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Effects of gibberellic acid and naphthalene acetic acid on saffron plant (*Crocus sativus* L.) under field conditions

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Abstract

An experiment was conducted to investigate the effect of growth regulator treatments on quantitative and qualitative yield of saffron. Growth regulator treatments included 50, 150 and 300 ppm gibberellic acid (GA3) and naphthalene acetic acid (NAA) . Saffron corms weighing 8 to 10 g were immersed in solutions of GA3 and NAA and then were planted in 1×1 meter plots with 15 cm within row and 20 cm between row spacing. At the end of the growing season (end of June), crop traits such as total number and weight of the produced corms and the number and weight of produced corms in weighing groups of less than 4 g, 4 to 8 g, and more than 8 g were measured by harvesting one third of the cultivated corms in each plot. At the flowering time of the remaining corms of each plot (November), weight and number of flower, fresh and dry weight of stigma, and the amount of active ingredients including crocin, picrocrocin and safranal were measured. The results showed that all studied traits were affected by growth regulator compounds and indicated significant ($P \leq 0.01$ and $P \leq 0.05$) differences with control. GA3 improved biological yield and corm weight, in addition it could increase the quality of stigma compared to the control treatment. Application of 150 ppm GA3 increased the weight of corms by 16.25 % and the number of flower plot^{-1} by 34.99 % compared to the control treatment. Also, 300 ppm GA3 increased the dry stigma yield by 37.5 % compared to the control treatment. NAA did not affect yield production significantly and at high concentrations, prevented the growth of corms and flower production. Results indicated that concentrations higher than 150 ppm GA3 can increase the yield of saffron, and can play an important role in enhancement of saffron farms productivity.

Key words: Gibberellic acid, growth regulators, naphthalene acetic acid, saffron, crocin, picrocrocin and safranal

Introduction

Saffron (*Crocus sativus* L.) belongs to the Iridaceae family (Mardani *et al.*, 2015). There are nine species of this genus in Iran, but only *C. sativus* is cultivated in Iran due to high economic value. *C. sativus* is an autotriploid plant and therefore, it is considered to be sterile in terms of reproductive and seed production (Behnia, 1996). The plant originated from West Asia and the Mediterranean region is distributed in the region of Iran and Turan. Cold autumns and winters, and hot summers with a low rainfall frequency are the significant characteristics of these regions (Negbi, 2003). Saffron is one of the most economical and valuable agricultural products in Iran and the most expensive spice in the world (Amini *et al.*, 2014). In addition to its use in the food industry as a natural dye, it also has many uses in medicine. Medically, it is considered as antispasmodic and anti-inflammatory, relaxing, decongestant, expectorant, strengthen the stomach strengthening, sexual stimulation, anti-depressant (Rios *et al.,* 1996; Karimi *et al.,* 2001).

The various environmental factors (light, moisture, and temperature) can affect growth and development of the plant. Growth regulator compounds, especially plant hormones, also affect the plant growth process (Immink, 2015). So far some studies have been done on the use of growth regulators to increase the flowering and yield of saffron. Size of mother corms of saffron directly affects the amount of flowering, and growth regulator compounds have direct effect on these traits. In saffron plants, usually larger and more developed corms produce higher-weighted flowers, thus preventing the growth of lateral buds and reducing the number of daughter corms which plays a significant role in flowering (DeMastro and Ruta, 1993). Studies have shown that the use of the combination of GA3 and Kinetin stimulated the growth and production of daughter corms and increased dry weight of stigma more than 150 % (Azizbekova, 1978). One-day immersion of saffron corms in 2,4-D solution also increased plant height, leaf to corm ratio and daughter to mother corm ratio (Kabdal and Joshi, 1978).

Considering the fact that increasing the number of saffron daughter corms reduces the corms weight, this study was planned to investigate the effect of applying growth regulating agents in increasing the apical dominance and decreasing the number of daughter corms, to achieve higher corm weight and flower yield.

Materials and methods

Experimental design and material: The field experiment was carried out as a completely randomized design with three replications at the Research Station of Agriculture Faculty, Ferdowsi University of Mashhad, Iran. Saffron corms were prepared from Torbat Heydarieh in September 2016. After cleaning and weight grading, corms with an average weight of 8 to 10 g with no contamination and mechanical damage were selected and then disinfected with 2 % benomyl solution for 20 minutes. Saffron corms were soaked for 24 hours in solutions of 50, 150 and 300 ppm GA3 and NAA (produced by Merck) using soaking method. After land preparation and plotting, saffron corms were planted at a depth of 15 cm in a 1×1 meter plots with 15 cm in row and 20 cm between row spacing (30 corms plot^{-1}). The irrigation of the farm was flooding during the growing season.

Measurements: Each plot was partitioned into two sections, one for study of corms traits and the second section used for study of flower traits. Number and weight of produced corms in each plot, total number and weight of produced corms in weighing groups of less than 4 g, 4 to 8 g, more than 8 g and the propagation coefficient (relative weight percentage of produced corms compared to the initial weight of cultivated corms) (Kumar *et al.,* 2013) were measured at the end of growing season, by harvesting one third of the cultivated corms. At the flowering time of plants growing in the remaining part of each plot (November), the flowers were harvested on a daily basis and the number and weight of flowers plot^{-1} were recorded. Stigma were separated from flowers by hand and kept for 48 h at room temperature of 25 °C. The amount of crocin, safranal and picrocrocin of stigma was measured based on absorbance of 1% ($E^{1\%}$) aqueous solution of dried saffron stigma at wavelengths of 440, 330 and 257 nm, respectively (ISO, 2003), by UV-Vis Spectrophotometer (i3, Hanon).

Statistical analysis: Data analysis was performed using JMP8 software and the mean differences were compared by Duncan's multiple range test at $P \leq 0.01$ and $P \leq 0.05$.

Results and discussion

Corms traits

Number and weight of produced corms: The effect of growth regulators on total number and weight of produced corms was significant ($P \leq 0.01$ and $P \leq 0.05$, respectively) (Table 1). The highest number of corms (55.67 and 54.33 plot^{-1}) were produced in 50 ppm GA3 and 50 ppm NAA, respectively and the lowest number of corms (22 number plot^{-1}) was observed in 300 ppm GA3 treatment. The highest and the lowest total weight of produced corms (242.3 and 97.19 g plot^{-1}) were obtained in 150 ppm GA3 and 150 ppm NAA, respectively. The number of daughter corms produced per mother corm is directly related to the weight of each daughter corm produced. It can be noted that the weight of each produced corm decreases with the increase of the number of lateral buds and daughter corms (Douglas, 2003) thus it is very important in the final yield (Fig. 2). Corms size and weight are one of the key indicators in determining the flowering or non-flowering daughter corms. Thus, the daughter corms do not start flowering process until they reach the base weight (about 8 g) (Mollafilabi, 2004; Turhan *et al.,* 2007; Renau-Morata *et al.,* 2012).

This experiment showed that with application of GA3 on saffron corms and increasing hormone concentration, the number of lateral buds and the production of daughter corms decreased from 34.26 in the control to 32.67 in 150 ppm GA3 and decreased to 22 in the 300 ppm GA3. This can be a very important point in the process of producing saffron. However, in addition to reducing the number of daughter corms in the GA3 treatment, the total weight of daughter corms in this treatment (242.3 g plot^{-1} in 150 ppm GA3) increased compared to the control (202.92 g plot^{-1}). In previous studies, it was shown that the use of some hormonal compounds, such as GA3, reduces the growth of lateral buds in the mother corms and the production of daughter corms in bulbous plants. This causes large mother corms and more flower yield (Azizbekova *et al.,* 1978; Azizbekova *et al.,* 1982; Azizbekova and Milyaeva, 1999).

Average and maximum weight of produced corms: The application of growth regulator compounds on both average weight of produced corms (total weight of produced corms per plot divided by number of produced corms per plot) and maximum weight of produced corms were significant at $P \leq 0.01$ level. The highest and lowest average weight of daughter corms

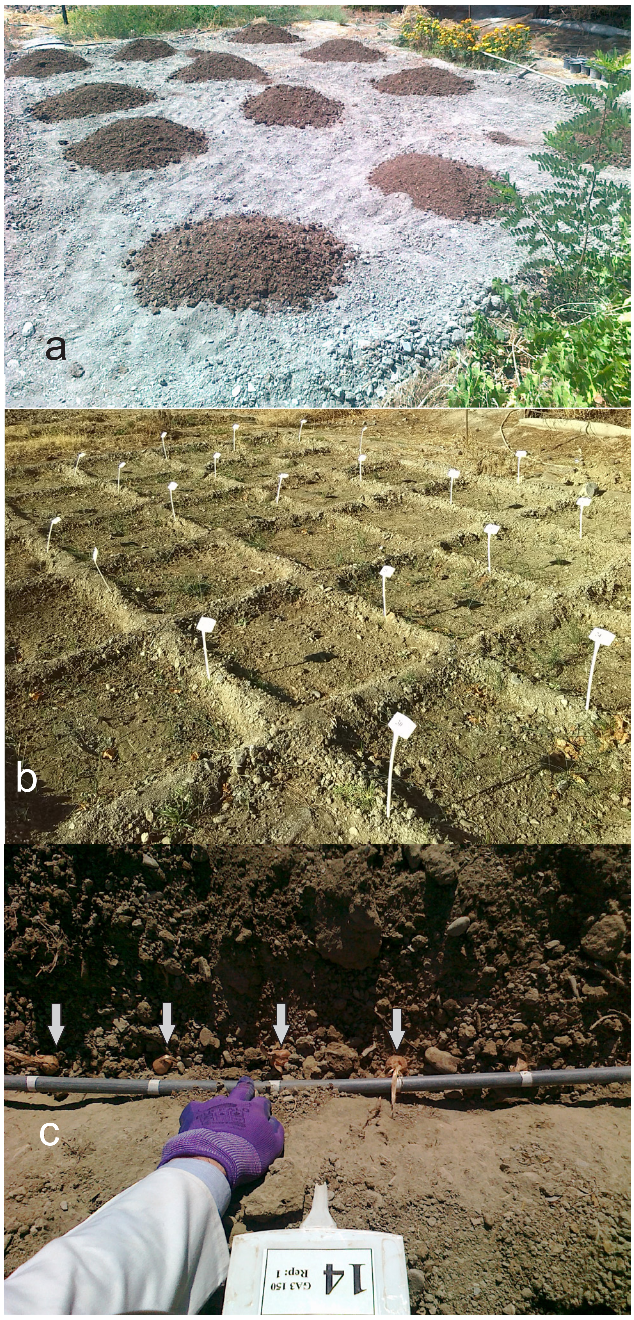

Fig. 1. Stages of land preparation and planting; a) land preparation, b) plotting, c) saffron corms planting.

Table 1. Effect of growth regulator treatments on saffron corms traits

Treatment	Corm number ($plot^{-1}$)	Total corm weight ($g\ plot^{-1}$)	Average weight ($g\ corm^{-1}$)	Maximum weight (g)	Number of corms > 8 g ($plot^{-1}$)	Weight of corms > 8 g ($g\ plot^{-1}$)	Average weight of corms > 8 g ($g\ corm^{-1}$)	Number of corms 4-8 g ($plot^{-1}$)	Weight of corms 4-8 g ($g\ plot^{-1}$)	Number of corms < 4 g (per plot)	Weight of corms < 4 g ($g\ plot^{-1}$)
Control	34.26 ^{ab}	202.92 ^{a}	8.01 ^{ab}	26.57 ^{ab}	9.66 ^{a}	147.44 ^{bc}	15.59 ^{abc}	6.00 ^{ab}	27.55 ^{bc}	18.66 ^{abc}	28.07 ^{ab}
50 ppm GA3	55.67 ^{a}	226.26 ^{a}	5.20 ^{bc}	21.39 ^{bc}	8.33 ^{a}	128.44 ^{c}	15.49 ^{abc}	9.66 ^{a}	54.98 ^{a}	29.00 ^{ab}	42.92 ^{a}
150 ppm GA3	31.67 ^{ab}	242.30 ^{a}	8.38 ^{ab}	28.09 ^{ab}	10.67 ^{a}	202.21 ^{a}	18.93 ^{a}	2.66 ^{b}	14.62 ^{c}	14.67 ^{bc}	25.48 ^{ab}
300 ppm GA3	22.00 ^{b}	230.07 ^{a}	10.24 ^{a}	28.97 ^{a}	10.33 ^{a}	189.30 ^{ab}	18.46 ^{ab}	4.33 ^{b}	28.77 ^{abc}	7.33 ^{c}	11.99 ^{b}
50 ppm NAA	54.33 ^{a}	201.16 ^{a}	3.76 ^{c}	22.78 ^{abc}	7.33 ^{ab}	107.17 ^{c}	15.07 ^{bc}	10.00 ^{a}	51.64 ^{ab}	37.00 ^{a}	42.37 ^{a}
150 ppm NAA	32.67 ^{ab}	97.19 ^{b}	2.96 ^{c}	16.71 ^{c}	4.33 ^{b}	56.16 ^{d}	14.51 ^{c}	2.66 ^{b}	14.48 ^{c}	25.66 ^{abc}	26.54 ^{ab}
300 ppm NAA	0	0	0	0	0	0	0	0	0	0	0
Significance	*	**	**	**	**	**	**	**	**	*	*
Standard error	10.13	18.11	1.26	2.19	1.27	15.41	1.16	1.42	8.53	6.27	7.67

Values followed by the same letters in each column are not significantly different. ($P<0.05$). **=P≤0.01; *= P≤0.05 probability level; and ns= non-significant at 0.05 probability level

were obtained in 300 ppm GA3 (10.24 g $corm^{-1}$) and 150 ppm NAA treatment (2.96 g $corm^{-1}$), respectively. 300 ppm GA3 and 150 ppm NAA treatments had the highest (28.97 g) and lowest (16.71 g) maximum weight of produced corms (Table 1). Since average weight of produced corms is related to the number and weight of produced corms, 300 ppm GA3 could reduce the number of daughter corms and increase the weight of daughter corms compared to the control as well as other treatments. In the case of maximum single corm weight, due to the limitations on the production of daughter corms in GA3 treatment, it was expected that new produced corms will have more weight. Reports show that there is a significant and direct correlation between the weight of mother corms and the number of produced flowers (Molina *et al.,* 2005; DeJuan *et al.,* 2009). For this reason, some researchers strongly recommend selection of appropriate weight of corms at the sowing time and use appropriate agronomic techniques to increase the corm weight in the field (DeMastro and Ruta, 1993; Omidbaigi, 2005). Typically, it is estimated that each saffron corm can produce 1 to 12 flowers, depending on its initial size and weight. Based on current study, it is clear that the minimum corm weight for starting the flowering process of saffron is between 6.5 and 7.5 grams. There is a possibility to produce 1 to 2 flowers in this weighting group (Mashayekhi and Latifi, 1997). The flower yield increases by increasing the weight of mother corms. In this regard, in order to produce more flowers in early years, corms with a weight more than 8 g are recommended for planting (HassanBeygi *et al.,* 2006). Although there is a limited research on the relationship between the amount of produced flowers and the weight of mother corms, it has been made clear that saffron corms in weighing groups of 20-30, 30-45 and more than 45 g can produce 6-8, 8-12 and more than 12 flowers per corm (DeMastro and Ruta, 1993; Mashayekhi and Latifi, 1997; Douglas, 2003). Considering the importance of this issue in the production process of saffron, Douglas (2003) designed a study based on cultivated corms in different weights and results indicated higher stigma yield (28.4 to 36.3 kg ha^{-1}) in fields with larger corms, which varies greatly with the average stigma yield of conventional cultivation. If this estimate is made in terms of the diameter of corms and the space occupied by corms, the importance of this issue will illustrate better. Since, corms with

Fig. 2. Produced corms in different treatments of growth regulator compounds; a) 300 ppm GA3, b) 50 ppm NAA, C) control.

a diameter less than 2 cm (about 8 g) are not able to produce flowers (DeMastro and Ruta, 1993), with increasing diameter (even one cm), the flowering rate increases several times. So that when the corm diameter reaches 3 cm, it can produce 6 flowers (Aga, 2008) and when its diameter reaches more than 4-5 cm, this amount increases to more than 10 flowers per corm (Douglas, 2003). In other words, by increasing the diameter of corms from 2 to 4 cm, the flowering of corms can be increased by 8 to 10 times. This proves the importance of increasing weight of saffron corms and preventing the increase in the number of daughter corms. In addition to physical occupation of plant growth space and absorption of resources and competition with larger corms, small daughter corms reduce farm yield and prevents the weight increase of other corms. On the other hand, due to the increasing number of corms, high competition of corms in the soil and the production of small corms will drastically reduce saffron yield in the fifth to seventh years after planting. As a result, farmers are forced to bring the corms out of the ground and re-plant. Increasing the weight of saffron corms and preventing excessive increase in the number of corms may be possible way to increase the length of saffron farms stability and the sustainability of yield over the years.

Number and weight of corms in different weight groups: The effect of growth regulator compounds was significant on number and weight of corms in different weight groups (Table 1). The highest number of corms weighing more than 8 g was observed in 150 ppm GA3 (10.67 plot^{-1}) and the lowest was obtained in 150 ppm NAA (4.33 plot^{-1}). The highest and the lowest weight of corms more than 8 g were observed in 150 ppm GA3 (202.21 g plot^{-1}) and 150 ppm NAA (56.16 g plot^{-1}), respectively. The treatments of 150 ppm GA3 and 150 ppm NAA had the highest (18.93 g plot^{-1}) and lowest (14.51 g plot^{-1}) average weight of corms more than 8 as well. Weight measurement and counting of corms between 4-8 g showed that the highest and the lowest number were observed in 50 ppm NAA (10 plot^{-1}) and 150 ppm GA3 (2.66 plot^{-1}). The highest weight of corms (4-8 g)was obtained in 50 ppm GA3 (54.98 g plot^{-1}) and the lowest was observed in 150 ppm NAA treatment (14.48 g plot^{-1}) and 150 ppm of GA3 (14.62 g plots^{-1}). 50 ppm NAA and 300 ppm GA3 had the highest (37 and 42.37 g plot^{-1}, respectively) and lowest (7.33 and 11.99 g plot^{-1}, respectively) number and weight of corms less than 4 g (Fig. 2).

The distribution of produced corms in different treatments is shown in Fig. 3. The corms more than 8 g had the highest proportion in 150 and 300 ppm GA3. High weighted corms have significant effects on the production of large corms and also the production of flowers and more yield in the future years.

The proportion of propagation coefficient was also investigated in this study (Fig. 4). The results showed that 150, 300 and 50 ppm GA3 were able to produce heavier corms (224.4, 213.1 and 209.5 % increase in production, respectively) compared to

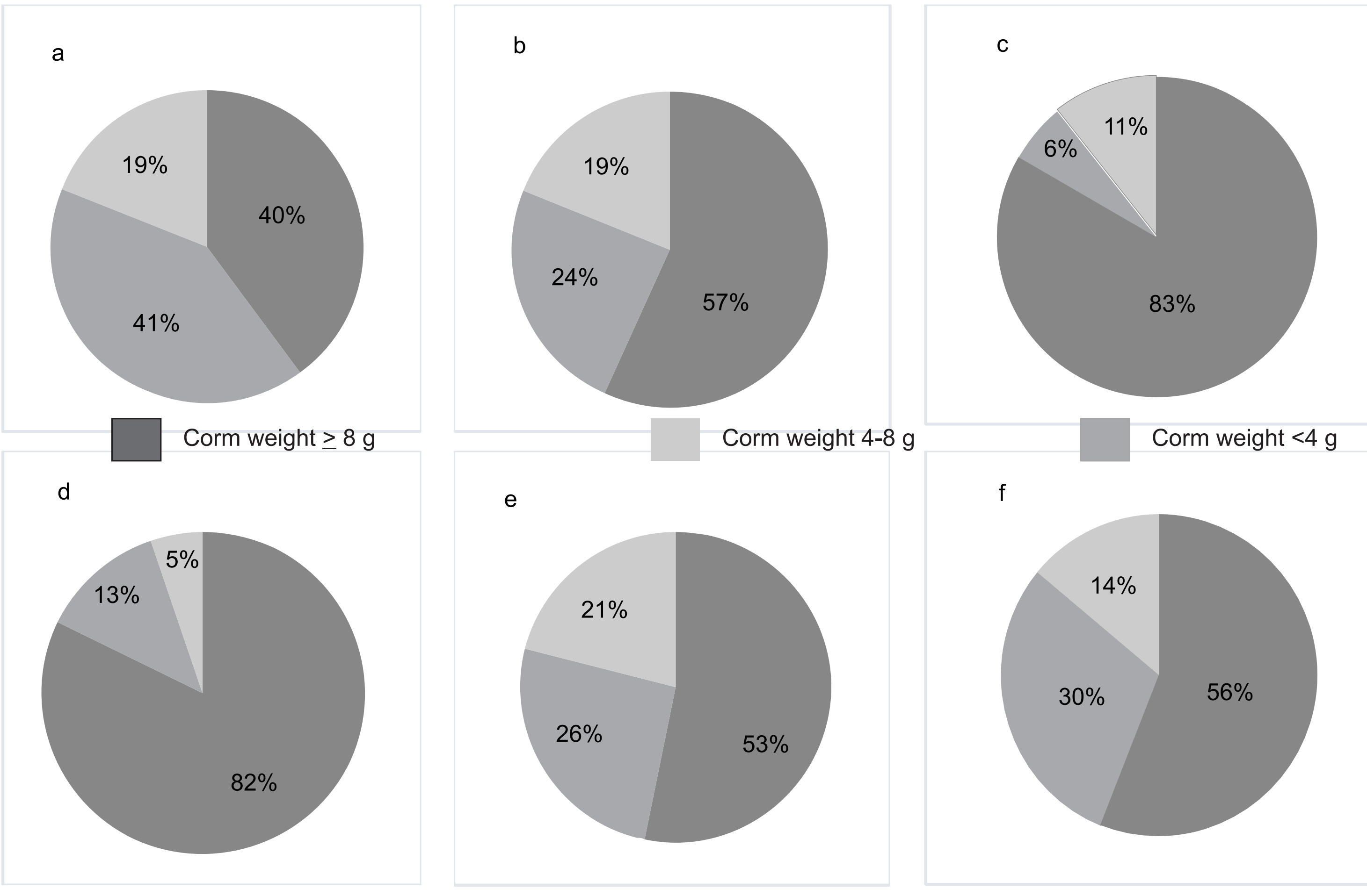

Fig. 3. The distribution of saffron corms in different treatments of the regulator compounds; a) control, b) 50 ppm GA3, c) 150 ppm GA3, d) 300 ppm GA3, e) 50 ppm NAA, f) 150 ppm NAA

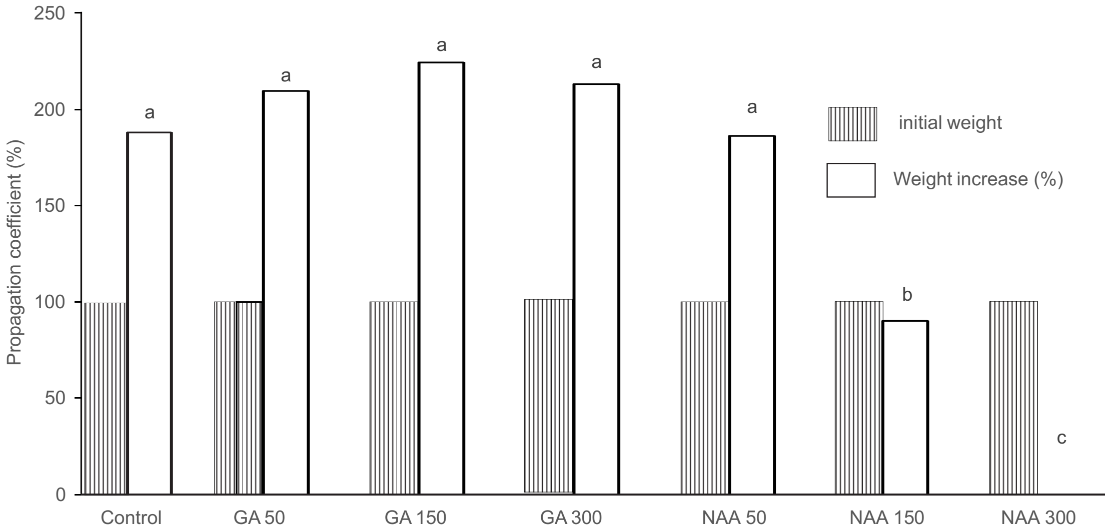

Fig. 4. The ratio (in percentage) of the weight of produced corms and planted corms.

control (187.9 %). The treatment of GA3 at medium and high concentrations had the greatest effect on increasing average weight of corms and the production of high weighted corms. It seems that at lower concentration of GA3 prevented production of smaller corms due to limited growth of lateral buds. In this study, the treatment of NAA did not have the positive effect on corm production, and at high concentrations completely stopped the growth of corms. After the cultivation of saffron corm, an average of six new corms are usually produced in the first year, and a total of 22 and 65 new corms are produced in the second and third years, respectively (Douglas, 2003). If these corms have a high weighted average, flower yield is very favorable, but usually a part of the corms cannot reach the desired weight due to competition for the absorption of nutrients and practically do not have acceptable yield in the farm. Therefore, maintaining the weight status of corms in the field can be a solution for yield stability in saffron production farms.

Flower traits

Number of flowers: The analysis of variance of the effect of growth regulator compounds on the number of flowers was significant at *P*≤0.01 (Table 2). The highest and lowest number of flowers were observed in the treatments of 150 ppm GA3 (56.67 plot^{-1}) and 150 ppm NAA (3.33 plot^{-1}), respectively. 150 and 300 ppm GA3 increased the number of flowers by 42.92 and 30.95 %, respectively, compared to the control treatment (42 plot^{-1}). The application of NAA did not have a positive effect on the flowering process of saffron and at high concentrations, flowering was completely stopped, in addition to limited vegetative growth Chrungoo and Farooq (1989) found that the application of NAA reduces vegetative growth and flowering, and also inhibits growth at high concentrations. Reports show that the use of GA3 in tulip increases the rate of flowering (Rudnicki *et al.,* 1976). Azizbekova (1978) showed GA3, by stimulating growth and flowering of saffron, can increases the number of flower and ultimately yield. This has a direct relationship with carbohydrate metabolism and starch content of corms. GA3 accelerates the development of physiological processes and flower production by increasing starch metabolism and producing simple sugars. Since saffron is a hysteranthous plants, the production and completion of flower buds is highly dependent on the metabolism of stored compounds, and GA3 plays an important role in this process (Chrungoo and Farooq, 1989).

Table 2. Effect of growth regulator treatments on saffron flower traits

Treatment	Flower number (plot^{-1})	Fresh weight of flower (g plot^{-1})	Dry weight of flower (g plot^{-1})	Fresh weight of stigma (g plot^{-1})	Dry weight of stigma (g plot^{-1})	Fresh weight flower to number ratio	Fresh weight of stigma to flower number ratio	Dry weight of stigma to flower number ratio	Fresh weight of flower to dry weight of stigma ratio
Control	42.00 ^{b}	17.25 ^{b}	2.32 ^{b}	1.59 ^{b}	0.280 ^{b}	0.410 ^{a}	0.037 ^{a}	0.010 ^{a}	61.402 ^{a}
50 ppm GA3	42.67 ^{b}	17.54 ^{b}	2.23 ^{b}	1.42 ^{b}	0.280 ^{b}	0.413 ^{a}	0.033 ^{a}	0.010 ^{a}	63.315 ^{a}
150 ppm GA3	56.67 ^{a}	24.24 ^{a}	3.11 ^{a}	2.09 ^{a}	0.356 ^{a}	0.427 ^{a}	0.037 ^{a}	0.010 ^{a}	68.297 ^{a}
300 ppm GA3	55.00 ^{a}	22.59 ^{a}	3.10 ^{a}	2.05 ^{a}	0.386 ^{a}	0.407 ^{a}	0.037 ^{a}	0.010 ^{a}	58.392 ^{a}
50 ppm NAA	28.00 ^{c}	10.94 ^{c}	1.47 ^{c}	0.98 ^{c}	0.170 ^{c}	0.397 ^{a}	0.036 ^{a}	0.010 ^{a}	64.233 ^{a}
150 ppm NAA	3.33 ^{d}	1.53 ^{d}	0.46 ^{d}	0.13 ^{d}	0.023 ^{d}	0.416 ^{a}	0.039 ^{a}	0.007 ^{a}	66.191 ^{a}
300 ppm NAA	0	0	0	0	0	0	0	0	0
Significant level	**	*	*	*	*	ns	ns	ns	ns
Standard error	2.156	1.362	0.183	0.124	0.018	0.033	0.002	0.001	3.904

Values followed by the same letters in each column are not significantly different. (*P*<0.05). **=P≤0.01; *= P≤0.05 probability level; and ns= non-significant at 0.05 probability level

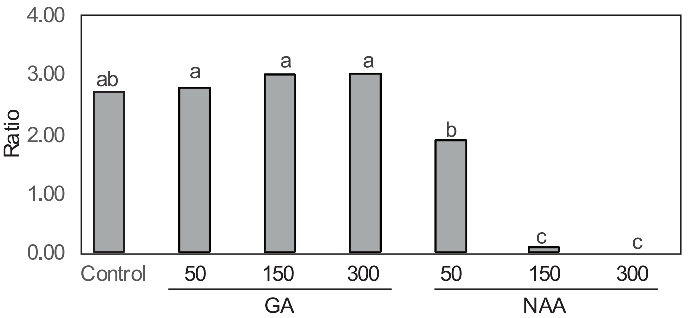

Fig. 5. The ratio of the number of harvested flowers to the weight of corms more than 8 g per plot.

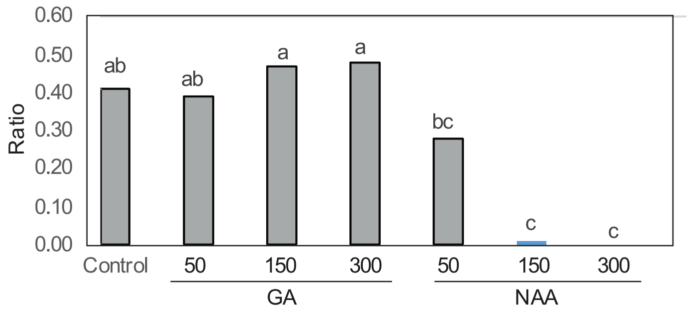

Fig. 6. The ratio of harvested flowers to the total weight of produced corms.

Fresh and dry weight of flower: The analysis of variance showed that there was significant difference ($P \leq 0.05$), among different treatments of growth regulator compounds based on fresh and dry weight of harvested flowers (Table 2). The highest and lowest fresh and dry weight of harvested flowers were observed in 150 ppm GA3 (24.24 and 3.11 g plot^{-1}) and NAA (1.53 and 0.46 g plot^{-1}), respectively. In 300 ppm NAA, no reproductive performance was evaluated due to lack of corm production. The flowering process of saffron corms has a direct relation to the weight of corms and their nutritional content, and any factor that can affect the corm weight influences the yield and weight of the produced flowers (DeMastro and Ruta, 1993; Omidbaigi, 2005). Due to the positive effects of GA3 on the produced corm, it has effect on the fresh and dry weight of the produced flower.

Fresh and dry weight of stigma: In this study, it was found that growth regulator compounds significantly ($P \leq 0.05$) affected the fresh and dry weight of stigma (Table 2). The highest and lowest fresh weight of stigma were observed in 150 ppm GA3 (2.09 g plot^{-1}) and NAA (0.13 g plot^{-1}), respectively. Also, the highest and lowest dry weight of stigma were obtained in 300 ppm GA3 (0.386 g plot^{-1}) and 150 ppm NAA (0.023 g plot^{-1}). As shown in (Table 2), 300 and 150 ppm GA3 had the highest amount of produced dry stigma with an increase of 37.5 and 27.14 %, respectively, as compared to the control (0.28 g plot^{-1}). Due to the effect of GA3 on the fresh and dry weight of the flower, increase in the yield of stigma was not unexpected. The fresh and dry weight of stigma is one of the most important determinants in the final yield of saffron. Any factor that can accelerate the transfer of nutrients and storage materials has a significant effect on the final yield. One of the factors that affect the yield of dry saffron stigma is the amount of stored material in saffron corms (DeMastro and Ruta, 1993; Mashayekhi and Latifi, 1997). Since, GA3 could have a significant effect on the weight of produced corms an increase in the weight of flower and stigma and thus increase the yield of saffron is expected.

Other traits

There was no significant difference between the control and treatments of growth regulator compounds in terms of the ratio of fresh weight to number of flower, the ratio of fresh weight of stigma to the flower number, the ratio of dry weight of stigma to flower number and the ratio of fresh weight of flower to dry weight of stigma (Table 2). However, there was no significant difference between control and treatments of 150 and 300 ppm GA3 in terms of flower yield, but 150 and 300 ppm GA3 had the highest value in most of the traits. This can be closely related to the number of produced corms in this treatment.

In order to investigate the relationship between number of produced corms and produced flowers, a comparison was conducted between the yield of dry stigma and produced corm. The results revealed that GA3 treatments had the highest yield at the concentration of 150 and 300 ppm, and they were able to convert more of the weight of corm to produce flower (Fig. 5 and 6). This issue has great importance in saffron farms, because a large part of the corms which are produced annually in saffron fields does not have the ability to produce flower due to lack of proper weight. So, the observed difference in each of the expressed indices was due to the production of this group of corms, which affects the next year yield. Munshi *et al.* (2003) suggested that large-sized corms (diameter greater than 4 cm) can produce more flowers than small-sized corms. Mariz (1996) also showed that large-sized corms (diameter greater than 4 cm) have high yield (dry weight of the stigma) than small-sized corms. The results of this study were consistent with the results of Mariz (1996), Munshi *et al.*(2003) and Mashayekhi *et al.* (2006).

Phytochemical indices: The quality grading of saffron samples

Table 3. Effect of different levels of growth regulators on phytochemical indices and quality grading of saffron stigma

Treatment	Absorption at 440 nm ($E^{1\%}$ 440)	INSO 259 Reference (Crocin)	Absorption at 330 nm ($E^{1\%}$ 330)	INSO 259 Reference (Safranal)	Absorption at 257 nm ($E^{1\%}$ 257)	INSO 259 Reference (Picrocrocin)
Control	200.1	II	40.9	I	87.6	I
50 ppm GA3	206.7	II	44.5	I	93.9	I
150 ppm GA3	208.5	II	48.3	I	98.4	I
300 ppm GA3	220.7	I	42.3	I	97.2	I
50 ppm NAA	198.1	III	39.3	I	84	II
150 ppm NAA	194	III	37.6	I	78	III
300 ppm NAA	-	-	-	-	-	-

was performed based on the Iranian National Standard No. INSO 259-1:1391. The concentration of each of the metabolite was determined according to the absorbance in each prepared solution in equal concentrations. The readings at wavelengths of 440, 330 and 257 nm, respectively, were related to the compounds of crocin, safranal and picrocrocin, respectively, which are responsible for the color, flavor and taste of saffron stigma (Amini *et al.*, 2014).

The results showed that GA3 had the greatest effect on the amount of produced metabolites as compared to the control. 300 ppm GA3 produced the highest amount of crocin (220.7 $E^{1\%}$ 440 nm) and 150 ppm GA3 had the highest amount of safranal (48.3 $E^{1\%}$ 330 nm) and picrocrocin (98.4 $E^{1\%}$ 257 nm) (Table 3). In contrast, the use of NAA was not able to produce favorable results. By increasing the concentration of this compound, the amount of active ingredients in saffron stigmas decreased. The lowest amount of crocin (194 E1% 440 nm), safranal (37.6 $E^{1\%}$ 330 nm) and picrocrocin (78 $E^{1\%}$ 257 nm) were obtained in 150 ppm NAA.

In the qualitative evaluation of the stigma in each treatment, only 300 ppm GA3 could be placed in the first grade of quality based on all the indices (including crocin, safranal and picrocrocin) and it was also the only treatment with a qualitative grade I in terms of the composition of crocin (Table 3). Other concentrations of GA3 (150 and 50 ppm) and control were placed in qualitative grade II, treatments of 50 and 150 ppm NAA had qualitative grade III, in terms of the composition of crocin. In case of safranal, all treatments were in qualitative grade I. Also, in terms of picrocrocin combination, control and all levels of GA3 treatment were placed in grade I, and 50 and 150 ppm NAA treatments were in qualitative grade II and III, respectively. GA3 treatment had the highest grade of quality, and the treatment of NAA failed to achieve the desired quality grade of saffron stigma. The results obtained in this section and the trend of increasing the quality of active ingredients by increasing concentrations of GA3 are consistent with the results presented by (Isfahani *et al.,* 2003). In general, limited research has been carried out on the relationship between growth regulating components and the quality of active ingredients of saffron. So, the identification of trends and the causes of such changes require more research.

Based on the results of the present study it seems that concentrations higher than 150 ppm GA3 is effective in increasing saffron yield. The mechanism involved in improved qualitative yield of saffron using GA3 and the effect of other plant growth regulators on the quantitative and qualitative yield of saffron are suggested line of work for future research.

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