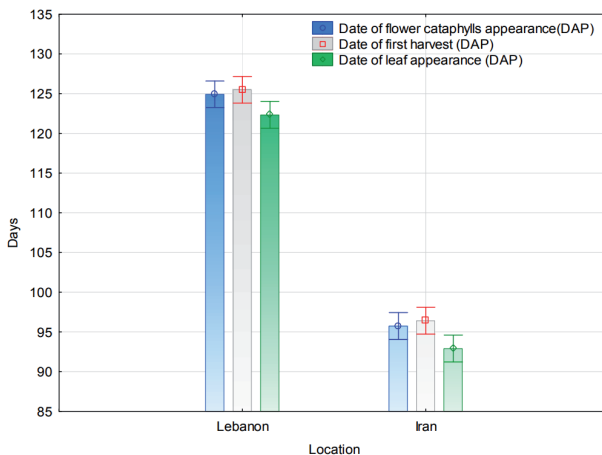
As shown in Table 7, Strong positive correlations were found between flower number per 1000 bulbs and fresh and dry stigmas yield (g/1000 bulb) (0.974 and 0.975 respectively) and between dry stigmas weight and fresh stigmas weight (0.938) (Table 7). Furthermore, high relationship was found between all qualitative absorbance readings.

**Table 2: Pearson correlation analysis of yield and quality characteristics of the saffron samples of both locations**

|                    | Fresh stigma yield | Dry stigma yield | Picrocrocin | Safranal | Crocin   |
|--------------------|--------------------|------------------|-------------|----------|----------|
| Flower number      | 0.974445           | 0.975812         | 0.947084    | 0.969079 | 0.987030 |
| Fresh stigma yield |                    | 0.938747         | 0.943080    | 0.941639 | 0.975326 |
| Dry stigma yield   |                    |                  | 0.866117    | 0.907944 | 0.936724 |
| Picrocrocin        |                    |                  |             | 0.992031 | 0.949999 |
| Safranal           |                    |                  |             |          | 0.959127 |

\*Fresh and dry stigma yield expressed in g/1000 bulbs; \*\*Flower number per 1000 bulbs

All recorded phenological dates were earlier in Mahallat-Iran than in Douma Lebanon (Fig. 7). Leaf and flower cataphylls appearance as well as harvest dates were earlier by around 30 days in Mahallat-Iran. It should be noted that the duration of harvest was also longer in Mahallat-Iran than in Douma-Lebanon with 12 and 9 days respectively.



**Fig. 7. Date of flower appearance, date of harvest and date of leaf appearance in DAP (date after plantation) (means  $\pm$  SE)**

Saffron quality depends on the concentration of its major metabolites. According to the absorbance readings, saffron grown in Mahallat-Iran resulted in significantly higher picrocrocin, safranal and crocin contents than those grown in Douma-Lebanon. Picrocrocin content was 58.2 and 50.9, Safranal content was 30.9 and 25.8 and crocin content was 115 and 96.7 in Mahallat-Iran and Douma-Lebanon respectively (Table 8). Moisture content of stigmas was the highest in Mahallat-Iran where it reached 13.5% compared to 9% in Douma-Lebanon. The major focus of saffron content measure is to establish whether the product meets the ISO standard parameters, to get an indication of the range of quality being produced under different environments and to have a comparison basis with saffron from other country.

As for coloring strength, which is related to the crocin content, Mahallat-Iran samples belong to category III, while those of Douma-Lebanon belong to the category IV. As for the taste, which is dependent on safranal content, Mahallat-Iran samples belonged to category II, while those of Douma-Lebanon belong to category III. Both samples met minimum requirement for aroma

strength, which is related to the picrocrin content. Finally, findings on saffron qualitative traits were positively correlated to quantitative ones (Table 8).

**Table 3. Quality characteristics of the saffron samples according to ISO 3632 (International Organization for Standardization, 2011) by site.**

| Active substance             |              | Mahallat | Douma |
|------------------------------|--------------|----------|-------|
| Picrocrocin ( $\lambda$ 257) | Reading      | 58.2     | 50.9  |
|                              | ISO category | II       | III   |
| Safranal ( $\lambda$ 330)    | Reading      | 30.9     | 25.8  |
|                              | ISO category | Min.     | Min.  |
| Crocine ( $\lambda$ 440)     | Reading      | 115      | 96.7  |
|                              | ISO category | III      | IV    |
| Moisture content%            |              | 13.4     | 9     |

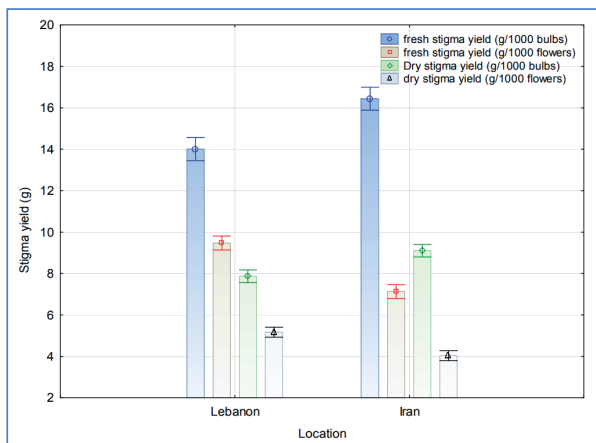
There was a great variability in saffron yields obtained in both investigated locations. Fresh and dry stigma yield per 1000 flowers were higher in Douma-Lebanon (9.5 and 7 g respectively) than in Mahallat-Iran (5 and 4 g respectively). On the contrary, when dry and fresh stigma weights were expressed in grams per 1000 bulbs, they were higher in Mahallat-Iran (9.1 and 16.4 g respectively) than in Douma-Lebanon (7.9 and 14 g respectively) (Fig. 8).

Average leaf number of saffron grown Mahallat-Iran was also significantly higher than in Douma Lebanon (i.e., 20 and 13.5 cm respectively). However, it was an opposite case regarding leaf length where it was 35 and 57 leaves respectively (Table 9).

**Table 4. Mean comparison of leaf number per plant, leaf length, and flower number per bulb according to experimental site.**

| Location | Leaf number per plant | Leaf length (cm) | Flowers number/bulb |
|----------|-----------------------|------------------|---------------------|
| Lebanon  | 13.58333 b            | 56.91667 a       | 1.5 b               |
| Iran     | 20 a                  | 35.41667 b       | 2.25 a              |

In effect, flower number per 1000 bulbs was higher by 35% in Mahallat-Iran compared to Douma-Lebanon, while flower number per square meter and flower number per bulb were both higher by almost 50% in Mahallat-Iran compared to Douma-Lebanon (Table 9).



**Fig. 8. Fresh and dry stigma yield of Saffron grown in Douma-Lebanon and Mahallat-Iran**

## IV.2. RESULTS OF PHASE 2: DNA RESULTS

Results of Pair-wise nucleotide sequence comparison (Table 10) showed that HE801161.1\_ *Crocus\_oreoreticus* only shared a 99.5% with AB699586.1:228-682\_ *Crocus\_sativus* and 1\_ *Crocus\_sativus\_Y*. The subsequent work in Phase 3 was conducted using the species *Crocus oreoreticus*.

## IV.3. RESULTS OF PHASE 3: FERTILIZERS AND SAP APPLICATION

### IV.3.1. Date of leaf appearance

The treatments CW2/Seamic, CW2/Seamic/SAP, and CW2/LIForte/SAP recorded significantly earlier date of leaf appearance (20.5, 20.3, and 22.0 DAP, respectively) compared to remaining treatments. The date of leaf appearance was similar in CW2/LIForte/SAP and CW2/ZFM++/SAP and recorded delayed values in the treatments CW1, CW1/SAP, and CW2 (42.2, 40, and 40 days, respectively), with the latest date in CW1. Also, this indicator was significantly earlier in treated corms (fertilizer + SAP) of CW2 compared to treated corms of CW1, although it did not significantly differ in non-treated corms of both weights. In all treatments with CW2, the date of leaf appearance was significantly earlier than control, while in all treatments with CW1, it was significantly earlier than control except for CW1/SAP. There was no significant difference in the date of leaf appearance among the treatments where fertilizers were applied alone on corms of 4-6 g (CW1/Seamic, CW1/ZFM++,



and CW1/LIForte), while, this phenological stage was significantly earlier in the treatment CW2/Seaumic compared to CW2/ZFM++, and CW2/LIForte (by 5.2 and 4.2 days, respectively). Concerning corm weight one (CW1), the combined use of SAP with tested fertilizers resulted in earlier leaf emergence dates compared to the use of fertilizers alone (by 10.8, 4.3, and 7.7 days in CW1/Seaumic/SAP, CW1/ZFM++/SAP, and CW1/LIForte/SAP compared to CW1/Seaumic, CW1/ZFM++ and CW1/ LIForte). On the other hand, in treatments with corm weight 2 (CW2), this was only applicable in the treatment CW2/LIForte/SAP with 2.7 days earliness compared to CW2/LIForte.

### **IV.3.2. Date of flower cataphylls appearance**

Earliest appearance of flower cataphylls was observed in CW2/Seaumic and CW2/Seaumic/SAP (20.2 and 19.2 DAP, respectively); with no significant difference between both treatments. The latest flower cataphylls appearance was in the treatment CW1 (47.3 DAP), with as significant difference compared with all remaining treatments. The date of flower cataphylls appearance was earlier by 5.0 days in CW2 compared with CW1, and by 5.7 days in CW2/SAP compared with CW1/SAP. The combination of fertilizers and SAP hastened the date of flower cataphylls appearance compared with control cases of both tested corm weights. Effectively this phenological stage was earlier by a range of 11.8-20.6 days concerning corms of weight 1 and by 16.3-22.8 days concerning corms of weight 2. Also, compared with the single use of SAP, the combination of SAP and fertilizers has hastened the date of flower cataphylls appearance by a range of 6.5-15.3 days with respect to corm weight 1 and by a range of 10.6-17.1 days with respect to corms of weight 2. Seaumic caused earlier appearance of flower cataphylls compared with ZFM++ and LIForte in corms of weight 2 (earliness of 7.5 and 6.3 days in CW2/Seaumic compared with CW2/ZFM++ and CW2/LIForte, respectively). Seaumic caused as well earliness in this indicator when combined with SAP, compared with ZFM++ and LIForte combined with SAP, and concerning both corm weights. Concerning corms of weight one, the use of Seaumic and LIForte in combination with SAP has hastened the appearance of flower cataphylls compared with the single use of each fertilizer (38.7, 40.3, 26.7, and 34.3 DAP in CW1/Seaumic, CW1/LIForte, CW1/Seaumic/SAP, and CW1/LIForte SAP, respectively). In corms of weight two, the use of ZFM++ combined with SAP (CW2/ZFM++/SAP) caused an earliness of 3.7 days in this indicator compared with ZFM++ without SAP (CW2/ZFM++).

### **IV.3.3. Date of first harvest**

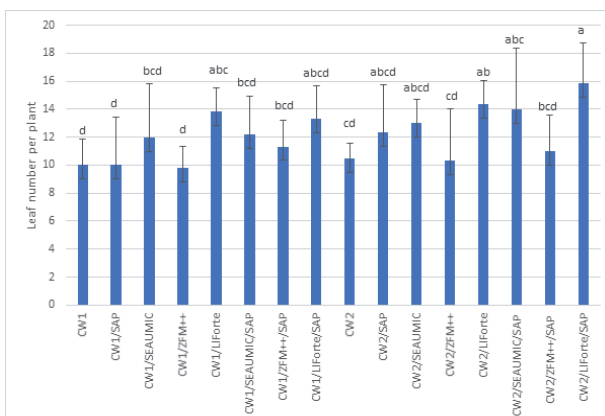
The treatments CW2/Seaumic and CW2/Seaumic/SAP recorded the earliest first harvest dates (20.8 and 20.0 DAP, respectively). On the other hand, the latest dates of first harvest were in CW1, CW1/SAP, and CW2 (45.0, 44.5, and 42.3 days respectively). All treated corms of both tested weights recorded earlier harvest dates than control cases, and all treated corms of weight 2 had earlier first harvest dates compared with those of weight 1. In addition, CW2/Seaumic had earlier first harvest from CW2/ZFM++ and CW2/LIForte by 7 and 6 days respectively, while CW1/Seaumic, CW1/ZFM++, and CW1/LIForte showed no significant difference with respect to this indicator. The combination of SAP with fertilizers had only caused hastening in first harvest dates in corms of weight one, in specific when combined with Seaumic and LIForte rather than with ZFM++; first harvest was earlier by 10.5 and 3.8 days in CW1/Seaumic/SAP and CW1/LIForte/SAP compared with CW1/Seaumic and CW1/LIForte, respectively.

### **IV.3.4. Leaf number per plant**

Leaf number per plant did not significantly differ in between non-treated corms of both sizes (CW1 and CW2). Also, fertilizers applied alone (Seaumic, ZFM++, and LIForte) had almost a similar effect on this indicator when comparing both corm weights (CW1 and CW2). The highest leaf number was reached in CW2/LIForte/SAP (15.8), followed by CW2/LIForte (14.3), CW2/Seaumic/SAP (14.0), CW1/LIForte (13.8), CW1/LIForte/SAP (13.3), CW2/Seaumic (13.0), and CW2/SAP (12.3), with no significant difference among the values recorded in these seven treatments (Fig. 9). Concerning corms of weight 1, LIForte applied alone caused a significant increase in leaf number compared to control (13.8 and 10.0 in CW1/LIForte and CW1, respectively). Considering corms of weight 2, LIForte applied alone and in combination with SAP caused an increase in leaf number by around 3.8 and 5.3 in CW2/LIForte and CW2/LIForte/SAP compared with CW2. Also, LIForte application caused a significantly higher number of leaves compared with ZFM++ with respect to both corm weights; 13.8 and 9.8 in CW1/LIForte and CW1/ZFM++, and 14.3 and 10.3 in CW2/LIForte and CW2/ZFM++.

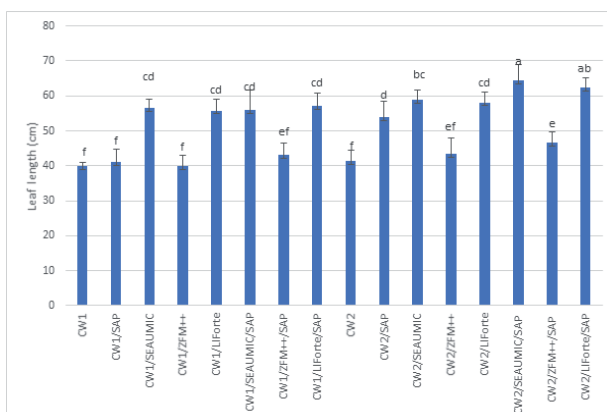
### **IV.3.5. Leaf length**

Concerning leaf length, CW2/Seaumic/SAP and CW2/LIForte/SAP recorded the highest average values among all treatments with a slight superiority for the first treatment (64.3 and 62.5 cm, respectively) (Fig. 10). Corm



**Fig. 9. Leaf number per plant (means  $\pm$  SE) (Means followed by different letters are significantly different according to Duncan's significant difference  $P < 0.05$ ).**

weight factor did not have a significant influence on this vegetative growth trait. The products Seaumic and LIForte significantly increased leaf length compared to ZFM++ (by 16.7 and 15.8 cm concerning CW1, and 15.5 and 14.7cm concerning CW2). Also, both products (Seaumic and LIForte) had superior effects compared to ZFM++ when combined with SAP and concerning both corm weights (56.0, 57.2, and 43.2 cm in CW1/Seaumic/SAP, CW1/LIForte /SAP, and CW1/ZFM++/SAP, and 64.3, 62.5, and 46.5 cm in CW2/



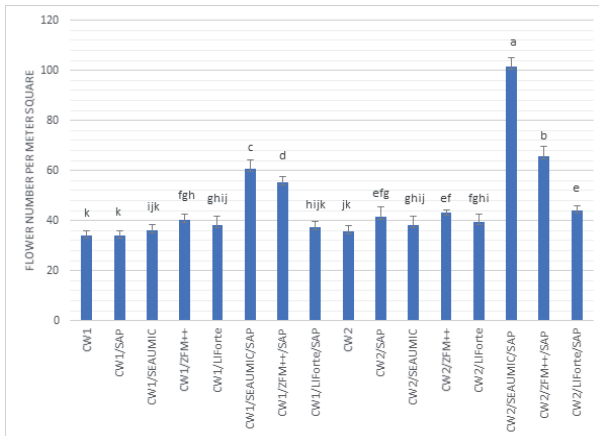
**Fig. 10. Leaf length (cm) (means  $\pm$  SE) (Means followed by different letters are significantly different according to Duncan's significant difference  $P < 0.05$ ).**

Seaumic/SAP, CW2/LIForte /SAP, and CW2/ZFM++/SAP, respectively). With respect to corms of weight 1, the combined use of fertilizers and SAP did not cause a significant difference on leaf length compared with the single use of each fertilizer. However, on corms of weight 2, leaf length increased by around 5.3 cm in CW2/Seaumic/SAP compared with CW2/Seaumic, and by around 4.3 cm in CW2/LIForte/SAP compared with CW2/LIForte.

### **IV.3.6. Flower number per meter square**

The treatment CW2/Seaumic/SAP recorded the highest number of flowers (101.7 flowers/m<sup>2</sup>). In contrast, both treatments CW1 and CW1/SAP recorded the lowest number of flowers (34 and 34.2 flowers/m<sup>2</sup> respectively); and they were significantly lower compared to all remaining treatments except for CW1/Seaumic (36.0 flowers/m<sup>2</sup>), CW1/LIForte/SAP (37.2 flowers/m<sup>2</sup>), and CW2 (35.5 flowers/m<sup>2</sup>). Evidently, non-treated corms of both weights produced almost similar number of flowers per m<sup>2</sup>, but heavier corms treated with SAP gave a significantly higher number of flowers compared with lighter ones (41.3 and 34.2 flowers/m<sup>2</sup> in CW2/SAP and CW1/SAP, respectively). SAP applied alone caused a significant improvement in flowers number per square meters only with respect to corms of weight 2; increase by 14% in CW2/SAP compared with CW2 (Fig. 11). When fertilizers were applied alone, and with respect to both tested corm weights, ZFM++ had a superior effect compared with Seaumic; flowers number per m<sup>2</sup> increased by 4.2 and 5.2 flowers in CW1/ZFM++ and CW2/ZFM++ compared with CW1/Seaumic and CW2/Seaumic, respectively. Also, ZFM++ and LIForte applied alone increased significantly this indicator in comparison with control by 6.2 and 4.2 flowers in CW1/ZFM++ and CW1/LIForte and by 7.7 and 4.0 flowers in CW2/ZFM++ and CW2/LIForte respectively compared to control. In treatments where fertilizers were tested in combination with SAP, flower number per m<sup>2</sup> was the highest with Seaumic, followed by ZFM++, and LIForte, respectively with regards to both corm weights. Besides, concerning corms of weight 1, combining SAP with Seaumic and ZFM++ improved flower number compared with the use of each fertilizer alone (increase by 24.7 and 15.1 flowers in CW1/Seaumic/SAP and CW1/ZFM++/SAP compared with CW1/Seaumic and CW1/ZFM++, respectively). On the other hand, the use of LIForte alone or combined with SAP resulted in an almost similar flower number per m<sup>2</sup>. Concerning corms of weight 2, the combination of SAP with the three tested fertilizers improved flowers number per m<sup>2</sup> compared with the single use of each fertilizer (increase by 63.7, 22.3, and 4.7 flowers in CW2/Seaumic/SAP, CW2/ZFM++/SAP, and CW2/LIForte/SAP compared

with CW2/Seaumic, CW2/ZFM++, and CW2/LIForte, respectively). Overall, compared with control cases, flowers number per square meters increased in all treatments, except in CW1/SAP, CW1/Seaumic, CW1/LIForte/SAP, and CW2/Seaumic. In the cases where there was a significant effect of treatments, improvement in flower number ranged between 10.9 and 43.9% in corms of weight 1, and between 10.1 and 65.0% in corms of weight 2.

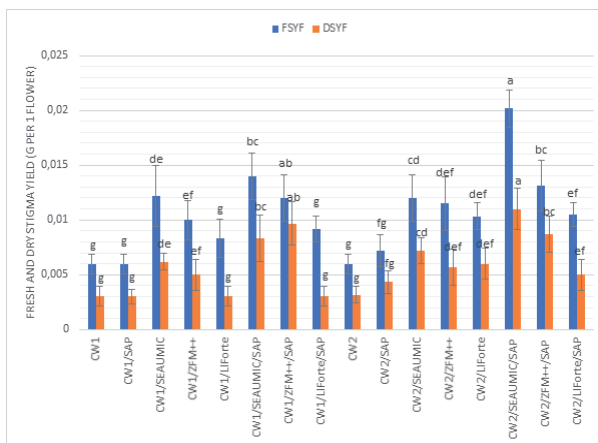


**Fig. 11. Flower number per meter square (means  $\pm$  SE) (Means followed by different letters are significantly different according to Duncan's significant difference  $P < 0.05$ ).**

### IV.3.7. Fresh and dry stigma yield per one flower

The comparison between corm weights shows that there was no significant difference in fresh and dry stigma yields of non-treated corms (CW1 and CW2) corms of both weights treated with SAP (CW1/SAP and CW2/SAP), and corms of both weights treated with fertilizers alone, except for the treatment CW2/LIForte the dry stigma yield of which was significantly higher compared with CW1/LIForte (0.006 and 0.003 g/flower, respectively) (Fig. 12). On the other hand, and in comparison, with corms of weight 1, corms of weight 2 produced significantly higher fresh and dry stigma yields when treated with SAP and Seaumic combined (0.02017 and 0.11 g/flower respectively in CW2/Seaumic/SAP compared with 0.014 and 0.0083 g/flower in CW1/Seaumic/SAP), and significantly higher dry stigma yield when treated with SAP and LIForte (0.005 g/flower in CW2/LIForte/SAP compared with CW1/LIForte/SAP). On corms of weight 1, the use of the three tested fertilizers alone caused a significant improvement in fresh stigma yield compared with control, but only Seaumic and ZFM++ significantly increased dry

stigma yield compared with control. Besides, LIForte applied alone had significantly decreased the dry stigma yield compared with Seaumic and ZFM++ (0.003, 0.00617, and 0.005 g/flower in CW1/ LIForte, CW1/ Seaumic, and CW1/ ZFM++, respectively). On corms of weight 2, using fertilizers alone caused a non-significant difference in the fresh and dry stigma yields recorded in between fertilizers, but a significant improvement in both indicators compared with control, with superior effect of Seaumic compared to ZFM++ and LIForte. On the other hand, dry stigma yield per flower increased significantly compared to control in all treatments where SAP was combined with fertilizers, except in the treatment CW1/LIForte/SAP; it increased by around 0.005 and 0.007 g in CW1/Seaumic/SAP and CW1/ZFM++/SAP respectively compared with CW1, and by around 0.008, 0.005, and 0.002 g in CW2/Seaumic/SAP, CW2/ZFM++/SAP, and CW2/LIForte/SAP respectively compared with CW2. Furthermore, the use of SAP+Seaumic and SAP+ZFM++ caused significantly higher fresh and dry yields compared with the use of SAP+LIForte and with respect to both tested corm weights. Overall, the highest fresh and dry yields of stigma were recorded in the treatment CW2/Seaumic/SAP (0.02017 and 0.011 g/flower, respectively).



**Fig. 12. Fresh and dry stigma yield (g per 1 flower) (means ± SE) (Means followed by different letters are significantly different according to Duncan's significant difference  $P < 0.05$ )**

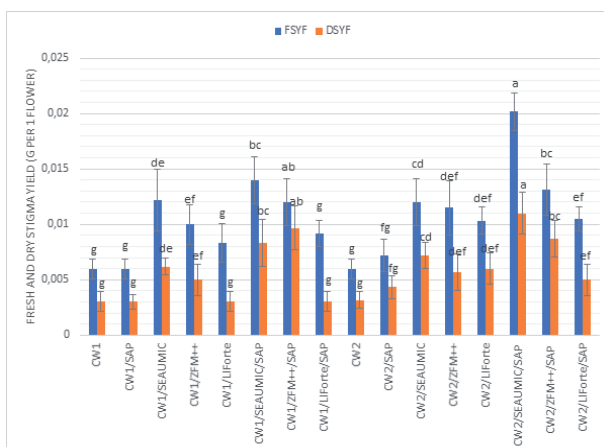
### IV.3.8. Fresh and dry stigma yield per m<sup>2</sup>

Results showed that there was no difference in fresh and dry stigma yields between CW1 and CW2. In contrast CW2/SAP recorded significantly

higher fresh and dry stigma yields per square meters (0.29 and 0.16 g/m<sup>2</sup> respectively) compared with CW1/SAP (0.2 and 0.1 g/m<sup>2</sup> respectively) (Fig. 13). The use of SAP alone improved the fresh and dry stigma yield of weight 2 corms only; by 27.9% and 35.8% respectively in CW2/SAP compared with CW2. With respect to both tested corm weights, fresh stigma yield per square meter was significantly higher in all treatments where fertilizers were applied alone, compared with control cases. The dry stigma yield was also significantly higher than control with respect to both corm weights, in all treatments where fertilizers were applied alone, except in the treatment CW1/LIForte. Effectively, compared with control cases, dry stigma yield of corms of weight 1 and 2 increased respectively by around 43.9 and 60% following the single use of Seaumic, and by 33.7 and 50.7% following the single use of ZFM++. The use of LIForte alone increased dry stigma yield per m<sup>2</sup> by 60.1% for corms of weight 2 compared with control.

The application of Seaumic and ZFM++ alone on corms of weight 1 resulted in significantly higher dry stigma yields per square meters compared with the use of LIForte; dry stigma yield was respectively higher by 0.13 and 0.09 g/m<sup>2</sup> in CW1/Seaumic and CW1/ZFM++ compared with CW1/LIForte. However, concerning corms of weight 2 the single use of the three tested fertilizers did not cause a significant difference in terms of dry stigma yield obtained in between the treatments CW2/Seaumic, CW2/ZFM++, and CW2/LIForte. Fresh stigma yield was significantly higher than control in all treatments where SAP was used in combination with fertilizers; improvement in this indicator was of 75.0, 69.2, and 31.8% in CW1/Seaumic/SAP, CW1/ZFM++/SAP, and CW1/LIForte/SAP respectively compared with CW1, and of 89.7, 75.6, and 53.2% in CW2/Seaumic/SAP, CW2/ZFM++/SAP, and CW2/LIForte/SAP respectively compared with CW2. On the other hand, dry stigma yield was significantly higher than control in all treatments where SAP was applied in combination with fertilizers, except the treatment CW1/LIForte/SAP; improvement in this indicator was of 71.4 and 72.3% in CW1/Seaumic/SAP and CW1/ZFM++/SAP respectively compared with CW1, and of 90.4, 79.6, and 51.9% in CW2/Seaumic/SAP, CW2/ZFM++/SAP, and CW2/LIForte/SAP, respectively compared with CW2. Eventually, the highest fresh and dry stigma yields were recorded in the treatment CW2/Seaumic/SAP (2.01 and 1.11 g/m<sup>2</sup>, respectively). The treatments CW2/ZFM++/SAP and CW1/Seaumic/SAP ranked in the second place in terms of fresh and dry stigma yields per square meters, with no significant difference between both treatments. On corms of weight 1, the use of SAP in combination with Seaumic or ZFM++ has significantly increased the fresh and dry stigma yields

compared with the single use of Seaumic and ZFM++. Effectively, fresh stigma yield improved by around 0.408 and 0.258 g and dry stigma yield increased by around 0.24 and 0.29 g in CW1/Seaumic/SAP and CW1/ZFM++/SAP compared with CW1/Seaumic and CW1/ZFM++. In a close pattern, the combination of SAP with Seaumic or ZFM++ significantly improved fresh and dry stigma yields of corms of weight 2 compared with the single use of both fertilizers; fresh stigma yield improved by 1.55 and 0.38 g and dry stigma yield increased by 0.84 around 0.30 g in CW2/Seaumic/SAP and CW2/ZFM++/SAP compared with CW2/Seaumic and CW2/ZFM++. On the other hand, the combination SAP and LIForte did only increase the fresh stigma yield but not the dry one.



**Fig. 13.** Fresh and dry stigma yield per m<sup>2</sup> (means ± SE) (Means followed by different letters are significantly different according to Duncan's significant difference P < 0.05).

### IV.3.9. Picrocrocin, crocin, and Safranal

Results showed that picrocrocin content increased significantly compared to control cases in all treatments except, CW1/SAP, CW2/SAP, CW1/LIForte/SAP and CW2/LIForte/SAP. The treatment CW2/ZFM++ recorded the highest value of picrocrocin content (88.1%) followed respectively by the treatments CW1/ZFM++ (87.1%), CW2/Seaumic (85.5%), and CW1/Seaumic (85.3%), with no significant difference between values recorded in these four treatments (Table 14). Seaumic and ZFM++ applied alone or in combination with SAP, had caused significantly higher picrocrocin content in stigmas compared to LIForte (applied alone or with SAP). The application of SAP in combination with Seaumic and ZFM++ resulted in higher picrocrocin content



compared with the single use of SAP; improvement by 6.3 and 7.5% in CW1/Seaumic/SAP and CW1/ZFM++/SAP compared to CW1/SAP, and by 6.9 and 8.3% in CW2/Seaumic/SAP and CW2/ZFM++/SAP compared to CW2/SAP. Results also showed that crocin content was significantly higher than control cases in all treatments except in CW1/SAP, CW2/SAP, and CW2/LIForte/SAP. The highest crocin content was recorded in the treatments CW2/ZFM++ (180.7%), followed by CW1/ZFM++ (178.5%) with no significant difference between both treatments.

**Table 5. Picrocrocin, safranal, and crocin content (means  $\pm$  SE) in each treatment (Means followed by different letters are significantly different according to Duncan's significant difference  $P < 0.05$ )**

|                 | PIC                 | SAF                | CRO                  |
|-----------------|---------------------|--------------------|----------------------|
| CW1             | 73.3 $\pm$ 4.9 j    | 24.1 $\pm$ 1.9 b   | 165.9 $\pm$ 1.5 i    |
| CW1/SAP         | 74.0 $\pm$ 2.9 ij   | 24.3 $\pm$ 2 ab    | 168.0 $\pm$ 2.2 hi   |
| CW1/SEAUMIC     | 85.3 $\pm$ 4.7 abcd | 26.9 $\pm$ 3.3 ab  | 175.7 $\pm$ 2.4 bcd  |
| CW1/ZFM++       | 87.1 $\pm$ 2.9 ab   | 27.9 $\pm$ 3 ab    | 178.5 $\pm$ 3 ab     |
| CW1/LIForte     | 78.0 $\pm$ 1.3 fghi | 25.0 $\pm$ 4.7 ab  | 171.9 $\pm$ 2.2 defg |
| CW1/SEAUMIC/SAP | 80.3 $\pm$ 5.8 efg  | 25.6 $\pm$ 1.1 ab  | 172.6 $\pm$ 6 def    |
| CW1/ZFM++/SAP   | 81.5 $\pm$ 1.8 def  | 25.9 $\pm$ 1.6 ab  | 173.3 $\pm$ 3.8 cdef |
| CW1/LIForte/SAP | 76.1 $\pm$ 2.3 hij  | 24.7 $\pm$ 3.8 ab  | 170.9 $\pm$ 2.2 fgh  |
| CW2             | 75.4 $\pm$ 4 hij    | 24.6 $\pm$ 2.3 ab  | 168.3 $\pm$ 2.8 ghi  |
| CW2/SAP         | 75.3 $\pm$ 2.2 hij  | 24.5 $\pm$ 1.8 ab  | 169.7 $\pm$ 2.2 fgh  |
| CW2/SEAUMIC     | 85.5 $\pm$ 1.8 abc  | 27.3 $\pm$ 3.7 ab  | 176.6 $\pm$ 2.4 bc   |
| CW2/ZFM++       | 88.1 $\pm$ 2 a      | 28.2 $\pm$ 1.8 a   | 180.7 $\pm$ 2 a      |
| CW2/LIForte     | 78.3 $\pm$ 2.4 fgh  | 25.0 $\pm$ 3.1 ab  | 172.2 $\pm$ 4.1 defg |
| CW2/SEAUMIC/SAP | 82.8 $\pm$ 3.6 cde  | 26.0 $\pm$ 2 ab    | 174.9 $\pm$ 2.1 bcde |
| CW2/ZFM++/SAP   | 83.6 $\pm$ 1.3 bcde | 26.2 $\pm$ 3 ab    | 175.2 $\pm$ 2.9 bcd  |
| CW2/LIForte/SAP | 76.5 $\pm$ 2 ghij   | 24.81 $\pm$ 2.8 ab | 171.2 $\pm$ 3 efg    |

Seaumic, ZFM++, and LIForte applied alone improved crocin content of weight 1 corms by 9.8 and 12.6% respectively compared to CW1 and that of corm weight 2 by 8.3 and 12.4%, respectively compared to CW2. LIForte applied alone improved crocin content of weight 1 corms by 6% compared to CW1. Crocin content decreased significantly when ZFM++ was applied in combination with SAP; by 5.2% in CW1/ZFM++/SAP compared with CW1/ZFM++ and by 5.5% in CW2/ZFM++/SAP compared with CW2/ZFM++.

Furthermore, safranal content did not significantly differ between treated and non-treated corms of both weights. The only exception is improvement

significantly higher value recorded in the treatment CW2/ZFM++ compared with CW1 (28.1 and 24.1%, respectively).

#### **IV.3.10. Yield of replacement corms**

Yield of daughter corms was significantly higher than control in all treatments. The highest average value of this indicator was recorded in the treatment CW2/Seaumic/SAP (850 g/m<sup>2</sup>), while the lowest value was in CW1 (560 g/m<sup>2</sup>). All treatments with CW2 recorded significantly higher values compared to treatments with CW1; yield of replacement corms ranged between 617.5 and 850.0 g/m<sup>2</sup> in the first case, and between 560.0 and 675.7 g/m<sup>2</sup> in the second case. In treatments where fertilizers were applied without SAP, LIForte showed a superior effect on this indicator compared with Seaumic and ZFM++ concerning both tested corm weights; average values were significantly higher in CW1/LIForte compared with CW1/Seaumic and CW1/ZFM++, and in CW2/LIForte compared with CW2/Seaumic and CW2/ZFM++. Further, treatments with fertilizers were combined with SAP recorded significantly higher average values than those with fertilizers were used alone. Besides, SAP combined with Seaumic caused a higher improvement in the yield of daughter corms than SAP combined with LIForte and ZFM++, respectively. Improvement of this indicator was of 17.1, 15.4, and 4.76% in CW1/Seaumic/SAP, CW1/LIForte/SAP, and CW1/ZFM++/SAP, respectively, compared to CW1, and of 27.3, 20.3, and 8.54% in CW2/Seaumic/SAP, CW2/LIForte/SAP, and CW2/ZFM++/SAP, respectively, compared to CW2.

#### **IV.3.11. Number of replacement corms per m<sup>2</sup>**

Results showed that compared with CW1, the average number of daughter corms increased significantly in all treatments, except CW1/SAP and CW1/Seaumic. Compared with CW2, this indicator significantly increased in all treatments, except CW2/SAP. Compared to control cases, the number of daughter corms was significantly higher in all treatments where fertilizers were applied alone, except in the treatment CW1/Seaumic. Further, this indicator did not significantly differ between CW1 and CW2 but it was significantly higher in CW2/SAP, CW2/Seaumic, CW2/Seaumic /SAP, and CW2/LIForte/SAP compared with CW1/SAP, CW1/Seaumic, CW1/Seaumic/SAP, and CW1/LIForte/SAP by 6, 8, 4, and 9 corms respectively. The number of daughter corms was also significantly higher in CW1/Seaumic/SAP and CW1/LIForte/SAP compared with CW1/Seaumic and CW1/LIForte by 7 and 4 corms, respectively. The highest number of daughter corms was in

the treatments CW2/LIForte/SAP and CW2/Seaumic/SAP, with around 15% improvement compared with CW2, and around 20% improvement compared with CW1.

#### **IV.4. DISCUSSION OF PHASE 1: SOIL AND CLIMATE**

##### **IV.4.1. Soil Parameters**

In term of pH, both soils in Douma and Mahallat are suitable for saffron growing, since according to Rahimi et al. (2017) a pH ranging from 6.8 to 7.8 is the optimal for saffron. However, Douma's soil was better in term of EC level since the electrical conductivity in the range of 0.09 to 0.30 ds/m is most suitable for increasing saffron productivity (Ganaie and Singh, 2019).

Regarding soil texture, the lighter texture and well-drained soil which is sandy loam to sandy is the optimal for saffron (Gresta et al., 2009a; Rahimi et al., 2017), thus the soil in Mahallat was more suitable than in Douma since the first was sandy loam and the second is rich in clay. The indicators are normally affected by soil texture (Gresta et al., 2009b). For instance, The higher number of flowers and the higher fresh weight of stigma per bulbs recorded in Iran's location compared to Lebanon could be due to the fact that planting saffron in lighter-textured soils increased flowering by 70% (Aghhavani Shajari et al., 2015); nonetheless, saffron stigma production increased by 18% when cultivated in sandy soil as opposed to clay soil (Khorramdel et al., 2015). In addition, Douma's soil was richest in OM which is better for growing saffron (Gresta et al., 2009a). The available and high levels of  $P_2O_5$  as well as the exchangeable and high levels of  $K_2O$ , have not posed any problem for the saffron crop which needs these quantities during the stages of its reproduction and development (Ramezani et al., 2009) which the case of Douma's soil. It is recommended that to grow saffron it is better to have 0.40% of total nitrogen in the soil (Shahandeh, 2020). Therefore, the soil in Douma is the most suitable since it contained this amount. In contrast Mahallat's soil only contained 0.05% so it is required applying mineral fertilizers at a rate of 40 to 60 kg/ha before the start of the vegetative cycle of the plant (Chaji et al., 2013).

##### **IV.4.2. Climate influence**

When comparing the stigma yield per 1000 flowers it was clearly found that this indicator was higher in Lebanon than in Iran. Even though, the yield of stigma per bulb was higher in Iran than in Lebanon. In fact, the increase in flower number leads to a decrease in stigma formation (Kumar et al., 2008). The dry stigma yield recorded in Lebanon (5.1 g/1000 flowers) was higher

than the one recorded in a previous study on saffron grown in Lebanon (1.8-4.9 g/flowers) (Yau et al., 2006). However the dry stigma yield recorded in Iranian location showed almost a similar yield (4 g/1000 flowers) to the ones recorded in Yau et al. (2006) study. Although water requirement of saffron is low, water stress affects its growth, development and yield (Slama et al., 2005). In fact, the altitude or elevation of the land with respect to the level of the sea surface influences plant growth and development primarily through temperature effect (Shokati et al., 2016). Mahallat-Iran was able to produce higher yield even though mean precipitations in this region were lower than those of Douma-Lebanon throughout the growing season (106.9 mm and 218.2 mm respectively) (Fig. 12). It could be suggested here that altitude may have played a role affecting saffron yield in both locations. Panwar et al. (1995) reported successful saffron cultivation between 1500 and 2000 m above sea level which could explain the highest yield recorded at Mahallat at 2000 m altitude compared to the one recorded at Douma at 1100 altitudes. Adding the effect of lighter soil texture in Mahallat, as mentioned before, which enhanced the saffron yield. In a study by Kothari et al. (2021b), fresh stigma yield and dry stigma yield increased with the corresponding increase in altitude where the highest yield was found at altitude 2,195 m with 742.7 mm total rainfall ; however, they recorded a decrease in yield with the increase in rainfall. Cultivation site conditions especially soil characteristics has been demonstrated to be associated with the variation in crocin, picrocrocin and safranal content (Cardone et al., 2019). In saffron, temperature plays a role in flowering induction, appearance and growth (Molin et al., 2004; Aghhavan Shajari et al., 2015). Cardone et al. (2019) used principal component analysis (PCA) to show that the cultivation location with the highest temperature and the least amount of rain during the flowering season had the best stigma yield with high-quality features. As a result, favorable environmental factors enhance the yield qualities to result in a higher saffron yield. The average temperature in Mahallat-Iran dropped from 22°C in September to 14°C in October (Fig. 12), and flower emergence required the transfer of the corms from a condition of higher temperature to conditions of markedly lower temperature. In addition, the optimal temperature for flower initiation lies in the range of 23-27°C, but long exposure to these temperatures results in delayed flowering (Molina et al., 2004) which was the case of Douma site. Global warming is expected to raise the average temperature of the Earth's climate system by 1.5 and 2.3° C in 2025 and 2050, respectively, contributing to the delay of saffron flower production (Koocheki et al., 2018).

Numerous studies (Behdani et al. 2004; Molina et al. 2004) have shown

that minimum temperature is main determinant of flower formation in saffron. In areas with earlier than normal onset of cold temperature (in cold season), saffron flowering begins earlier. Due to strong correlation between saffron flowering behavior and ambient temperature, it seems that future climate change will influence flowering patterns of this plant (Koocheki, 2004). Kouzegaran et al. (2011) in their study on the effects of minimum, mean, and maximum temperatures on saffron yield showed that yield increases where maximum temperature is lower, and that there is a reverse correlation between maximum temperature and yield. All recorded phenological dates were earlier in Mahallat than in Douma. Leaf and flower cataphylls appearance as well as harvest dates were earlier by around 30 days in Mahallat. According to Koocheki et al. (2016b), leaf emergence can take place before, during, or after flowering depending on the planting date and the first irrigation date. In addition, early irrigation promotes corm activity and hastens the appearance of leaves before flowering, making harvesting challenging. Summer irrigation, however, boosts not only flower induction but also cell division, which leads to daughter corm growth and a decrease in the number of small corms (Koocheki and Seyyedi 2016). Cultivation site conditions especially soil characteristics has been demonstrated to be associated with the variation in crocin, picrocrocin and Safranal content (Cardone et al., 2019). The lower mean temperature during the trial period from May to October at Douma-Lebanon (19.5°C) compared to Mahallat-Iran (21.4°C) might have been the reason of the lower quality of the stigmas in Douma. According to Gresta et al. (2009b) a colder environment was able to reduce the amount of picrocrocin and other qualitative indicators giving lower stigmas quality. High altitudes may have higher levels of crocin and picrocrocin due to factors including total rainfall, air temperature, solar radiation, and soil properties, which have a big impact on how many marker compounds accumulate in plants (Mykhailenko et al., 2020). Mild water stress may, depending on time, increase the amount of crocin and picrocin in the stigma by slightly slowing development, potentially as a defensive mechanism (Koocheki et al. (2016b).

Previous research suggested that the amount of crocin increased with altitude. As a result, agronomic and climatic conditions influence saffron quality (Lage and Cantrell, 2009). The two growth factors, number and length of the leaves, were greatly affected by various geographic locations (Kothari et al., 2021b). Sand-particles usually cause higher pores, improving the soil's permeability (Banihabib and Iranpour, 2012). This increases the root growth, improves the production and development of leaves (Fallahi et al., 2017).

## IV.5. DISCUSSION OF PHASE 2: DNA RESULTS

As for the DNA analysis, perhaps the most relevant result of this study is the identification of saffron cultivated in Douma as *C. oreoreticus*. Research results provide more arguments in favour of the possibility of existence of different species of the saffron spice that are cultivated but recognized for their real identity. Moreover, this is the first report on the existence of another species of *Crocus* other than *Crocus sativus* being cultivated and propagated. In this study, the genetic characterization based on molecular study of cultivated *Crocus* is presented for the first time in Lebanon. In a study by Golshani et al. (2019) to identify relationship between *Crocus* species, analysis indicated a close relationship between saffron crop cultivated in Iran, two crop saffron species of NCBI (AB699586 and DQ094185) and two wild species of *C. cartwrightianus* and *C. oreoreticus*. Accordingly, the wild species of *C. cartwrightianus* and *C. oreoreticus* were considered as the closest relatives of saffron (*C. sativus*) and probably *C. cartwrightianus* the ancestor of this cultivated species, based on genetic distance and dendrogram obtained by analyzing ITS nuclear sequences. Nemati et al. (2018) analyzed sequences of two chloroplast (trnL-trnF, matK-trnK) and three nuclear (TOPO6, ribosomal DNA ETS, and ITS) marker regions to infer phylogenetic relationships among series *Crocus*, making the probability for the existence of an autotriploid origin of *C. sativus* from *C. cartwrightianus* very likely, also stated that *C. oreoreticus*, is the closest relative of *C. cartwrightianus* and *C. sativus*. Schmidt et al. (2019), by comparative FISH (fluorescent in situ hybridization) of six *Crocus* species from 11 accessions, indicated that *C. sativus* is an autotriploid hybrid derived from heterogeneous *C. cartwrightianus* cytotypes. Another study by Nemati et al. (2018) showed that *C. sativus* shares about 93% of its alleles with *C. cartwrightianus*, 88% with *C. oreoreticus* and 83% with *C. hadriaticus*. More molecular studies should be done to explore the differences between different genotypes of saffron.

## IV.6. DISCUSSION OF PHASE 3: FERTILIZERS AND SAP APPLICATION

### IV.6.1. Date of leaf appearance

Based on the results of the experiment, a higher mother corm did not show an effective role in hastening the leaf emergence date, but a higher mother corm combined with SAP or fertilizers caused earliness in this phenomenon. According to Heydari et al. (2014) increasing nutrient availability causes corms to perform better than solely depending on food storage, because it helps roots to grow faster and thus encourages a faster vegeta-

tive development. On the contrary, Kumar et al. (2008) had earlier reported a direct relationship between the size of the mother corm and the emergence of saffron leaves. Gresta et al. (2008) observed as well a significant effect of corm weight on growth characteristics and performance of saffron. Also, Molina et al. (2005) explained that cell division and subsequent leaf growth occur earlier in larger corms than in smaller corms, which is due to earlier and more leaf utilization of light which increases the production of photosynthetic material and the production of larger corms.

Smaller corm weights had earlier dates when SAP was applied, suggesting that they require higher water availability for earlier leaf emergence. In this concern, Kouchaki et al. (2006) reported that irrigation of smaller mother corms can reduce the time interval from planting to emergence. The use of SAP accelerates cell division in corm by providing more moisture. These materials can enhance leaf growth and consequently more photo-assimilates partitioning to corms (Khorramdel et al., 2013; Fallahi et al., 2014a). The application of fertilizers hastened leaf emergence. Appropriate plant nutrition is an important factor in stimulating growth and development. According to Heydari et al. (2014), the use of fertilizer containing organic substances as humic acid is an effective factor in improving soil fertility, due to the increase in the supply of available nutrients, and causing an improvement in the vegetative growth.

#### **IV.6.2. Date of flower cataphylls appearance**

Saffron is a subhystranthous plant, meaning that flowers can appear after leaf appearance (Mathew, 1977; Gresta et al., 2009a). As results have shown, the date of flowering was affected by corm weight in both treated and non-treated cases; and was always earlier in heavier corms. Eventually, the size of corms is one of the main factors that determine the capacity of saffron for flowering. Studies have shown that there is a close relationship between corm size and flowering in saffron (Molina et al., 2005; Mashayekhi et al., 2006; Nassiri Mahallati et al., 2007). According to Hosseini et al. (2004), the nutritional elements of corms are very important; rich and large corms begin to flower at a faster rate and with a higher quality. Experiments of Çavuşoğlu and Erkel (2005), Çavuşoğlu et al. (2009), and Ghobadi et al. (2015) showed that flowering dates were affected by corm size where bigger corms began to flower earlier than smaller ones. On the contrary, Çavuşoğlu and Erkel (2009) observed that smaller corms begun to flower earlier than bigger corms and attributed such a finding to the fact that smaller corms tried to grow bigger while bigger corms used the potential larger nutrient reserves and stored power to increase daughter corm production.

### **IV.6.3. Leaf number**

The interaction of a big corm x fertilization did not cause an increase in leaf number per corm; leaf number ranged between 10.3 and 14.3 in bigger corms and between 9.8 and 13.8 leaves in smaller corms. These findings contradicted those of Gresta et al. (2017) who reported a significant increase in the leaf number per corm as a result of big corm x fertilization, reaching values of 40.5. According to Golzari Jahan Abadi et al. (2017), the use of biofertilizers based on humic acid had a significant effect on leaf number; however, in the current study Seaumic- a product containing humic acid- did not cause a significant improvement in leaf number in treated cases compared with control. On the contrary, LIForte had a prominent effect on increasing leaf number. According to Rostami et al. (2019), improving leaf traits means increasing the photosynthesis in the plant. The number and length of saffron leaves have a great effect on determining the photosynthetic capacity of the plant (Kafi et al., 2006). It is noteworthy that there was almost a similar improvement in leaf number compared to control as a result of LIForte used alone or combined with SAP. Effectively, highly energized LIForte particles, sprayed finely onto the leaf surface, are taken up directly through the stomata and converted into carbon dioxide. In this way LIForte can considerably increase the photosynthesis rate, since the essential factor limiting photosynthesis outdoors is the natural CO<sub>2</sub> content of the air. This leads to a reduced water requirement, since with LIForte, the plants are able to keep the stomata closed longer in case of water stress. LIForte application increases the processes of chlorophyll formation and can significantly boost the rate of photosynthesis, which in turns may have caused the formation of higher number of leaves in saffron treated by the product. Further, LIForte contains trace elements and micronutrients including manganese, copper, and zinc that have a good impact on plant physiology. Akbarian et al. (2012) reported an increase in leaf number coupled with increased doses of application of trace elements, like iron, zinc and potassium Hokmabadi et al. (2006) found also that the application of iron nano-chelate caused a significant increase in leaf number in treated corms compared with control.

### **IV.6.4. Leaf length**

According to Mahmoodi et al. (2021) increasing the size of saffron mother corms had an effective role in the significant increase of the maximum length of saffron leaves. Also, Tavakkoli et al. (2014) found that by planting large corms, the leaf length and the number of leaves increased. However,



results of the current experiment showed no significant difference in terms of leaf number among treated and untreated corms weight 1 and 2. Results demonstrated a positive effect of Seaumic- containing humic acid and seaweed extract- on leaf growth. Increased leaf length was also reported by Kamel et al. (2018) as a result of using micronutrient fertilizer containing seaweed extract. On the contrary, Golzari Jahan Abadi et al. (2017) who had concluded that the use of biofertilizers containing humic acid was not significant on leaf length. Besides, the product Seaumic is rich in all kinds of nutritive elements and plant growth regulators, including nitrogen, phosphate, potassium, calcium, magnesium, microelement (Cu, Fe, Zn, Mo, and B), alginic acid, cytokinin, betaine, mannitol, polysaccharides, iodine and amino acids, it causes a greater root development and a greater microbial activity in the soil, and this in turn leads to increased nutrient availability, thus a better leaf growth. It also contains humic acid, the beneficial impact of which on plant growth is attributed to a variety of variables, including greater water and nutrient uptake, increased nutrient availability, better root system development, higher chlorophyll content, and improvements in the plant's enzyme activity (Barea et al., 2005; Sabzevari et al., 2010). Also, LIForte caused a great leaf development due to its effect on stimulating the photosynthesis process, and consequently leaf growth. The product also provided micronutrients which may have played a role as well in the development of leaves. Earlier, Rostami et al. (2019) observed an increased leaf length following the application of nano-zinc. Also, Akbarian et al. (2012) found that foliar application of zinc increased leaf length compared with control cases and presumed that the reason of leaf length reaction to Zn application is that, the Zn is necessary for producing chlorophyll and forming carbohydrate, also it is closely involved N-metabolism of the plant. Furthermore, Baghai and Maleki Farahani (2013) obtained a longer leaf length as a result of increasing levels of nano-chelate applied.

#### **IV.6.5. Flower number**

According to Kumar et al. (2008) and Rezvani-Moghaddam et al. (2014), corm size is the main agent for determining the capacity of bulbous plants to flower. Aghhavani Shajari et al. (2015) and Koocheki et al. (2016b) added that the percentage of flowering increase significantly with enhancement of corm weight. In the current study, non-treated corms produced close number of flowers per m<sup>2</sup>, which contradicts the findings of Koocheki et al. (2019a) where corms of 6-8 grams increased flower yield by 1.94, 2.15, and 1.75 times compared with corms of 4-6 grams in one-, two-, and three-

year old fields. Also, Vurdu et al. (2002), Molina et al. (2004), Molina et al. (2005), Nassiri Mahallati et al. (2007), and Ghobadi et al. (2015) reported that the number of flowers increased when larger corms were used. Arslan et al. (2007), Çavuşoğlu et al. (2009), Mohammad-Abadi et al. (2011), and Douglas et al. (2014) considered as well that flowering capacity in saffron is highly dependent on corm weight. The number of flower is one of the most economically important attributes of saffron (Agayev et al., 2009). According to Temperini et al. (2009), about 16 to 80% of flower yield changes depend on soil variables. The number of flowers per square meters obtained from corms of weight 2 treated with SAP alone increased by 14% compared with control, matching the percent increase of this indicator obtained in the study of Falahi et al. (2017a) following the use of SAP. However, the number of flowers per square meters obtained with 40 kg/ha SAP was lower than that reported by same authors using same SAP rate. Azizi et al. (2020) reported a 37% increase in flowers number following the foliar treatment of seaweed compared with control, but in the current study, Seamic, containing seaweed extracts, applied alone did not cause a significant difference in terms of flowers, but when combined with SAP, it caused an increase in this indicator by 43.9% and 65.1% in CW1/Seamic/SAP and CW2/Seamic/SAP compared with control. According to Osmani Roudi et al. (2015) and Mollafilabi and Khorramdel (2016), humic acid application was found to have a good influence on the quantity of saffron flowers and to improve saffron yield. Further, Azizi et al. (2020) noted a 32.0% increase in flowers number following the application of micronutrients. In the current experiment, products rich in micronutrients like ZFM++ and LIForte applied alone improved the flower number per square meter by 15.4 and 10.9% respectively for lighter corms and by 17.8 and 10.1% for heavier corms. According to Zheng et al. (2005), nano-fertilizers increase the rate of photosynthesis and thus increase the number of flowers in the plant. Besides, micronutrients play a vital role in crop productivity, and their deficiency affects saffron yield. For instance, iron and zinc play a role in the reproductive growth yield (Moosavi and Ronaghi, 2010) and their application could affect saffron flowering (Koocheki and Seyyedi, 2016). The supply of elements effectively increases the number of flowers (Koocheki et al., 2011). In the study of Baghai and Maleki Farahani (2013), the use of nano-chelated iron fertilizer improved the number of flowers per unit area. According to Azarpour et al. (2013) foliar spraying of nano iron fertilizers resulted in the highest amount of fresh flower yield. Also, foliar application of nano Zinc Oxide (6 g/L) had significant effects on the number of flowers of saffron (Rostami et al., 2019). Superiority in flowers number obtained with

ZFM++ compared with Seaumic when products were applied alone could be thus attributed to the higher zinc and iron contents in the first product compared with the second. Various studies have reported the positive effect of complete fertilizer use on saffron yield as compared to other fertilization regimes so that the higher the amount of complete nutrients in the fertilization regime is, the higher the flower yield will be (Jahan and Jahani, 2007).

#### **IV.6.6. Fresh and dry stigma yield**

Stigma yield is one of the most important yield components on which the economic value of the saffron product depends (Baghai and Maleki Farahani, 2013). According to Rostami et al. (2019), saffron yield can be considerably affected by changes of nutrient uptake, mother corm weight, and fertilizer applications. Yield is a complex plant characteristic and is influenced by many yield components and their interactions Fageria and Baligar (2005). Lundmark et al. (2009) stated that the mother corms in saffron could supply up to 20% of the yield and biomass. Several researches have reported that stigma yield increases significantly with enhancement of corm weight (de Juan et al., 2009; Çavuşoğlu and Erkel, 2009, Aghhavani Shajari et al., 2015). In the current experiment, dry stigma yield obtained from untreated corms of 4-6 grams was of 0.137 g/m<sup>2</sup>, and that obtained from corms of 6-8 grams was of 0.1058 g/m<sup>2</sup>, thus, increasing maternal corm weight did not cause a significant increase in dry saffron yield, contradicting the results of Amirnia et al. (2014) where dry yield increased 5.17 times as a result of increasing corm weight from 6 to 12 grams, and those of Koocheki and Teimouri (2014) where it was shown that with the increase of the corm weight from 4 grams to 8 to 12 grams, the yield of flowers and stigma increased. Also, Ghobadi et al. (2015) reported higher stigma yield (0.180 g/m<sup>2</sup>) obtained from bigger corms (weighing 10-14 grams) compared with yield (0.061 g/m<sup>2</sup>) obtained from smaller corms (weighing 5-9 grams). According to Amiri (2008) both vegetative and reproductive growth phases of saffron are highly dependent on nutrient availability. Gholami et al. (2017) added that biomass partitioning in saffron has a near relation with good water and nutrient availability. Eventually, the vegetative growth of the plant including the production of leaves and stems and followed by the formation of flowers is significantly influenced by the availability of water and nutrients required by the plant (Ramezani et al., 2020). In particular, the availability of adequate moisture will lead to a better growth and flowering of saffron (Falahi et al., 2014a; Khorramdel et al., 2013).

The use of SAP alone in 40 kg/ha increased fresh and dry stigma yields of heavier corms (6-8 grams) by 27.9% and 35.8% respectively compared

with control. Fallahi et al. (2017a) had earlier reported an increase of 24% in fresh and dry yields as a result of applying SAP with a rate of 40 kg/ha compared with control. They reported a dry stigma yield of 4.88 kg/ha in such treatment, which was higher than the one obtained in the current study; 1.65 kg/ha (equivalent to 0.165 g/m<sup>2</sup> in CW2/SAP). The positive effect of SAP on dry weight of stigma was also observed in the study of Khorramdel et al. (2013) where increasing SAP rates caused increasing dry stigma yield. Fallahi et al. (2017a) observed that usage of SAP increased fresh stigma yield. By enhancing soil water retention and nutrient use efficiency; SAP application may successfully improve plant growth. The penetration rate, density, soil structure, soil compaction, soil texture, aggregate stability, and evaporation rate are all properly impacted by these polymers (Abedi-Koupai and Asadkazemi, 2006).

The single use of Seaumic and ZFM++ could compensate the low weight of mother corms; effectively 43.9% and 33.7% increase in dry stigma yield obtained in corms of low weight following the application of both products respectively is of high economic importance at the level of saffron production. Further, combining Seaumic and ZFM++ with SAP was also effective on smaller corms, causing improvements of 71.4 and 72.3% in terms of dry stigma yield compared with control. Therefore, the performance of smaller saffron corms may be ameliorated by the use of Seaumic and ZFM++ alone or in combination with SAP, allowing enhancement in yield obtained since the first season of growth.

A noteworthy advantage of using SAP is that it may decrease water need and increase WUE (water-use efficiency) of saffron by reducing the amount of water consumption during its life cycle (Fallahi et al., 2016), consequently, the combination of SAP with Seaumic or ZFM++ seems more advantageous and is recommended in regions with drought stress conditions.

Superior flower number and dry stigma yields were obtained when SAP was used in combination with Seaumic on heavier corms, with 65.1 and 90.4% increase respectively compared with control. In this concern, Omidi et al. (2009) had found a strong positive correlation between the number of flowers and stigma yield, which is consistent with the findings of the present study with respect to the treatment CW2/Seaumic/SAP. Seaumic is a combined fertilizer of seaweed and humic acid. In their study, Azizi et al. (2020) recorded a 65.2% increase in dry stigma yield as a result of foliar application of seaweed. According to MacDonald et al. (2012), seaweed extract improves soil ventilation and foliar application leads to increased growth, yield and production in many plants which is related to the content of amino acids that

stimulate different growth attributes. Humic acid application was found to improve saffron yield (Osmani Roudi et al., 2015; Mollafilabi and Khorramdel, 2016).

Ahmadi et al. (2018) reported the highest stigma dry (0.23 g/m<sup>2</sup>) yield in plants treated with 10 kg/ha humic acid. Gerdakaneh et al. (2020) reported that the use of 20 kg/ha of solid humic acid and 3 l/ha of foliar humic acid has increased flower number dry stigma (by 78.61%) compared to control. Armak et al. (2021) also obtained higher stigma dry weight following the application of Super Humic treatment (improvement by 86.49% relative to control). Khorramdel et al. (2022) reported an enhancement in dry stigma weight as a result of applying humic acid as soil fertilizer with a level of 30 kg/ha.

#### **IV.6.7. Picrocrocin, crocin, and Safranal**

The value of saffron (dry stigma) is due to its qualitative properties and the presence of three main secondary metabolites: crocin, picrogrosin, safranal and their derivatives (Moraga et al., 2009). Seaumic and ZFM++ had almost a similar positive effect on picrocrocin content. Both products were rich in micronutrients, and according to Akbarian et al. (2012), the increase of K, Fe, and Zn elements can increase picrocrocin content. Also, humic acid, present in Seaumic, is known to play an important role in increasing the quality of medicinal plants Ahmadiyan et al. (2010). Humic acid can chelate elements such as nitrogen, phosphorus, potassium and iron and increase their absorption and increase the activity of enzymes involved in the center attributed organic compounds in plants (de Santiago et al., 2008). Ahmadi et al. (2017) found that humic acid increased significantly the amount of picrocrocin, crocin, and safranal compared to control. Golzari (2016) reported also a significant effect of humic acid on the secondary metabolites of saffron. Crocin content increased to the highest values in treatments where ZFM++ was applied alone. On the contrary, Akbarian et al. (2012) reported that increasing concentrations of Zn and Fe fertilizers decreased the crocin content. Picrocrocin increased in almost all treatments, but safranal did not. According to Ahmadi et al. (2017), because the biosynthesis of safranal start from cleavage of zeaxanthin to produce cyclic carotenoid (safranal and picrocrocin), it seems that decrease in safranal with increase in picrocrocin are due to competition. Furthermore, Seaumic and ZFM++ applied alone had a superior effect on qualitative indicators (higher picrocrocin and crocin contents) compared to their use in combination with SAP, while they had superior effect on dry stigma yield when combined with SAP compared to their use alone.

According to Heydari et al. (2014), in larger flowers or heavier stigmas, we should expect a lower percentage of safranal, crocin and picrocrocin and therefore a reduction in the aroma and taste of the stigma. So, it is possible that the plant growth speed is higher than the speed and amount of essential oil production by the plant and it causes its concentration to decrease in the stigma. On the contrary, Khoshpeyk et al. (2022) concluded that if a product leads to the improvement of the quantitative performance of one or more traits, it may be effective in increasing the qualitative performance of other substances such as secondary metabolites.

#### **IV.6.8. Yield and number of replacement corms**

Saffron can grow like a perennial plant on the field for several years and be multiplied by increasing the number of replacement (daughter) corms (Koocheki et al., 2015). In the current study, larger mother corm weight increased the weight but not the number of daughter corms. Golzari Jahan Abadi et al. (2017) reported higher number and weight of daughter corms obtained from larger mother corms. Also, according to Arslan et al. (2007) corm weight has a positive effect on the production and growth of daughter corms. Hassanzadeh Aval et al. (2013) found that with the increase in the weight of the corm from 1.1-3 grams to 7.1-9 grams, larger number of corms was produced. In this regard, Tavakkoli et al. (2014) also obtained larger daughter corms from larger mother corms. Eventually, the amount of materials transferred to the corms and other underground organs depends on the photosynthetic level and the photosynthetic efficiency of the leaf (Kafi et al., 2006). In this concern, Golzari Jahan Abadi et al. (2017) explained that the larger the reserves of larger maternal corms and the possibility of allocating a larger volume of it provided photosynthetic materials to the vegetative buds, which has led to an increase in the number and weight of the corms through the faster emergence and a better growth of leaves.

The treatment CW2/Seamic/SAP improved daughter corms number by 15% and daughter corm yield by 27.3% compared with control. Golzari Jahan Abadi et al. (2017) found 56.7 and 61.6% increase in the number of daughter corms and fresh weight of mother corms respectively compared to control following humic acid treatment. According to Nehvi et al. (2010), humic acid stimulates corm growth by improving the nutrient conditions of the planted corms, which ultimately determines the potential yield of saffron. Furthermore, the use of SAP combined with fertilizers caused superior yield of daughter corms compared with the use of fertilizers alone. According to Islam et al. (2011), the formation of daughter corms is significantly affected

by both the availability of water and soil nutrients.

Therefore, SAP provide suitable conditions for the production of daughter corms, by increasing the water retention capacity soil, improving porosity, increasing ventilation, and providing essential nutrients required by the plant in the rhizosphere environment (Wu et al., 2008; Ramezani et al., 2020). In their study, Khoshpeyk et al. (2022) found that the combined use of nano-silicon and SAP has increased the production of daughter corms from water-stressed saffron plants.

## V. WORK CONTRIBUTIONS

The work presents several contributions, both scientific and practical:

I. It has been proven that climate and soil conditions in Douma (North Lebanon) are suitable for saffron cultivation allowing the production enough stigma yield per flower.

II. It has been found that Spanish *Crocus* cultivated in North Lebanon in Douma was *Crocus oreocreticus* according to the DNA study

III. It has been demonstrated that nano-fertilizers and Seaumic, applied alone, can improve all quantitative and qualitative traits of saffron in comparison to non-treated cases.

IV. It has been clearly proven that the use of SAP can complement the effect of the tested fertilizers improving the overall plant performance; higher yield and better quality.

V. The use of the tested fertilizers and SAP in combination may compensate the smaller size of saffron corms allowing the production of enough yield and good quality from corms of 4-6g.

## VI. CONCLUSION AND RECOMMENDATIONS

After testing the effect of climate, fertilizers, corm weight, and SAP on different saffron growth attributes, different conclusions were reached:

I. Lower temperature and precipitation during flowering in Mahallat region enhanced production and quality of the saffron cultivated

II. It has been found that saffron cultivated in North Lebanon in Douma was *Crocus oreocreticus* and this species can be successfully cultivated in this region.

III. Seaumic and ZFM++ fertilizers applied alone enhanced dry yield of stigma compared to control by 84% and 54% respectively, while LIForte alone did not have an effect.

IV. The three fertilizers enhanced saffron quality except for safranal and

Seamic had superior effect on the indicators tested – Seamic, LIForte, and ZFM increased picrocrocin content by 16%,19%, and 7% respectively, while increased crocin content by 5%, 8%, and 4% respectively.

V. Mutual effect of the different fertilizers types and SAP showed the improvement and synergy of the use of the two products together. SAP combined with Seamic was able to increase dry stigma yield in heavier corms three times ( $1.1\text{g/m}^2$ ) more compared to using Seamic alone ( $0.26\text{ g/m}^2$ ), while it was doubled in lighter corms (from  $0.24\text{ g/m}^2$  to  $0.48\text{ g/m}^2$ ). However, fertilizers applied alone had a better effect on saffron quality compared to their use with SAP in both corm weights.

VI. The performance of smaller saffron corms has been ameliorated by the use of Seamic and ZFM++ alone or in combination with SAP, allowing enhancement in the saffron yield obtained which is needed for the first season of growth.

VII. The combination of SAP with Seamic or ZFM++ is more advantageous and is recommended in regions with drought and low soil fertility conditions

VIII. The use of nano-fertilizers and organic fertilizers reduces the need for chemical fertilizers and allows optimizing saffron quantity and quality while minimizing the negative effects of fertilizers on the environment

IX. The current study provided a strategy to adopt by the Lebanese farmers to cultivate saffron in North Lebanon

Many recommendations arise from the findings of this study:

1. Further testing of the tested nano-fertilizers and Seamic in other doses and timing of application is required to understand the optimal nutritional management for the saffron crop using nano-fertilizers and organic fertilizers.

2. It is recommended to use the identified *C. oreocreticus* in the cultivation of saffron by the Lebanese farmers as it shows unique and promising potential, and further experiments should investigate the success rate of its cultivation in other regions of Lebanon.

3. It is recommended to conduct study in order to find a way to induce earliness in flowering in Lebanon to mitigate the effect of high precipitation on quality and production of saffron.

4. To recommend the use of SAP on saffron in Baalbek-Hermel; the main growing region of saffron in Lebanon, due to the drought conditions found there.