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ORIGINAL RESEARCH PAPER in HORTICULTURAL PLANTS

Effects of Inorganic and Foliar Fertilizers on Antioxidant Capacity and Flower Yield of Saffron (*Crocus sativus* L.)

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Abstract

Saffron (*Crocus sativus* L., Iridaceae) is a highly valued species in the food, medicinal, and nutraceutical industries as a coloring, flavoring, and therapeutic agent. Its productivity and flower production vary depending on different factors, including fertilizer treatment. This study was conducted to evaluate the effects of inorganic (NK) fertilizer combined with organic matter in different plant densities as well as the influence of split foliar fertilizer application on flower yield of saffron. The performance of saffron plants revealed that the combined application of inorganic fertilizer NK and vegetal organic matter (1%) was generally better than the effect of foliar treatment. Treatment with split foliar fertilizers at the recommended optimal concentration prolonged the flowering period of saffron plants.

Keywords

antioxidants; bioactive compounds; plant density; plant treatment; FRAP; DPPH; MDA; flavonoids

1. Introduction

Crocus sativus L. (saffron) is a highly valued species owing to its unique qualities as a plant that provides color, taste, and flavor to the food as well as its medicinal properties (Abdullaev & Espinosa-Aguirre, 2004; Baba et al., 2015; Samarghandian et al., 2010). The natural color and aroma of saffron are derived from stigmas after appropriate drying; the color is derived from crocin, whereas picrocrocin and safranal are responsible for the taste and aroma (Lahmass et al., 2018).

Although saffron is a low nutrient demanding crop, inorganic fertilizers containing mineral nitrogen (N), phosphorus (P), and exchangeable potassium (K) are the most important factors affecting the yield of saffron (Jahan & Jahani, 2007). However, the overuse of inorganic fertilizers damages soil structure, increases soil acidity, causes nutrient imbalance, and decreases crop sustainability, yield, and quality (Khan et al., 2008; Nazli et al., 2014). Therefore, the use of organic materials in agricultural lands to restore soil fertility and prevent potential environmental problems is of considerable interest (Alridiwirsah et al., 2021; Fard et al., 2020; Hirzel & Walter, 2008; Koockeki & Seyyedi, 2015). In addition, the effect of organic matter is attributed to the enhancement of physical characteristics of the soil, including better aeration, better water-holding capacity, good balance between nutrients in the soil, and improvement of nutrient exchange in the soil. The slow release of nutrients from organic matter during the growth period and hence the low leaching of nutrients could also play a role in enhancing the soil quality (Amiri, 2008). The efficiency of fertilizer utilization by a crop is determined by the method of application, time of incorporation, and the rate of decomposition in the soil (Achieng et al., 2010). The application of various fertilizers by foliar spraying is a new approach to supply nutrients through the leaf, which is increasingly used in many saffron farms (Khorramdel et al., 2015).

In Bulgaria, a program for the promotion of *C. sativus* as an alternative to traditional tobacco areas started in 2014, and it is limited to the regions of Kardzhali and Silistra, but saffron-cultivated areas have gradually increased in other non-traditional areas. Owing to the high economic value of saffron and its commercial significance, Bulgarian farmers are interested in growing *C. sativus* on the site of tobacco plantations.

The current study aimed to: (***i***) investigate the effect of inorganic (NK) fertilizer combined with organic matter in different plant densities; (***ii***) evaluate the influence of a split of foliar fertilizers on flower yield; and (***iii***) study the influence of foliar fertilizers on total antioxidant capacity and bioactive compound content during the second growing season.

2. Material and Methods

2.1. Plant Growth Conditions and Treatments

The experiment was conducted in the experimental field of the Institute of Plant Physiology and Genetics, Sofia, Bulgaria (42°50′ N, 23°40′ E, altitude 595 m) during the 2019 and 2020 growing seasons. The climate of the area is classified as marine west coast, with a mild and no dry season and warm summer. Heavy precipitation occurs during mild winters, which are dominated by mid-latitude cyclones (Köppen–Geiger climate classification: Cfb). The soil of the plots where the trial was conducted is catalogued as a sandy loam (0–15 cm topsoil).

In the current study, the Bulgarian Association of Saffron Producers kindly provided saffron corms. The trial area was cultivated in late spring, and soil was prepared using conventional tilling equipment to form the seedbed. Each trial was conducted in a randomized complete block design with three replicates. Corms (3.0–3.5 cm horizontal diameter) were planted 15 cm deep by hand in 1 m × 2-m plots, with 20-cm spacing between rows and two plant densities (high density, 75 corms/m^2 and low density, Ld – 55 corms/m^2). Between blocks and plots, a 2-m alley was kept to eliminate any influence of the treatments. The saffron corms were planted a year ago (2019, for adaptation) in the second half of August and covered the range normally used for saffron in Bulgaria.

Two types of soil treatments were used: (***i***) inorganic fertilizer (nitrogen and potassium) application and (***ii***) treatment with a mixture of inorganic (Ultrasol K plus) fertilizer and vegetal organic matter (VOM, a peat substrate). Three different doses of Ultrasol K plus (KNO_3, a unit equal to 1.25 g/m^2), that is, 5, 10, and 15 units and 0 NK (control), were applied to the soil prior to planting the mother corms. Before corm planting, in cases of combined treatment, 1% of VOM was used to improve the efficiency of chemical fertilizer, and soil aeration and draining were scattered onto the soil surface, followed by mixing into the soil.

The optimal concentrations (OC) of three commercial foliar fertilizers, Shigeki, Kaishi, and Boronia, are presented in Table 1. In our experiment, we used three concentration levels of foliar fertilizer treatment: half of the optimal concentration (0.5 OC), optimal concentration (OC), and 1.5 times the optimal concentration (1.5 OC).

In the control treatment, distilled water was sprayed on the plants. Kaishi, Sumiagro, is a liquid organic fertilizer containing an amino acid of herbal origin. It contained 6% organic carbon, 2% organic nitrogen, and 12% free amino acids (pH 4–6). Shigeki, Sumiagro, is a liquid organo-mineral fertilizer with additive trace elements that contains 15% boron, water-soluble iron (0.20%) chelated with EDTA, soluble manganese chelated with EDTA (0.18%), water-soluble zinc (0.20%), and 0.5%

Table 1 Foliar fertilizers and type of treatment.

Fertilizer	Type of treatment	Optimal amount for treatment (OAT)
Shigeki	Seventh day from the beginning of growing season	200 mL/1,000 m^2
Kaishi	Fourteenth day from the beginning of growing season	500 mL/1,000 m^2
Boronia	Fourteenth day from the beginning of growing season	100 mL/1,000 m^2

alginic acid (pH 5.5–7.5). Boronia Mo, a soluble fertilizer, prevents and corrects boron and molybdenum deficiencies in many crops. To minimize evaporation from both the plant and soil surfaces, irrigation and foliar fertilizer spraying were performed in the early morning while the relative humidity was high and the temperature was quite low. A fine mist spray was used to ensure the full coverage of all foliage surfaces for maximum absorption.

The number of flowers and total dry weight of the stigmas were recorded daily for each plot. Whole flowers were manually picked daily early in the morning, in the first hours after sunrise, before the flower had completely opened. Immediately after flower picking, stigmas were separated by hand and dried in a forced air oven at 30 °C for 24 hr. Dry stigmas were stored at room temperature (18–22 °C) in closed glass jars and kept in the dark until the analyses of antioxidant capacity and malondialdehyde content were performed.

For all analyses, the leaves were harvested 1 week after the last flowering day. Extract preparation for the determination of antioxidant activity and flavonoid content was done with 80% ethyl alcohol and with 1% trichloroacetic acid for the determination of malondialdehyde (MDA).

2.2. Antioxidant Activity, Total Flavonoids, and Lipid Peroxidation

The free radical scavenging ability against DPPH (2,2-diphenyl-1-picryl-hydrazyl) was evaluated with a method described by Brand-Williams et al. (1995). Antiradical activity is represented as μmol Trolox/g fresh weight (FW). Antioxidant activity was estimated using the ferric reducing antioxidant power assay (FRAP) (Benzie & Strain, 1999). The antioxidant activity results are expressed as μmol FRAP/g FW. Total flavonoid content was measured according to the method described by Rivas et al. (2014) using a standard curve with rutin as a standard. The results were expressed as μmol rutin/g FW. Lipid peroxidation was measured by determining the MDA content (Hodges et al., 1999). The MDA concentration was calculated according to the method described by Hodges et al. (1999).

2.3. Statistical Analysis

A completely randomized design was used to collect leaf material for each treatment. Three to four replicates (plant samples) were used for each experiment. Data were statistically analyzed using ANOVA followed by Tukey's HSD test (SPSS 14.0; SPSS Chicago, IL, USA). Significant differences between the three means for each treatment were analyzed based on $p < 0.05$.

3. Results

3.1. Effects of NK Levels and Plant Density on the Productivity of Saffron

Combined or separate treatments with NK and/or VOM on low-density plots did not affect the flower number and dried stigma yield of saffron plants among the tested groups. An increase in the flower number and stigma yield was observed in the high-density plot (Table 2).

Treatment with all concentrations of inorganic (NK) fertilizer led to an increase in the flower number and dried stigma yield in the low-density plot (Table 2). A similar

Table 2 Effect of different type of fertilization on number of flowers per corm and stigma yield.

Fertilizers	No. flowers/corm (mean ± *SE*)	Stigma yield (mg/flower)
Planting corms with low density		
Control	11.10 ± 0.33 a	6.73 ± 0.06 a
5 units NK*	12.10 ± 2.00 a	6.86 ± 0.15 a
10 units NK	12.65 ± 1.99 a	7.12 ± 0.34 a
15 units NK	11.62 ± 0.86 a	7.17 ± 0.35 a
1% organic matter	9.92 ± 2.59 a	7.07 ± 0.42 a
5 units NK + 1% organic matter	10.46 ± 2.58 a	7.32 ± 0.37 a
10 units NK + 1% organic matter	10.90 ± 3.47 a	6.77 ± 0.03 a
15 units NK + 1% organic matter	10.22 ± 3.04 a	7.00 ± 0.01 a
Planting corms with high density		
Control	12.72 ± 0.21 a	6.89 ± 0.04 d
5 units NK	13.50 ± 0.50 b	5.99 ± 0.12 a
10 units NK	14.66 ± 0.54 c	6.11 ± 0.11 a
15 units NK	13.00 ± 0.29 d	6.13 ± 0.05 a
1% organic matter	11.82 ± 0.51 e	6.39 ± 0.09 b
5 units NK + 1% organic matter	12.30 ± 0.11 f	6.53 ± 0.08 b,c
10 units NK + 1% organic matter	13.18 ± 0.16 g	6.77 ± 0.08 c,d
15 units NK + 1% organic matter	12.27 ± 0.30 h	6.77 ± 0.08 c,d

Values are represented as mean ± *SE* (standard error of three repetitions). The mean values marked by the same letter are not significantly different at $p < 0.05$, as determined by Tukey's test. * NK – N and K.

effect was observed in the plot with high corm density for flower number, but not for dried stigma yield. The most significant effect was observed in plots treated with 10 units of NK, where the flower number increased by 15.3%. Field observations showed that treatment with 1% VOM negatively affected flower number and stigma yield compared with the control. The combined treatment with 10 units of inorganic (NK) fertilizer and 1% VOM increased the flower number by 3.6% compared with the control.

With regards to plant density, high-density plots showed significantly better results than the low-density plots in terms of both yield attributes monitored. In the current study, NK 10 units + 1% VOM and high plant density (75 corms/m^2) was determined to be the best combination among inorganic NK levels and VOM treatment for improved flower production and dried stigma yield.

3.2. Effects of Foliar Fertilizers on the Productivity of Saffron Under Open-Field Conditions

The number of saffron flowers reached the highest level (17.5% increase compared to the control) after treatment with foliar fertilizers at a concentration of 1.5 times (1.5 OC) the recommended optimal amount of treatment (OAT) (Table 3). Since the three foliar fertilizers were applied sequentially and uniformly (see Material and Methods), and at different concentrations, it was difficult to determine the individual impact of each of them. There were no significant differences in stigma yields between the tested variants (Table 3).

As indicated by the effects of the foliar treatment, an extension of the flowering period during the growing season was observed. Consequently, saffron flowering began after 30–40 days and continued for 5–6 weeks. According to our results, foliar treatment influenced reproductive growth between the fifth and twenty-fifth days of flowering as compared to the control (Figure 1). A stronger effect on the delay of flowering (days of flowering/number of flowers per plant) was observed after treatment with foliar fertilizers at the recommended optimal concentration; the monitored parameter decreased gradually with time. This could be because of

Table 3 Effect of foliar fertilizers on number of flowers per corm and stigma yield.

Fertilizer	No. flowers/corm	Stigma yield (mg/flower)
Control	10.25 ± 0.62 a	6.50 ± 0.29 b
Foliar treatment, 0.5 OC*	9.24 ± 0.33 a	6.11 ± 0.31 a
Foliar treatment, OC	10.51 ± 0.50 a,b	6.90 ± 0.09 b
Foliar treatment, 1.5 OC	12.04 ± 0.46 b	6.25 ± 0.22 a,b

Values are represented as mean ± *SE* ($n = 3$). The mean values marked by the same letter are not significantly different at $p < 0.05$, as determined by Tukey's test. * OC – optimal concentration.

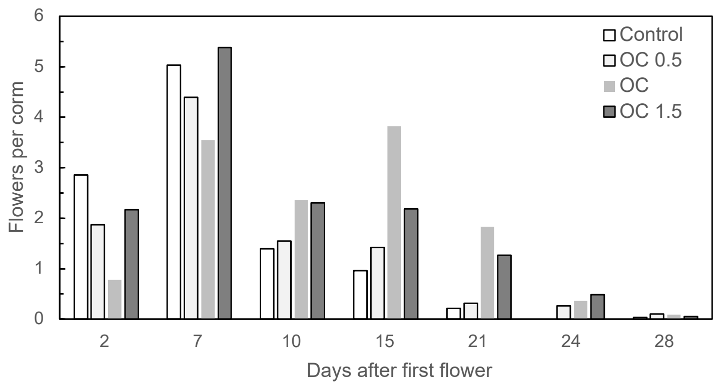

Figure 1 Flower formation in saffron plants (*Crocus sativus* L.) during the flowering period. OC – optimal concentration.

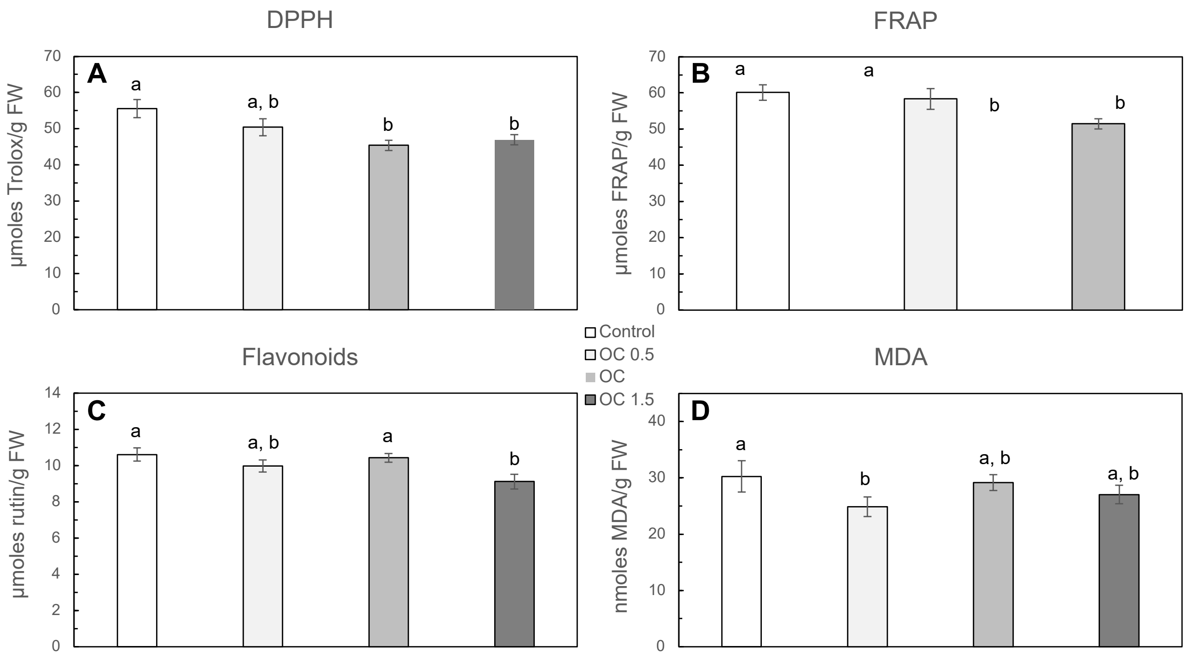

Figure 2 Effect of foliar fertilizer treatment on the antioxidant capacity of saffron leaves (*Crocus sativus* L.). Values are represented as mean ± *SE* ($n = 3$). The mean values marked by the same letter are not significantly different at $p < 0.05$ as determined by Tukey's test. FW – fresh weight; OC – optimal concentration; DPPH – 2,2-diphenyl-1-picryl-hydrazyl; FRAP – ferric reducing antioxidant power assay; MDA – malondialdehyde.

the overall improvement in plant growth and development due to foliar fertilizer application.

There were slight differences in the antioxidant activity, as measured by both the DPPH radical scavenging method and FRAP reducing power (assay), between foliar fertilizer plants and the control (Figure 2). In addition, the flavonoid and malondialdehyde (MDA) contents were slightly affected by the treatment (Figure 2). It was noted that the application of foliar fertilizers did not cause changes in the antioxidant potential of plants, as well as in the content of flavonoids or damage to cell membranes and lipid peroxidation, that is, the antioxidant compounds and bioactive capacity in saffron plants remained stable after foliar fertilizer application.

4. Discussion

For saffron, plant density is a cultural management factor that predominantly influences plant productivity (Karra et al., 2017; Temperini et al., 2009). Saffron yield is also attributed to soil chemical characteristics, such as organic content, mineral nitrogen, exchangeable potassium, available phosphorus, and C/N ratio (Jahan & Jahani, 2007). Positive effects of composted cattle manure on saffron production have been reported (Kafi, 2006). Regarding the beneficial effect of mineral fertilizers (NK) in combination with 1% VOM on the stigma yield attributes of *C. sativus*, the present results are in agreement with the findings of other studies on saffron plants (Jahan & Jahani, 2007).

In this study, the effect of foliar fertilization on saffron yield indicated an increase in the flower number in the highest concentration treatment only. These results do not completely agree with the findings of other researchers who observed a beneficial effect of foliar fertilization on the yield components and growth characteristics of saffron (Khorramdel et al., 2015; Rabani-Foroutagheh et al., 2013). Instead, the present results revealed that a one-time treatment with foliar fertilizers did not significantly affect saffron yield. Therefore, it can be assumed that, in the case of saffron, it is necessary to determine the best combination of fertilizers to attain maximum saffron yield and improved yield components.

In the present study, foliar fertilization extended the flowering period of saffron. Many operations are required for the production of saffron, from flower picking to stigma separation, all of which are performed during the flowering stage. Therefore, an opportunity to modify the flowering time would be beneficial for saffron growers, providing them a chance to fix an appropriate time for picking flowers.

Analysis of lipid oxidation and antioxidant capacity in leaves indicated no harmful effects of the selected foliar fertilizers, which is of special importance for saffron plants as a source of compounds with high antioxidant capacity (Lahmass et al., 2018). It was demonstrated the potential of saffron stigma and spats as a source of compounds with high antioxidant capacity (Lahmass et al., 2018). However, additional data is required to support this interpretation. The results of the present study are of interest for testing different agronomic management strategies to increase the yield and productivity of saffron.

5. Conclusion

In conclusion, the performance of saffron plants revealed that the combined application of inorganic fertilizer NK and vegetal organic matter (1%) exerted superior effects than foliar treatment. Treatment with split foliar fertilizers at the recommended optimal concentration prolonged the flowering period of saffron plants. Thus, investigation of the effects of different concentrations of foliar fertilizers and a mixture of essential nutrients on the growth, flower, and corm yield of saffron needs to be continued in future research. In Bulgaria, appropriate crop management can improve the quantitative characteristics of saffron.

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