The Journal of Medicinal Plants and By-Products
Original Article

Effect of Salicylic Acid and Foliar Application of Calcium, Potassium and Zinc Fertilizers on Quantitative and Qualitative Traits of Saffron

Running Title: Saffron responses to Salicylic acid and Fertilizers

Mohammadjavad Seghatoleslami[1*], Gholamreza Mousavi[1] and Hassan Feizi[2, 3]

1 Agricultural, Medicinal Plants and Animal Sciences Research Center, Birjand branch, Islamic Azad University, Birjand, Iran
2 Saffron Institute, University of Torbat Heydarieh, Torbat Heydarieh, Iran
3 Department of Plant Production, University of Torbat Heydarieh, Torbat Heydarieh, Iran

*Corresponding author: Email: mjseghat@yahoo.com, mjseghat@iaubir.ac.ir

Article History: Received: 03 November 2024/Accepted in revised form: 04 January 2025

ABSTRACT

The use of some chemical compounds and nutrients can postpone saffron leaf senescence and increase photosynthesis and yield. A two-year experiment was conducted to evaluate the effect of salicylic acid (SA) and solu potash (FP), nitro calcium (Fca) and zinc sulfate (FZn) fertilizers on saffron in Birjand, Iran. The experiment was conducted as a factorial based on a randomized complete block design with three replications. The results indicated that in the condition of SA_0, the treatment FP+ FCa+ FZn had the highest fresh weight and flower number. Also, in these conditions, the use of each fertilizer alone could not have a significant effect on fresh weight and number of flowers compared to the control. Under conditions of SA_0, fresh weight and flower number did not significantly differ between fertilizer treatments in the first year. But in the second year, the treatment FP+ FCa+ FZn had the highest fresh weight (51.23 $g.m^{-2}$) and the number of flowers per square meter (123.73). The consumption of salicylic acid significantly increased leaf chlorophyll a, chlorophyll b and total chlorophyll content. In conclusion, although the use of salicylic acid (without fertilizers) improved the amount of chlorophyll in the leaves, but did not have a positive effect on the economic yield of saffron. On the other hand, the qualitative traits of the stigma including the amounts of crocin, picrocrocin and safranal did not show significant changes with the use of fertilizer or salicylic acid.
Keywords: Chlorophyll, Crocin, Picrocrocin, Safranal, Stigma

INTRODUCTION

Saffron (*Crocus sativus* L.) from the Iridaceae family is the most valuable crop plant in the world, which has a special place among Iran's export products [1]. Climate change is one of the phenomena that has affected the production of this plant in its main cultivation areas. Two important phenomena caused by climate change, i.e. heat stress and drought, caused the reduction of saffron yield. The biggest impact of global climate change is climatic drought and lack of irrigation water, especially in arid and semi-arid areas [2].

The phenology and growth period of saffron is such that in the spring season, as the air temperature increases, the leaves gradually turn yellow and dry. The yellowing of saffron leaves is affected by various factors such as drought stress, tuber age, plant nutrition status, corm infestation by acari, tuber density and irrigation water quality. In general, any factor that accelerates the aging of the plant will also result in the yellowing of the leaves. Postponing leaf senescence can increase photosynthesis and final yield. Meanwhile, the use of some chemical compounds as well as different nutrients, can be effective. For example, zinc and potassium fertilizers increase chlorophyll production and delay plant aging [3].

Salicylic acid is one of the growth regulators that acts as a chemical messenger and plays an important role in the tolerance of biotic and abiotic stresses [4]. Salicylic acid can stimulate the plant's defense reactions to create resistance to environmental stresses and quickly increase the activity of the relevant enzymes in plant tissues [5].

This compound plays an important role in regulating plant physiological processes such as photosynthesis, growth and development, flowering and absorption and transfer of nutrients and membrane permeability and creating resistance mechanisms [6,7]. Salicylic acid also improves plant photosynthesis and its growth, through increasing the activity of Rubisco enzyme and chlorophyll content [8].

Salicylic acid is effective in some plant metabolic processes such as stomatal closure, glycolysis and seed germination [9]. It has been reported that salicylic acid plays an important role in modulating the response of plants to abiotic stresses, including drought [10] and cold [11]. Positive effects of salicylic acid application on the growth and yield of rosemary [12] and also *Lippia citriodora* [13] have been reported. Also, the study of Asle Zaeem *et al*. (2018) in different saffron populations showed that the use of salicylic acid had a reducing effect on catalase enzyme activity and also caused a decrease in malondialdehyde content [14].

Ansarian Mahabadi *et al*. (2019) investigated the effect of different levels of salicylic acid on saffron. Their results showed that salicylic acid application significantly improved the physiological indices of the plant [15]. In another experiment, Jabbari *et al*. (2017) investigated the role of salicylic acid and potassium nitrate in improving different traits in saffron [16]. In general, their results showed that foliar application of solution 2 mM salicylic acid and then using 1000 $mg.l^{-1}$ of potassium nitrate, increased the growth of leaves, bulbs and leaf chlorophyll content and decreased the amount of electrolyte leakage in the saffron plant.

Improving the nutritional status of saffron can increase its yield in various ways, including reducing the effect of heat stress and the speed of leaf aging [17]. Among the different methods of inducing heat tolerance, foliar application of different compounds is common [18]. In the conditions of heat stress, the plant's need for calcium increases to overcome the adverse effects of stress. Kolupaev *et al*. (2005) reported that external consumption of calcium ions increases heat tolerance, through increasing the resistance of the chloroplast membrane and maintaining the speed of photosynthesis [19].

Among the nutrients, nitrogen plays an important role in temperature stress tolerance. In high temperatures, the light intensity is usually high. This affects the nutrients absorption and harms plant growth. Plants that receive enough nitrogen can tolerate excess and high light intensity by maintaining photosynthesis and creating protective mechanisms [18].

Potassium also plays an essential role in plant survival under stress conditions. This element is involved in processes such as photosynthesis and activation of enzymes under stress conditions [18]. Potassium also neutralizes the effect of reactive oxygen species by increasing the activity of antioxidant enzymes [20].

Zinc element is also one of the essential elements needed by plants, which has a great effect in reducing the adverse effects of drought stress in plants. This element regulates plant growth by interfering with the formation of auxin hormones. In addition, it causes the activation of many plant enzymes. Plant nutrition with this element can increase leaf area duration, and as a result, increase the photosynthesis of the plant [21]. In an experiment on cotton, Sarwar *et al*. (2019) showed that the application of zinc and potassium fertilizers delayed leaf senescence, increased its chlorophyll, and improved photosynthesis and yield through increasing the production of antioxidant enzymes [3].

The supply of essential nutrients plays an important role in flowering induction and improving the growth of saffron corms. Using the appropriate method and chemical fertilizer rate, in addition to increasing product quality, prevents the pollution of the environment, especially underground water [22]. The main route of nutrients absorption in plants is their root system. Although the saffron plant has less foliar absorption due to the low leaf surface and the waxiness of the leaves, in arid and semi-arid regions with alkaline soil where soil moisture is usually low, foliar application of elements is more effective compared to their soil application. [23].

Increasing temperature in the spring, saffron leaves gradually turn yellow and cannot photosynthesize. Therefore, one of the reasons for the low yield potential of saffron can be the short greening period of its leaves and the short period of photosynthesis. This issue can also affect nutrient absorption and cause a severe decrease in yield. Therefore, the simultaneous use of salicylic acid and various nutrients such as zinc, potassium and calcium to reduce the effects of heat stress and improve the nutrient absorption may improve plant growth and yield. According to the above, this experiment was conducted to evaluate the effect of different levels of salicylic acid, and solu potash, nitro calcium and zinc sulfate fertilizers on the quantitative and qualitative characteristics of saffron flowers in Birjand.

MATERIAL AND METHODS

This experiment was conducted for two years from autumn 2020 to autumn 2022 on a two-year-old saffron farm at Agricultural, Medicinal Plants and Animal Sciences Research Center, Birjand Branch, Islamic Azad University, Birjand, Iran. The longitude, latitude and altitude of the experiment location were 59°13'54.5"E, 32°50'06.9"N, and 1550 m, respectively. The experiment was conducted as a factorial based on a randomized complete block design with three replications. The first factor was a foliar application of salicylic acid in two concentrations (0 ppm as control and 5000 ppm). The second factor was a foliar application of fertilizers in six levels (control, solu potash, FP- nitro calcium, FCa-, zinc sulfate, FZn- solu potash+ nitro calcium, FP+ FCa- solu potash + nitro calcium + zinc sulfate, FP+ FCa+ FZn). The fertilizer concentration for foliar application was 2000 ppm. The plot's dimensions were 2 * 3 m. The distance between the blocks was considered one meter to prevent the interference of fertilizer treatments.

Before the experiment, to determine soil characteristics, sampling was done from a depth of 0 to 30 cm. The physical and chemical characteristics of the soil are listed in Table 1.

Table 1 The result of soil analysis in the experiment site.

Sand (%)	Silt (%)	Clay (%)	pH	EC ($dc.m^{-1}$)	Lime T.N.V (%)	Organic matter (%)	Nitrogen (%)	Phosphorus ($mg.kg^{-1}$)	Potassium ($mg.kg^{-1}$)	Zinc ($mg.kg^{-1}$)
19	51	30	7.65	0.81	14.5	0.7	0.07	19	185	0.47

Foliar application of fertilizers was done in two stages, on 15 February and 5 March. Salicylic acid was also sprayed in two stages (5 and 19 March) in the corresponding treatments. Irrigation was done according to the common pattern in the region. The first irrigation was done on 10 October. The weeds were controlled manually in all stages of the experiment if needed. To determine the traits related to flowers, two square meters of each plot were marked concerning marginal effect. Flowers were collected, counted and weighed daily when the harvest season arrived in late October. Means for the length of the style and stigma were determined by measuring 5 samples from each plot. Then the stigma+ style was carefully separated from the flowers and weighed after drying.

The leaf dryness index was determined visually by monitoring the leaves in the early and middle of April based on odd numbers from 1 to 9. Number 1 had the lowest and number 9 had the highest level of leaf dryness. In late March five leaves from 5 plants in each plot were collected randomly, to determine leaf chlorophyll content. The leaf chlorophyll content was determined using Arnon's method [24].

Flower quality traits, including crocin, picrocrocin and safranal content, were measured in the saffron laboratory of the Saffron Research Institute of Torbat Heydarieh University. The determination of crocin, picrocrocin and safranal values was carried out according to the Iranian National Standard No. 2-259, which is adapted from the ISO 3632 method (ISO/TS 3632-1/2, 2003).

Data analysis was done using Minitab statistical software. Means were compared using Duncan's test at the 5% probability level.

REDULTS AND DISCUSSION

Quantitative Traits of Saffron Flowers

Means comparison for the interaction of salicylic acid and fertilizer on flower quantitative traits of saffron are shown in Table 2. When salicylic acid was not used, the treatment FP+ FCa+ FZn had the highest fresh weight and the number of flowers per square meter. Also, in these conditions, the use of each fertilizer alone could not have a significant effect on fresh weight and number of flowers compared to the control (no fertilizer) (Table 2). In the first year of the experiment, fresh weight and number of flowers per square meter were not significantly different between this treatment and the treatments of nitro calcium, zinc sulfate and FP+ FCa. In the SA_0 treatment, in the first year, the lowest fresh weight (26.39 $g.m^{-2}$) and the number of flowers per square meter (62.67) were related to the control (no fertilizer). But in the second year, the least of these traits in these conditions was related to the treatment FCa (23.19 $g.m^{-2}$ and 61.87, respectively) (Table 2).

Ansarian Mahabadi *et al.* (2019) and Jabbari *et al.* (2017) in experiments on saffron showed that the use of different levels of salicylic acid improves the growth characteristics of saffron [15, 25]. Regarding the mechanism of the effect of salicylic acid, Khan *et al.* (2010) reported that this compound plays a role in increasing the physiological and biochemical aspects, increasing the amount of nitrogen, phosphorus, potassium and calcium and increasing the activity of the antioxidant enzyme [26]. There are also reports about the role of salicylic acid in reducing the effects of stress on plants. In this context, El-Taher *et al.*, (2022) in an experiment on cowpea showed that the use of salicylic acid with a concentration of 100 ppm could reduce the adverse effects of salinity stress on various aspects of plant growth, including its adverse effects on photosynthetic pigments, especially chlorophyll [27]. This reduction occurred through the reduction of ROS and the activation of the antioxidant defense system.

The results of different experiments on the effects of fertilization on saffron are different. Ayoubi *et al.* (2023) investigated the effect of foliar application of iron and zinc elements and zinc+ iron on saffron [28]. Their results showed that zinc and zinc+ iron foliar application treatments respectively had the highest and lowest number and fresh weight of flower and dry weight of stigma. They stated that the combination of elements according to their formulation probably harmed the absorption of nutrients.

Table 2 Means comparison for the interaction of salicylic acid and fertilizer on flower quantitative traits of saffron.

Treatments		Flower fresh weight ($g.m^{-2}$)		Flower number per m^{-2}		Stigma length (mm)		Style length (mm)		Stimga+Style dry weight ($g.m^{-2}$)	
Salicylic acid	Fertilizer	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
S0	F0	26.39 b	28.49 ef	62.67 c	70.67 ef	26.17 ab	26.01 a	16.84 ab	16.79 abc	0.2884 c	0.3574 def
	FP	26.29 b	40.38 bcd	67.87 bc	95.33 cde	25.71 ab	25.31 a	16.95 ab	16.07 abcd	0.3027 c	0.4247 cde
	FCa	31.22 ab	23.19 f	83.77 abc	61.87 f	25.36 ab	25.75 a	17.53 a	17.13 ab	0.3334 bc	0.2528 f
	FZn	36.95 ab	49.46 abc	95.40 ab	119.60 abc	25.62 ab	25.20 a	16.32 ab	15.07 cd	0.4034 abc	0.4999 abc

	FP+FCa	34.41 ab	42.84 bcd	83.40 abc	105.03 bcd	25.96 ab	25.29 a	16.83 ab	16.16 abcd	0.3701 abc	0.4458 bcd
	FP+FCa+FZn	42.74 a	57.25 a	111.07 a	139.93 a	26.08 ab	25.60 a	15.87 b	15.21 cd	0.4963 a	0.5983 a
S1	F0	41.42 a	39.51 cde	106.83 a	90.97 de	26.01 ab	25.64 a	15.87 b	14.67 d	0.4777 ab	0.4161 cde
	FP	41.99 a	28.73 ef	106.87 a	72.03 ef	25.77 ab	26.42 a	16.42 ab	15.37 bcd	0.4579 ab	0.3107 ef
	FCa	34.35 ab	34.98 de	87.00 abc	85.70 def	25.96 ab	25.83 a	17.26 ab	16.82 abc	0.3946 abc	0.3692 def
	FZn	42.13 a	38.34 cde	108.30 a	92.20 de	24.75 b	25.54 a	15.97 b	16.04 abcd	0.4627 ab	0.3844 cde
	FP+FCa	37.58 ab	43.96 bcd	91.20 abc	106.47 bcd	26.57 a	26.16 a	17.15 ab	17.25 a	0.4283 abc	0.4618 bcd
	FP+FCa+FZn	32.96 ab	51.23 ab	82.63 abc	123.73 ab	25.80 ab	25.58 a	17.16 ab	16.68 abc	0.3626 abc	0.5450 ab

S0: No Salicylic acid, S1: Salicylic acid (5000 ppm), F0: No fertilizer, FP: Solu Potash (2000 ppm), FCa: Nitro Calcium (2000 ppm), FZn: Zinc Sulfate (2000 ppm)

- Means with at least one same letter in each column, are not significantly different

Asadi *et al*. (2013) compared the effects of soil nutrition and foliar application of nutrients on saffron and showed that the use of 150 kg. ha^{-1} of complete fertilizer in the form of soil nutrition resulted in the highest number of flowers, fresh and dry weight of flowers, and stigma yield [29]. Akbarian *et al*. (2012) reported that two stages of potassium, zinc and iron foliar application caused a significant increase in saffron flower yield compared to the control treatment [30]. The results of Akrami *et al*., (2015) showed that the consumption of zinc sulfate caused a significant increase in the number and weight of flowers and stigma yield of saffron [31]. While Khorasani *et al*. (2013) showed that foliar application of nutrients could not improve the growth characteristics of saffron [32]. Azizi *et al*. (2019) showed that the foliar application of 2% seaweed extract combined with 7% urea and 2% micronutrients increased the most of growth traits [33].

In this experiment, the lack of significant effect of some fertilizer treatments on the growth parameters of saffron may be due to the consumption of low concentrations. On the other hand, the simultaneous use of solu potash, zinc sulfate and nitro-calcium fertilizers has probably created suitable nutritional conditions for saffron, especially after two years of using these fertilizers, and has caused growth indicators such as the number and fresh weight of flowers as well as dry weight of stigma + style show a significant increase.

Although salicylic acid and fertilizer had a significant effect on various traits, changes in the length of the stigma and style mostly were not significant (Table 2). This shows that these traits are genetically controlled. Various factors often affect the economic yield of saffron through the effect on the number of flowers.

In general, the difference in important traits such as flower fresh weight between fertilizer treatments at different levels of salicylic acid was more obvious in the second year and less in the first year. The insignificant effects of fertilizers as foliar application, in the first year can be caused by low absorption of elements. The spatial distribution of saffron leaves and the waxiness of its leaf surface is such that the amount of absorption through the leaves is associated with low efficiency [32]. Despite this issue, considering that foliar application was done in two stages in each year, after the second year, the difference between the treatments has been revealed due to the increase in the total absorption of nutrients.

Under conditions of salicylic acid consumption, fresh weight and number of flowers per square meter did not differ significantly between different fertilizer treatments in the first year. But in the second year, the treatment FP+ FCa+ FZn had the highest fresh weight (51.23 g.m^{-2}) and the number of flowers per square meter (123.73). It is noteworthy that the use of salicylic acid, compared to its non-use, increased the fresh weight of flowers per square meter (57 and 39% in the first and second year, respectively) and the number of flowers per square meter (70 and 29%, respectively in the first and second year) that these increases were significant in the first year (Table 2).

In general, in the conditions of combined consumption of fertilizers, the balance between nutrients is better established and the effectiveness of fertilizers increases. Atarodi *et al*. (2022) in a study investigated the nutritional status of saffron farms in South Khorasan province. Their results showed that there was a nutritional imbalance problem in all 23 studied saffron farms [34].

Using different fertilizers, the effectiveness of salicylic acid decreased, while when the fertilizer was not used, the use of salicylic acid had a significant effect on the growth characteristics of saffron. The reason for this issue can be attributed to the effect of salicylic acid in improving the absorption of nutrients, which has also been mentioned in the results of various research [6, 26, 35].

The interaction of salicylic acid and fertilizer in the second year on the dry weight of stigma + style shows that the highest amount was related to the treatment of FP+ FCa+ FZn (Table 2). Salicylic acid improves plant growth by regulating physiological processes such as photosynthesis and absorption of nutrients. Improvement of growth and yield with the use of salicylic acid in other plants such as fennel [36], sweet pepper [37] and saffron [38] also has been reported. However in another experiment on saffron populations, Asle Zaim *et al*. (2018) indicated that the application of salicylic acid did not have a special restorative effect on the fresh weight of saffron leaves under drought stress conditions [14].

Leaf Chlorophyll Content and Dryness

Leaf chlorophyll a, chlorophyll b, total chlorophyll and dryness index were significantly affected by the simple effects of salicylic acid and fertilizer (Table 3). The consumption of salicylic acid could significantly increase the amount of chlorophyll a, chlorophyll b and total chlorophyll in both years of the experiment compared to the control. The rate of increase for these traits respectively was 28.2, 51.2 and 36.3 percent in the first year and 22.8, 28.2 and 24.7 percent in the second year (Table 3). On the other hand, the use of salicylic acid reduced the leaf dryness index from 7.22 to 5.11 and from 5.11 to 3.22 in the first and second year of the experiment, respectively, compared to the control (Table 3). Salicylic acid prevents the destruction of chlorophyll and increases the content of chlorophyll by preventing the activity of ACC (1-aminocyclopropane-1-carboxylic acid) oxidase preventing the production of ethylene and regulating the function of ABA (abscisic acid) hormone [16].

Table 3 Means comparison for the simple effect of salicylic acid and fertilizer on leaf chlorophyll content and dryness index of saffron.

Treatments	Chlorophyll a (mg.g^{-1})		Chlorophyll b (mg.g^{-1})		Total chlorophyll (mg.g^{-1})		Leaf dryness index	
	2021	2022	2021	2022	2021	2022	2021	2022
Salicylic acid								
S0	0.3508 b	0.4527 b	0.1900 b	0.2646 b	0.5408 b	0.7173 b	7.222 a	5.111 a
S1	0.4497 a	0.5557 a	0.2873 a	0.3391 a	0.7370 a	0.8947 a	5.111 b	3.222 b
Fertilizer								
F0	0.3008 c	0.3730 c	0.1752 c	0.2550 c	0.4760 c	0.6280 d	7.667 a	6.000 a
FP	0.3772 b	0.4818 b	0.2230 b	0.2890 bc	0.6002 b	0.7708 bc	6.333 b	4.667 ab
FCa	0.3042 c	0.4200 bc	0.1858 c	0.2322 c	0.4900 c	0.6522 cd	7.667 a	6.000 a
FZn	0.4813 a	0.5633 a	0.2940 a	0.3238 ab	0.7753 a	0.8872 ab	4.667 c	3.333 bc
FP+FCa	0.4732 a	0.5765 a	0.2793 a	0.3402 ab	0.7525 a	0.9167 a	5.333 c	3.000 c
FP+FCa+FZn	0.4648 a	0.6105 a	0.2747 a	0.3707 a	0.7395 a	0.9812 a	5.333 c	2.000 c

S0: No Salicylic acid, S1: Salicylic acid (5000 ppm), F0: No fertilizer, FP: Solu Potash (2000 ppm), FCa: Nitro Calcium (2000 ppm), FZn: Zinc Sulfate (2000 ppm)

- Means with at least one same letter in each column, are not significantly different

Researchers in experiments on beans [39] and spinach [40] showed that the consumption of salicylic acid increased the amount of chlorophyll in leaves. Maintaining the content of chlorophyll, especially in adverse environmental conditions, helps stabilize photosynthesis and reduces damage caused by stress [16].

However, increasing the leaves' chlorophyll content with the use of salicylic acid could not have a clear and consistent effect on increasing flower production or the economic yield of saffron. This shows the complexity of yield relationships in saffron and its effectiveness from various environmental factors. Since the yield of flowers and stigma as reproductive structures is closely related to the growth condition of corms (flowers appear from buds on corms), all activities that lead to the improvement of corm growth and their reproduction, will increase the economic yield of saffron. Therefore, it is possible that repeating the use of salicylic acid in consecutive years will improve economic yield.

The photosynthesis of the whole plant depends on the rate of photosynthesis and the length of the photosynthesis period. In the treatment SA_0, although the duration of photosynthesis has decreased, due to the decrease in leaf chlorophyll content, the rate of photosynthesis may have increased.

The use of different fertilizers led to the improvement of leaf chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll). The highest amounts of chlorophyll a, chlorophyll b and total chlorophyll were related to the treatments zinc sulfate, FP+ FCa, as well as FP+ FCa+ FZn, which were not significantly different. The control treatment without fertilizer had the lowest chlorophyll a, chlorophyll b and total chlorophyll (Table 3).

The highest leaf dryness index was related to the control treatment without fertilizer and the lowest one, was related to the treatments of zinc sulfate, FP+ FCa, as well as FP+ FCa+ FZn (Table 3). The nutritional status of the plant strongly affects the leaf chlorophyll content. Various nutrients such as zinc, potassium and calcium play an important role in improving photosynthesis. The noteworthy point in Table 3 is that potassium and calcium fertilizer compounds when used alone did not have a significant effect on increasing the index of chlorophyll a, chlorophyll b and total chlorophyll, but when they were used together, their influence on these traits increased.

Qualitative Traits of Flower

Crocin, picrocrocin and safranal are the most important secondary metabolites of saffron, which are responsible for the color, flavor and aroma of saffron, respectively [41]. Means comparison for the interaction of salicylic acid and fertilizer on saffron qualitative traits are shown in Table 4. In the first year, the control treatment without fertilizer had the highest amount of crocin in the absence of salicylic acid; while this treatment had the lowest amount of crocin in the presence of salicylic acid (Table 4). The amount of safranal in the first year and the absence of salicylic acid application did not differ significantly between fertilizer treatments, but the use of salicylic acid caused a significant difference in the amount of safranal between fertilizer treatments. At this condition, the control treatment without fertilizer had the highest and the fertilizer treatment solu potash+ nitro-calcium, and zinc sulfate had the lowest amount of safranal (Table 4).

Ansarian Mahabadi *et al*. (2019) showed that priming saffron corms with salicylic acid at a level of 2 mM increased the amount of saffron crocin. Priming saffron corms with salicylic acid (1 mM) also increased the amount of saffron picrocrocin and safranal [15].

In general, the results of the effects of different fertilizers on the qualitative characteristics of saffron stigmas varied depending on the type and concentration of fertilizers. Ayoubi et al. (2023) in an experiment investigated the effect of foliar application of micronutrients iron and zinc on saffron [28]. Their results showed that zinc foliar application significantly increased the amount of crocin, picrocrocin and safranal, but foliar application of iron and a combination of iron and zinc had no significant effect on the amount of these compounds in saffron stigmas. Akbarian *et al*. (2012) reported that two stages of foliar application of potassium, zinc and iron improved the qualitative characteristics of saffron flowers [30]. Tabatabaeian *et al*. (2019) in an experiment on saffron investigated the effects of potassium nitrate and zinc [20]. Their results showed that the effect of zinc on crocin, picrocrocin and safranal was not significant, but the use of potassium nitrate (10000 ppm) compared to the control significantly increased these compounds in saffron stigmas.

Table 4 Means comparison for the interaction of salicylic acid and fertilizer on saffron qualitative traits.

Treatments		Crocin ($E^{1\%}_{1cm}$)		Picrocrocin ($E^{1\%}_{1cm}$)		Saffranal ($E^{1\%}_{1cm}$)	
Salicylic acid	Fertilizer	2021	2022	2021	2022	2021	2022
S0	F0	259.67 a	242.00 ab	95.33 a	91.33 ab	26.33 bc	24.67 b
	FP	253.33 ab	246.67 a	93.00 abc	91.67 ab	27.00 b	24.33 b
	FCa	247.33 b	243.67 ab	90.33 c	92.67 a	27.00 b	25.67 ab
	FZn	247.00 b	233.33 ab	91.33 bc	90.33 ab	27.33 b	26.00 ab
	FP+FCa	254.33 ab	224.67 ab	93.33 abc	89.33 ab	27.00 b	27.00 ab
	FP+FCa+FZn	253.00 ab	223.00 ab	94.00 ab	88.67 b	27.00 b	25.67 ab
S1	F0	247.00 b	213.67 b	93.00 abc	90.33 ab	29.00 a	29.33 a
	FP	254.00 ab	231.00 ab	93.33 abc	88.67 ab	27.00 b	26.00 ab
	FCa	263.33 a	226.00 ab	94.00 ab	90.00 ab	27.00 b	26.67 ab
	FZn	260.33 a	239.00 ab	93.67 ab	91.67 ab	26.00 bc	26.67 ab
	FP+FCa	256.33 ab	241.00 ab	94.00 ab	91.33 ab	26.33 bc	26.00 ab
	FP+FCa+FZn	261.33 a	235.00 ab	94.00 ab	89.33 ab	25.33 c	25.33 ab

S0: No Salicylic acid, S1: Salicylic acid (5000 ppm), F0: No fertilizer, FP: Solu Potash (2000 ppm), FCa: Nitro Calcium (2000 ppm), FZn: Zinc Sulfate (2000 ppm)

- Means with at least one same letter in each column, are not significantly different

CONCLUSION

The results showed that the highest yield and number of flowers as well as the highest dry weight of stigma+ style (economic yield) were related to the treatment of solu potash + nitro- calcium + zinc Sulphate and in the absence of salicylic acid. In addition, although the use of salicylic acid alone improved the amount of chlorophyll in the leaves, this did not have a positive effect on the economic yield of saffron. On the other hand, the qualitative traits of the stigma including the amounts of crocin, picrocrocin and safranal did not show significant changes with the use of fertilizer or salicylic acid. This indicates that, given the complexities of functional relationships in saffron, the widespread use of hormone-like compounds such as salicylic acid requires extensive and further research and should be done with caution.

Conflict of Interests

The authors have not declared any conflict of interests.

Acknowledgement

This research has been financially supported by the Saffron Institute, University of Torbat Heydarieh. The grant number was 137897.

REFERENCES

1. Abrishami M.H. Knowledge of Iranian Saffron, Toos Publisher. 1987.
2. Abd El Gayed M.E. Influence of foliar application of salicylic acid on growth and flowering of calendula officinalis L. under levels of salinity. Menoufia Journal of Plant Production. 2020; 5: 325-341.
3. Sarwar M., Saleem M.F., Najeeb Ullah, Ali S., Rizwan M., Rizwan Shahid M., Alyemeni M.N., Alamri S.A., Ahmad P. Role of mineral nutrition in alleviation of heat stress in cotton plants grown in glasshouse and field conditions. Scientific Reports. 2019; 9:13022.
4. Hayat S., Ahmad A. Salicylic acid: A plant hormone, Springer. 2007.
5. Yadav V., Singh H., Singh A., Hussain I., Singh N.B. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress in maize (*Zea mays* L.) grown under cinnamic acid stress. Russian Agricultural Sciences. 2018; 44: 9–17.
6. Simaei M., Khavari-Nejad R.A., Bernard F. Exogenous application of salicylic acid and nitricoxide on the ionic contents and enzymatic activities in NaCl-stressed soybean plants. American Journal of Plant Sciences. 2012; 3: 1495-1503.
7. Fathi S., Najafian S. Morpho-physiological and biochemical properties of *Carum copticum* (L.): effects of salicylic acid. Iranian Journal of Plant Physiology. 2020; 10 (2): 3103- 3112.
8. Hassanzadeh K., Hematie K.H., Alizadeh M. The effect of organic fertilizers and salicylic acid on yield and some secondary metabolites of common balm (*Melissa officinalis* L.). Journal of Plant Production Research. 2016; 23(1): 107-130.
9. Rathor Y., Kumar Mishra P., Kumar P. Zinc and salicylic acid mediated response in crops grown under abiotic stress. Biological Forum – An International Journal. 2022; 14(1): 1647-1652.
10. Mohi-Ud-Din M., Talukder D., Rohman M., Ahmed J.U., Jagadish S., Islam T., Hasanuzzaman M. Exogenous Application of methyl jasmonate and salicylic acid mitigates drought-induced oxidative damages in french bean (*Phaseolus vulgaris* L.). Plants. 2021; 10: 2066.
11. Ignatenko A., Talanova V., Repkina N. Titov A. Exogenous salicylic acid treatment induces cold tolerance in wheat through promotion of antioxidant enzyme activity and proline accumulation. Acta Physiologiae Plantarum. 2019; 41: 1–10.
12. Abbaszadeh B., Layeghhaghighi M., Azimi R., Hadi N. Improving water use efficiency through drought stress and using salicylic acid for proper production of *Rosmarinus officinalis* L. Industrial Crops and Products. 2020; 144: 111893.
13. Dianat M., Saharkhiz M.J., Tavassolian I. Salicylic acid mitigates drought stress in *Lippia citriodora* L.: effects on biochemical traits biologia and essential oil yield. Biocatalysis and Agricultural Biotechnology. 2016; 8: 286– 293.
14. Asle Zaeem F., Niknam V., Ebrahimzadeh H., Sharifi G. Comparative study of drought stress and salicylic acid effects on different accessions of saffron (*Crocus Sativus* L.). Journal of Plant Research. 2018; 31(3): 483-498.
15. Ansarian Mahabadi S., Alahdadi I., Ghorbani Javid M., Soltani E. Effect of corm priming with salicylic acid and mother corm weight on flowering and qualitative characteristics of saffron stigma. Saffron Agronomy and Technology. 2019; 7(1): 41-53.
16. Jabbari1 M., Khayyat M., Fallahi H.R., Samadzadeh A. Influence of saffron corm soaking in salicylic acid and potassium nitrate on vegetative and reproductive growth and its chlorophyll fluorescence indices. Saffron Agronomy and Technology. 2017; 5(1): 21-35.
17. Janni M., Gullì M., Maestri E., Marmiroli M., Valliyodan B., Nguyen H.T., Marmiroli N. Molecular and genetic bases of heat stress responses in crop plants and breeding for increased resilience and productivity. Journal of Experimental Botany. 2020; 71(13): 3780–3802.
18. Waraich E.A., Ahmad R., Halim A., Aziz T. Alleviation of temperature stress by nutrient management in crop plants: a review. Journal of Soil Science and Plant Nutrition. 2012; 12(2): 221-244.
19. Kolupaev Y., Akinina G., Mokrousov A. Induction of heat tolerance in wheat coleoptiles by calcium ions and its relation to oxidative stress. Russian Journal of Plant Physiology. 2005; 52: 199–204.
20. Tabatabaeian J., Hassanian Badi S., Kadkhodaee A. Effect of micronutrient foliar application on quantitative and qualitative traits of saffron (*Crocus sativus* L.). Saffron Agronomy and Technology. 2020; 8(2): 147-163.
21. Zahmatkesh Y., Pessarakli M., Souhani darbani A. Morpho-physiological and yield responses of quinoa genotypes (*Chenopodium quinoa* Willd) to the application of potassium and zinc chelates under drought stress conditions. Iranian Journal of Field Crops Research. 2022; 22(3): 45-54.
22. Malakouti M.J., Homayi M. Fertile soils of arid regions, "Problems and Solutions". second edition. Tarbiat Modarres University Press. Iran. 2004.
23. Arnon D.I. Copper enzyme in isolated chloroplast and polyphenoloxidase in *Beta vulgaris*. Plant Physiology. 1949; 24(1): 1-15.
24. Jabbari M., Khayyat M., Fallahi H.R., Samadzadeh A. Effects of foliar application of salicylic acid and potassium nitrate on chlorophyll content, electrolyte leakage and daughter corm growth of saffron. Journal of Saffron Research. 2018; 6(1):27-49.
25. Khan W., Balakrishnan P., Smith D.L. Photosynthetic responses of corn and soybean to foliar application of salicylates. Journal of Plant Physiology. 2003; 160 (5): 485-92.
26. El-Taher A.M., Abd El-Raouf H.S., Osman N.A., Azoz S.N., Omar M.A., Elkelish A., Abd El-Hady M.A.M. Effect of salt stress and foliar application of salicylic acid on morphological, biochemical, anatomical, and productivity characteristics of cowpea (*Vigna unguiculata* L.) plants. Plants. 2022; 11: 115.
27. Ayoubi K.A., Eisvand H.R., Heydari S., Mousavi-Fard S. Effects of foliar application of iron and zinc micronutrient elements on quantitative and qualitative yield of saffron (*Crocus sativus* L.). Journal of Saffron Research. 2023; 11(1): 66-78.

28. Asadi G.A., Rezvani Moghaddam P., Hassanzadeh Aval F. Effects of soil and foliar applications of nutrients on corm growth and flower yield of saffron (*Crocus sativus* L.) in six-year-old farm. Saffron Agronomy and Technology. 2014; 2(1): 31-44.
29. Akbarian M.M., Heidari Sharifabad H., Noormohammadi G.h., Darvish Kojouri F. The effect of potassium, zinc and iron foliar application on the production of saffron (*Crocus sativa*). Annals of Biological Research. 2012; 3(12): 5651-5658.
30. Akrami M.R., Malakouti M.J., Keshavarz P. Study of flower and stigma yield of saffron as affected by potassium and zinc fertilizers in Khorasan Razavi Province. Journal of Saffron Research. 2015; 2(1): 85-96.
31. Khorasani R., Rezvani Moghaddam P., Hassanzadeh Aval F. Effect of nutrients concentration, time and frequency of foliar applications on leaves and growth of replacement corms of saffron (*Crocus sativus* L.). Iranian Journal of Field Crops Research. 2015; 13(1): 193-202.
32. Azizi G., Mousavi S.G., Seghatoleslami M., Fazeli Rostampour M. Effects of foliar application of seaweed, urea and micronutrient fertilizers on yield and its components of saffron (*Crocus sativus* L.). Journal of Saffron Research. 2020; 8(1): 141-159.
33. Atarodi B., Zabihi H.R., Zangiabdi M. Evaluation of nutritional status of saffron (*Crocus Sativus* L.) in South Khorasan province by "deviation from optimum percentage" (DOP) Method. Saffron Agronomy and Technology. 2022; 10(2): 117-127.
34. Es-sbihi F.Z., Hazzoumi Z., Amrani Joutei K. Effect of salicylic acid foliar application on growth, glandular hairs and essential oil yield in *Salvia officinalis* L. grown under zinc stress. Chemical and Biological Technologies in Agriculture. 2020; 7:26.
35. Ghilavizadeh A., Hadidi Masouleh E., Zakerin H.R., Valadabadi S.A.R., Sayfzadeh S., Yousefi M. Influence of salicylic acid on growth, yield and macro-elements absorption of fennel (*foeniculum vulgare* mill.) under water stress. Journal of Medicinal Plants and By-Products. 2019; 1: 67-75.
36. Sadeghian F., Hadian J., Hadavi M., Mohamadi A., Ghorbanpour M., Ghafarzadegan R. Effects of exogenous salicylic acid application on growth, metabolic activities and essential oil composition of *Satureja khuzistanica* Jamzad. Journal of Medicinal Plants. 2013; 47(12).
37. Shekofteh H., Shahrokhi H., Solimani E. Effect of drought stress and salicylic acid on yield and mucilage content of the medicinal herb *Plantago ovata* Forssk. Desert. 2015; 20(2): 245-252.
38. Popova L., Ananieva V., Hristova V., Christov K., Geovgieva K., Alexieva V., Stoinova Z. Salicylic acid and methyl jasmonate-induced protection on photosynthesis to paraquat oxidative stress. Bulgarian Journal of Plant Physiology. 2003; 133-152.
39. Eraslan F., Inal A., Gunes A., Alpaslan M. Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. Scientia Horticulturae. 2007; 113: 120-128.
40. Kumar R., Singh V., Devi K., Sharma M., Singh M.K. Ahuja P.S. State of art of saffron (*Crocus sativus* L.) agronomy: a comprehensive review. Food Reviews International. 2009; 25: 44–85.