The effects of fulvic acid application on seed and oil yield of safflower cultivars

Payman MORADI, Babak PASARI* and Farzad FAYYAZ

Department of Agronomy and Plant Breeding, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran, *correspondence: bpasary@yahoo.com

Abstract

This study was carried out as split split plots in a randomized complete block design with three replications at Research Field of Agriculture Faculty, during 2014-2015 in Iran. In this study, the main factor was two safflower cultivars including: Sina and Faraman and subplot were foliar application of fulvic acid in 2 stages as: stem elongation and flowering and also sub sub plot were 3 different fulvic acid concentrations including as: 0: control (distilled water), 0.5 and 1 $kg^{*}ha^{-1}$. The results showed that the characters including: numbers of seed in head, 1,000 seed weight, seed yield, harvest index and oil percent were affected significantly by safflower cultivars, and also head weight were affected in different growth stages. Also the results of fulvic acid were showed significant different about head numbers, numbers of seed in head, biological yield, harvest index and oil percent. In this study the maximum seed yield and oil percent were achieved by Faraman cultivar in comparison with Sina, as it produced 14.33 and 19.5 percent more seed yield and oil percent in arrangement. Foliar application in stem elongation stage obtained 6.02 percent more seed yield but in flowering stage, fulvic acid spraying were achieved 35.5 percent more oil percent. Finally the results showed the positive effects of foliar application of fulvic acid in 1 $kg^{*}ha^{-1}$, as it produced 85.67 percent more oil percent in comparison with control.

Keywords: cultivar, fulvic acid, growth stages, oil percent, safflower seed, spraying

Introduction

Increasing of oil production in Iran is vital due to growing population and also trends to consuming vegetable oil instead of animal oil as well. Among 40 different oil seeds whose oil can be consumed (Sharma et al., 2011), Safflower (*Carthamus tinctorius)* is grown in 60 countries around the world. Although safflower is considered minor crop with less than 1 million hectares planted, producing around 500,000 mt each year, it plays however an important role in the farming systems (Gilbert, 2008). Safflower is one of the oldest cultivated crops, usually grown at a small scale. It is grown for flowers used for coloring, flavoring foods, dyes, medicinal properties, livestock and bird feed. Under lower rainfall and limited input farming system, Safflower planting is a good choice. This plant have a great oil yield potential and able to grow under high temperatures, drought, and salinity (Hussain et al., 2015). It has also found for industrial applications such as biofuel (Dajue and Mundel, 1996). Safflower oil contains about 75% linoleic acid that is essential for human nutrition (Weiss, 2000).

Totally one of the critical inputs in achieving high productivity of safflower is nutrient management (Mündel et al., 2004). Chemical Fertilization is a current practice for maintain and increase soil fertility and consequently higher crop production. But currently some adverse effects of excessive chemical fertilization were reported such as: soil salinity, heavy metal accumulations, water eutrophication, accumulation of nitrate in soil and plant, air pollution and also greenhouse effect (Savci, 2012). On the other hands, Tilman et al. (2002) were announced that the global use of nitrogen and phosphorus fertilizers increased by 7 and 3.5 fold, respectively, in the past six decades; both fertilizers are expected to increase further threefold by 2050. Therefore introducing new approaches to delete or decreases such problems is necessary. The positive effects of organic amendments or plant biostimulants based on humic substances is an alternative method for improvement of crop production and soil fertility maintenance (Canellas et al., 2015).

Humic and fulvic acids as plant biostimulants are mainly produced by biodegradation of lignin containing plant organic matter (Malan, 2015). Fulvic acids that are always in solution, especially at the pH of productive agricultural soils, also contribute towards cation exchange capacity of the soil (Yamauchi et al.,1984; Malan, 2015). Due to the solubility of fulvic acids in water and the fact that it can be easily leached out, it is usually only present in very low concentrations (0.2-1% w/v) in leonardite, peat, and compost etc, sources. Therefore some companies will dry fulvic acids to a powder (Malan, 2015). Fulvic acid as an organic fertilizer, is a non-toxic mineral chelating additive and water binder that maximizes uptake through leaves and stimulates plant productivity (Malan, 2015). It attracts water molecules, helping the soil to remain moist and aiding the movement of nutrients into plant roots. Fulvic acid easily binds or chelate minerals such as iron, calcium, copper, zinc and magnesium, as it can deliver this elements to plant directly (Yamauchi et al., 1984). Lotfi et al. (2015) were studied the physiological responses of *Brassica napus* subjected to fulvic acid application under water stress and found that application of fulvic acid improved the maximum quantum efficiency of PSII (Fv/Fm) and performance index (PI) of plants under both well-watered and limited-water conditions. In various studies, other effects of fulvic acid application were reported such as: enhanced root activity, increase in ion uptake, high rate of transport of phosphorus to the grains (Xudan, 1987), increasing the number and length of root hairs of Arabidopsis plants (Schmidt et al., 2005), ameliorative growth of rice and radish and higher plant height (Khang, 2011), limiting the development of some pathogens, e.g. Fusarium spp (Yigit and Dikilitaş, 2008), promote plant growth and increase marketable yield in tomato production (Suh et al., 2014), improved plant growth and yield quantity and quality as well as controlling powdery and downy mildews of cucumber plants and enhanced the activity of soil microorganism (Kamel et al., 2014), enhanced effectively the physiological activities and yield production of tomato plants, as antitranspirants via conserving soil water and thereby reduce the applied water by 25% of irrigation water (Aggag et al., 2015), improve the white rot disease resistance of grape, the quality of berry fruit and more absorption of calcium by grape (Huanpu et al., 2004), enhanced potassium levels in leaves of tobacco (Priya et al., 2014). Also Yang et al. (2013) have demonstrated that fulvic acid is optimum choice for the improvement of P availability and soil physicochemical conditions. Xudan (1987) has demonstrated that spraying with fulvic acid can decrease the water stress or the stress imposed by hot, dry winds during ear development and it can increase the grain yield by 7.3-18.0% (Xudan, 1987). Anjum et al. (2011) have reported that fulvic acid increased chlorophyll and water content of leaves. It also increased photosynthesis, reduced stomata opening status and transpirations, thus led to growth stimulation and water loss reduction (Li et al., 2005). Also they have found that fulvic acid and humic acid have been used to regulate the plant growth under well watered and drought conditions. Furthermore, fulvic acid as metabolic antitranspirations is an organic acid, nontoxic, not expensive and did not cause pollution problems as a result of extensive use (Nardi et al., 2002).

Therefore, this study were conducted to estimate the effects of fulvic acid application on seed and oil yield of safflower cultivars in semiarid condition of northwest Iran.

Materials and methods

This experiment was carried out as split split plots in a randomized complete block design with three replications at Research Field of Agriculture and Natural Resources Faculty, Islamic Azad University-Sanandaj Branch, during 2014-2015 growing season, in the northwest of Iran. In this study, the main factor was two safflower cultivars including: Sina and Faraman and subplot were foliar application of fulvic acid in 2 growing stages as: stem elongation and flowering and also sub sub plot were 3 different fulvic acid concentration including as: control (distilled water), fulvic acid (0.5 $kg^{*}ha^{-1}$) and fulvic acid (1 $kg^{*}ha^{-1}$).

Before the seed bed preparation a composite sample of soil was taken in different site of field and sent to the laboratory for measurement of physical and chemical soil properties (Table 1). The field preparation was done first by a 30 cm depth plough in May followed by disc and furrowing. Plot size was 6 m^2 (1.5*4) and contained 3 rows 4m long and 50 cm apart. The seeds were sown by hand in the row with 10 cm distance and in a depth of about 5 cm in 4 May 2015. During the growth period, the necessary operations such as irrigation and weed control was done. The fulvic acid was prepared by Fanavari Sabz Shargh Co, Tehran, Iran. It diluted with distilled water as 0.5 and 1 $kg^{*}ha^{-1}$ and foliar application was conducted at beginning of stem elongation and flowering stages. At the end of the experiment, five plants from each plot were selected and some plant growth parameters such as: head numbers, head weight, numbers of seed in head and 1,000 seed weight were recorded. In order to measure the head numbers, the total of head in five harvested plants were counted and averaged. Also the all head of five plant weighted and averaged. Than the all heads were trashed and cleaned seed counted and divided by numbers of head and numbers of seed in head was achieved. For determine 1,000 seed weight, four sample were counted and weighted. Biological yield were obtained by harvesting and weighing of plant in middle sowing rows and then the plant trashed and total weight of seeds were consider as seed yield. Harvest index were obtained by dividing seed yield by biological yield. Oilseed percent were achieved from Soxhlet Extraction apparatus. Finally data were analyzed according to the experimental design by SAS 9.1 and trait means comparisons were done according to Duncan's multiple range test method at the 0.05 probability level.

Table 1. Characteristic of soil test in the field of study

Soil depth (cm)	Clay (%)	Silt (%)	Sand (%)	EC (ds*m-1)	pH	TNV%	OM%	NT%	P (mg*kg-1)	K (mg*kg-1)
0-30	34.2	26	39.8	0.84	7.52	46.7	1.131	16	9.35	192
30-60	35.2	22	42.8	0.72	7.68	48.9	0.682	20.5	5.2	122

EC= Electrical Conductivity as decisiemens per meter, TNV= Total Neutralizing Value, OM= Organic Matter, NT= Total Nitrogen.

Results and discussion

Head numbers

Results in Table 2 reveal that head numbers were affected significantly by foliar application of fulvic acid. As seen in Table 3 the maximum head numbers were found by 0.5 $kg^{*}ha^{-1}$ fulvic acid. Numbers of head in safflower plant is the most important character were subjected to seed yield. It seems that the stimulatory effects of fulvic acid have been directly correlated with enhanced uptake of macronutrients, such as nitrogen, phosphorus, potassium and micronutrients (Priya et al., 2014; Silva et al., 2016). Also Yamauchi et al. (1984) were found that fulvic acid easily binds or chelate minerals such as iron, calcium, copper, zinc and magnesium, as it can deliver this elements to plant directly. The same results were found by other researcher. Esringü et al. (2015) were used two dose (0, 40 mg^{*} kg^{-1}) of fulvic acid and humic acid on *Impatiens walleriana,* and showed that 40 $mg^{*}kg^{-1}$ both fulvic and humic acid dose treatment was the most effective in particular on flowering and plant growth parameter (number of buds, number of main shoots, the number of secondary shoots, plant diameter, flowers number, root length, fresh root weight, dry root weight, fresh leaf weight and dry leaf weight) when compared to with control plant.

Table 2. Anova analyses of the effects of fulvic acid on safflower cultivars traits

SOV	df	Head number	Head weight	Seed in head	1,000 seed weight	Seed yield	Biological yield	Harvest index	Oil percent
Block	2	199.1*	389.2 ns	13.72 ns	22.5 ns	153,517.1 ns	486,052.7 ns	8.1 ns	106.4 ns
Cultivar	1	0.06ns	0.24 ns	235.79 *	2,312**	1,088,370.5 *	1,060,900 ns	97.5*	65.6 *
Ea	2	3.8	57.5	81.51	9.6	733,763.1	211,525	46.4	43.5
Growing stage	1	14.1 ns	1.3 ns	0.917 ns	24.8 ns	206,979.5 ns	1,006,677 ns	3.7ns	189.5 *
C * G	1	23.1 ns	20.2 ns	71.82 ns	0.61 ns	175,295.7 ns	115,600 ns	11.4ns	82.8 ns
Eb	4	70.3	65.4	11.46	32.8	568,006.8	809,405	37.3	32.1
Fulvic	2	91.8**	6.4 ns	70.23 *	16.6 ns	422,774.3 ns	29,222,919**	146.8**	271.7**
C * F	2	32.9 ns	1.1ns	27.47	11.8 ns	494,564.2 ns	502,308 ns	38.2ns	39.1 ns
G * F	2	10 ns	139.1*	9.51 ns	18 ns	27,739 ns	1,888,502 ns	4.5ns	18.3 ns
C * G * F	2	14.3 ns	148.3*	14.02 ns	11.3 ns	263,487.2 ns	1,152,658 ns	23.6ns	0.523 ns
Experiment error	16	12.1	33.4	21.98	13.96	228,818.4	965,955.5	15.9	31.8
CV (%)		13.34	14.85	27.82	10.46	18.39	7.25	20.37	37.12

**, * Significant at 1% and 5% level an arrangement, ns non-significant. SOV = Sources of Variation. CV = coefficient of variation.

Table 3. Mean comparison of the effects of fulvic acid on safflower cultivars traits

Treatment\ characters	Head number	Head weight (g)	Seed in head	1,000 seed weight (g)	Seed yield (kg^*ha^{-1})	Biological yield (kg^*ha^{-1})	HI (%)	Oil (%)
Cultivar:								
Faraman	26.16[a]	39[a]	14.28[b]	43.73[a]	2,774.1[a]	13,370.6[a]	21.21[a]	16.54[a]
Sina	26.07[a]	38.84[a]	19.4[a]	27.7[b]	2,426.4[b]	13,713.9[a]	17.92[b]	13.84[b]
Growing stage:								
Stem elongation	25.49[a]	39.12[a]	17[a]	36.55[a]	2,676[a]	13,709[a]	19.89[a]	12.9[b]
Flowering	26.74[a]	38.73[a]	16.68[a]	34.88[a]	2,524[a]	13,375[a]	19.24[a]	17.48[a]
Fulvic:								
0 (control: distilled water)	23.34[b]	38.28[a]	19.32[a]	34.55[a]	2,707.5[a]	11,805 [b]	23.09[a]	9.7[b]
0.5 kg^*ha^{-1}	28.87[a]	39.73[a]	14.49[b]	36.9[a]	2,709.7[a]	13,993[a]	19.51[b]	17.86[a]
1 kg^*ha^{-1}	26.13[ab]	38.75[a]	16.71[ab]	35.7[a]	2,383.5[a]	14,827[a]	16.09[b]	18.01[a]

Mean with the common letters in each column have not significant differences at 0.05 probability level by Duncan’s multiple range test.

Head weight

This character were affected significantly by interaction of growing stage * fulvic acid and cultivar * growing stage * fulvic acid. As shown in Figure 1 the maximum head weight was achieved in flowering stage by 1 $kg^{*}ha^{-1}$ fulvic acid, but adversely in stem elongation it had negative effect on head weight, it seems that application of fulvic acid in stem elongation were increased the number of head, hence due to competition between them, the head weight decreased.

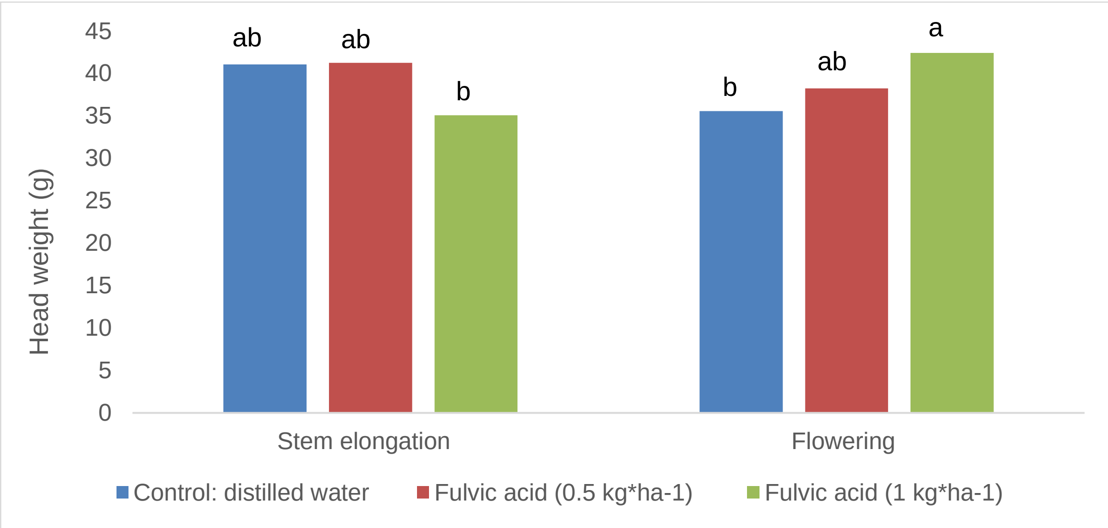

Figure 1. Mean comparison of head weight as affected by growing stage and fulvic acid

Also according Figure 2 in this study, the maximum head weight were obtained by sina in flowering stage and 1 $kg^{*}ha^{-1}$ fulvic acid. The maximum head weight in faraman were found in 0.5 $kg^{*}ha^{-1}$ and stem elongation stage. It were occurred maybe due to have some genetically different traits between cultivar such as: total growing cycle, tolerant to hot and drought, nutrient needs, etc. In an experiment, Kamel et al. (2014) were found that the foliar application of fulvic acid improved plant growth and yield quantity and quality of cucumber plants. Additionally, the yield components such as weight of fruits and mean of fruit weight /plant recorded the same values with all fulvic acid concentrations.

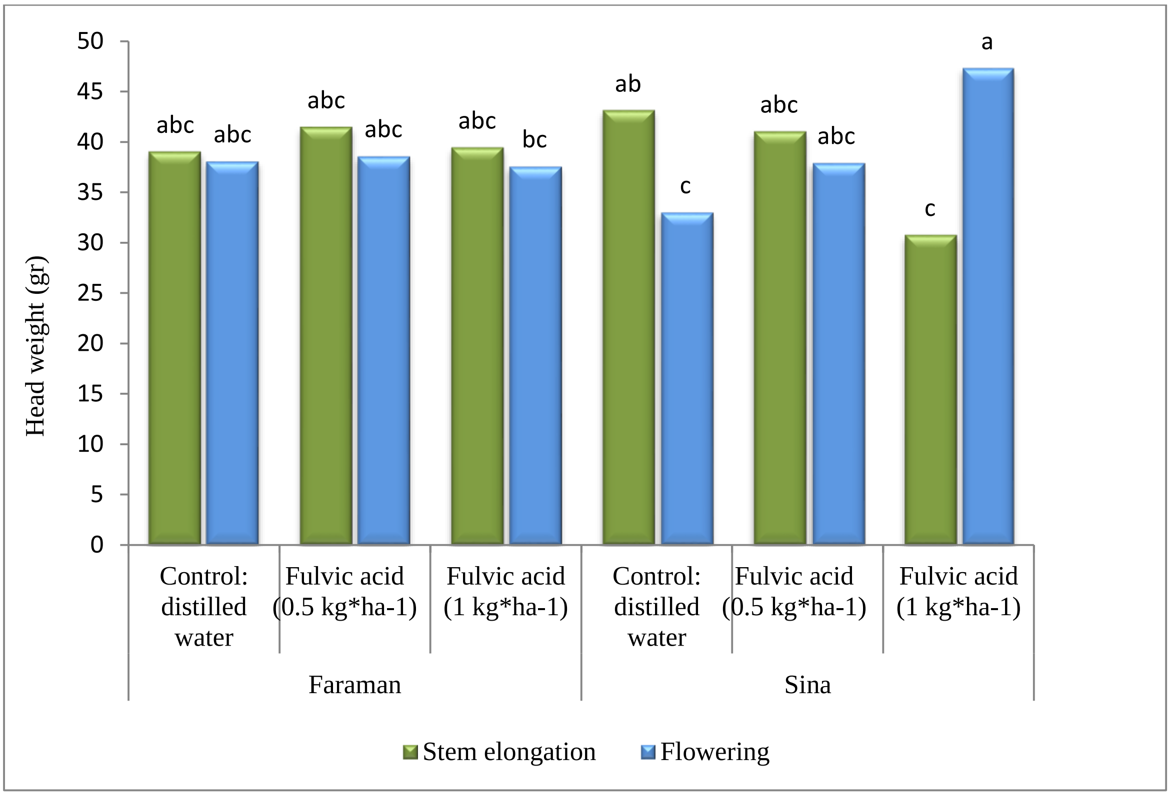

Figure 2. Mean comparison of head weight as affected by cultivar *growing stage* fulvic

Numbers of seed in head

Numbers of seed in head were different significantly by cultivar. In this study sina cultivar were produced more seed in head (Table 3). According to negative relationship between seed component in safflower and on finding in previous results about head numbers and weights it was logical. In this study numbers of seed in head were affected by fulvic acid, as shown in Table 3 the maximum seed in head were achieved by control treatment, it seems decreasing the numbers and weights of head in previous findings, results to increasing numbers of seed in head. Çopur et al. (2010) were found an increase in the number of seeds per cotton fruit with the application of gibberellin-based biostimulants.

1,000 seed weight

It affected significantly by cultivars, on the mean comparison data in Table 3, the maximum seed weight were obtained by faraman. It seems the decrease number of seed in head in faraman, induced the ameliorative effects on seed weight. Other treatment had not significant effects. Başalma (2014) concluded that the significant differences were found a positive seedling growth by treatment of seeds using 60-120 g humic acid per 100 kg seed before sowing in safflower cultivars. They indicated that seedlings of cv. Dinçer had the best response to humic acid applications compared to other cultivars. Additionally, application of 60 g per 100 kg seed humic acid dose has positive effects on seedling growth and safflower seed germination when applied before sowing.

Seed yield

The same trend was observed on seed yield of faraman, as it achieved the maximum seed yield (Table 3). The effects of fulvic acid on seed yield weren't significant. In this study in each growing stage (stem elongation or flowering) foliar application of fulvic acid carried out one time, therefore the expected effects of them weren't found, maybe if fulvic acid application were carried out in two or more times in growth stage or in higher value of fulvic acid, the positive effect of them were showed. Also maybe priming of safflower by fulvic acid could increase the seed yield as reported by Başalma (2014). Also treatment of maize with fulvic acid increased net photosynthesis, transpiration rate, the intercellular concentration of CO_2 and ameliorate plant growth (Anjum et al., 2011).

Silva et al, 2016 were studied four crop biostimulate (Booster®, Stimulate®, Improver® and Biozyme®) on cotton and found that application of biostimulants in the seeds increased the N, K, S and Fe contents in the cotton leaf, but there was no influence on the crop yield, therefore they concluded that maybe the effect of this products is localized when applied to the seed, restricted only to the crop establishment stage. Oosterhuis and Robertson (2000) also did not find any effect of foliar spraying application of growth promoters on cotton yield. However, the positive effects of fulvic acid were reported in many studies.

Li et al. (2005) concluded that falvic acid foliar spraying resulted to 7.2% increase of grain yield at the optimal concentration of fulvia acid (1.5 $mL^{*}L^{-1}$). Aggag et al. (2015) were studied the kaolin and fulvic acid as antitranspirants on tomato plants under three water regimes in the two seasons and revealed that both kaolin and fulvic acid enhanced effectively the physiological activities and yield production of tomato plants, These led to conserving soil water and thereby reduce the applied water by 25% of irrigation water. Anjum et al. (2011) reported that fulvic acid increased chlorophyll and water content of leaves. Fulvic acid also increased photosynthesis, reduced stomata opening status and transpiration, thus led to growth stimulation and water loss reduction. Zancani et al. (2011) suggested that fulvic acid applied to cell cultures of Greek fir interacted with the signaling pathway for plant hormones and increased intercellular levels of ATP and glucose-6-phosphate, physiological effects that were related to growth promotion.

Biological yield

Fulvic acid had significant effect on biological yield. The maximum biological yield were found by $1kg^{*}ha^{-1}$ fulvic acid foliar application. It seems that the positive effects of fulvic on foliage growth of safflower cultivar was famous in comparison with seed yield. Probably in case of increasing the foliar value and replication of fulvic acid and also selecting different safflower cultivars or accelerating sowing time in companion with limited irrigation, the positive effects of fulvic on seed yield were obvious. Also increases by fulvic acid have been reported in tomato plant growth above ground by Lulakis and Petsas, (1995).

Harvest index

It was affected significantly by cultivar. As faraman were obtained more harvest index. In addition to seed yield and biological yield data in Table 3 it seems that despite producing more biological yield by sina cultivar it can’t success in remobilization and transport of nutrient towards seed, hence it had lower harvest index. The effect of fulvic acid on harvest index were different significantly. In control treatment maximum value of harvest index were found, because lower biological, therefore concluded that the positive effect of fulvic acid was more on biological yield versus seed yield.

Oil percent

Oil percent were affected significant by cultivar, growing stage and fulvic acid. As shown in Table 3, the faraman cultivar were produced more oil percent. Oil percent were increased in flowering stage. On the negative relationship between numbers of seed in head and oil percent, this results is logical. In this study the maximum oil percent were obtained by 1 $kg^{*}ha^{-1}$ fulvic acid foliar application. Maybe spraying with fulvic acid could binds or chelate minerals such as iron, calcium, copper, zinc and magnesium, as it can deliver this elements to plant directly (Yamauchi et al., 1984).

These results are in accordance with those obtained by Yazdani et al. (2014), whom found higher nutrient uptake and accumulation of N, P, K, Ca, Fe and Zn in both leaves and scapes of gerbera by fulvic acid. Hendawy et al. (2015) were suggested that foliar application of humic acid or amino spot fertilizer (Algae extract) had a significant effect on essential oil percentage and oil constituents of Mint plant. They concluded that increasing nutrient absorption can induce enzyme activity and metabolism of essential oil production. They also were stated that phosphorous can activate coenzymes for amino acid production, photosynthesis, glycolysis, respiration and fatty acid synthesis. On the other hands, increasing potassium absorption by fulvic acid may affect the metabolism of N and carbohydrates and the synthesis of lipid, starch and protein as reported by Zahra et al. (1984).

Huanpu et al. (2004) showed that fulvic acid could improve the white rot disease resistance of grape, the quality of berry fruit and more absorption of calcium by grape. Suh et al. 2014 demonstrated that foliar application of fulvic acid at about 0.8 $g^{*}L^{-1}$ could be used to promote plant growth and increase marketable yield in tomato production. Priya et al. (2014) were studied several agronomic inputs such as soil amendments, fertilizer application, and plant growth regulators, and found that foliar application of fulvic acid, were one of the most bioactive humate molecules, that enhanced potassium levels in leaves of tobacco. These researchers suggested that “fulvic acid acts in a manner similar to the plant hormone auxin in tobacco, influencing expression of key genes encoding transporters and enzymes involved in potassium uptake and starch metabolism. While fulvic acid has beneficial effects on plant growth, its mechanism of action is still unclear”. Silva et al. (2016) were found that application of biostimulants in the seeds increased the N, K, S and Fe contents in the cotton leaf and they concluded that these products caused changes in the fiber characteristics, related to length uniformity, micronaire, length and strength of the fiber. Therefore, “the exogenous application of biostimulants may increase the gibberellin content of the plant, resulting in changes in fiber formation”. Çopur et al. (2010) were found an improvement in the technological quality of the cotton fiber with the application of gibberellin-based biostimulants. Also Aminifard et al. (2012) were reported that fulvic acid enhanced multiple parameters of fruit quality, including total soluble solids, antioxidant activity, total phenolics, carbohydrates, capsaicin, and carotenoids of pepper. Bocanegra et al. (2006) were concluded that “the combined capacity of fulvic acids both to chelate nutrients such as Fe and move through membranes has suggested the fulvic acids may play similar roles as natural chelators in the mobilization and transport of Fe and other micronutrients”.

Conclusion

In this study the priority of faraman cultivar in term of producing more seed yield and oil percent was obvious, as it produced 14.33 and 19.5 percent more seed yield and oil percent in arrangement. Foliar application in stem elongation stage obtained 6.02 percent more seed yield but in flowering stage, fulvic acid spraying were achieved 35.5 percent more oil percent. Finally the results showed the positive effects of foliar application of fulvic acid in 1 $kg^{*}ha^{-1}$, as it produced 85.67 percent more oil percent in comparison with control. Also following that, the 0.5 $kg^{*}ha^{-1}$ fulvic acid were obtained 84.12 percent more oil percent in versus with control. Therefore by considering oil yield in hectare (oil percent * seed yield) the maximum oil yield in hectare were achieved as: 487.03 kg in 0.5 $kg^{*}ha^{-1}$ fulvic acid.

References

Aggag, A. M., Alzoheiry, A. M., Abdallah, A. E. (2015) Effect of kaolin and fulvic acid antitranspirants on tomato plants grown under different water regimes. Alexandria Science Exchange Journal, 36 (2), 169-179. Available at http://connection.ebscohost.com/c/articles/109049784.

Aminifard, M. H., Aroiee, H., Nemati, H., Azizi, M., Jaafar, H. Z. E. (2012) Fulvic acid affects pepper antioxidant activity and fruit quality. African Journal of Biotechnology, 11 (68), 13179-13185. http://dx.doi.org/10.5897/AJB12.1507.

Anjum, S. A., Wang, L., Farooq, M., Xue, L., Ali, S. (2011) Fulvic acid application improves the maize performance under well-watered and drought conditions. Journal of Agronomy and Crop Science, 197 (6), 409- 417. http://onlinelibrary.wiley.com/doi/10.1111/j.1439-037X.2011.00483.x/abstract.

Başalma, D. (2014) Effects of humic acid on the emergence and seedling growth of safflower (*Carthamus tinctorius* L.). Turkish Journal of Agricultural and Natural Sciences, Special Issue: 2, 1402-1406. Available at http://dergipark.gov.tr/download/article-file/142280.

Bocanegra, M. P., Lobartini, J. C., Orioli, G. A. (2006) Plant uptake of iron chelated by humic acids of different molecular weights. Communications in Soil Science and Plant Analysis, 37, 1– 2. http://dx.doi.org/10.1080/00103620500408779.

Canellas, L. P., Olivares, F. L., Aguiar, N. O., Jones, D. L., Nebbioso, A., Mazzei, P., Piccolo, A. (2015) Humic and fulvic acids as biostimulants in horticulture. Scientia Horticulturae, 196, 15- 27. http://www.sciencedirect.com/science/article/pii/S0304423815301771?via%3Dihub

Çopur, O., Demirel, U., Karakuş, M. (2010) Effects of several plant growth regulators on the yield and fiber quality of cotton (*Gossypium hirsutum* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38 (3), 104-110. http://www.notulaebotanicae.ro/index.php/nbha/article/view/4588.

Dajue, L., Mundel, H. H. (1996) Safflower (*Carthamus tinctorius* L.). Gatersleben: Institute of plant genetics and crop plant research.

Esringü, A., Sezen, I., Aytatli, B., A., Ercişli, S. (2015) Effect of humic and fulvic acid application on growth parameters in *Impatiens walleriana* L. Akademik Ziraat Dergisi, 4 (1), 37-42. Available at http://azd.odu.edu.tr/files/4_1/37-42.pdf.

Gilbert, J. (2008) International safflower production - an overview. In: Australian Oilseeds Federation, 7th International safflower conference. Wagga Wagga, 3-6 November 2008. Agri-MC.

Hendawy, S. F., Hussein, M. S., El-Gohary, A. E., Ibrahim, M. E. (2015) Effect of foliar organic fertilization on the growth, yield and oil content of *Mentha piperita* var.citrata. Asian Journal of Agricultural Research, 9 (5), 237-248. http://scialert.net/abstract/?doi=ajar.2015.237.248.

Huanpu, M., Baoyan, L., Zhimin, L. (2004) Effects of fulvic acid foliar spray on growth and development of grape. Journal of Beijing Agricultural College, 19 (4), 1-3. Available at http://europepmc.org/abstract/CBA/573439.

Hussain, M. I., Dionyssia-Angeliki, L., Farooq, M., Nikoloudakis, N., Khalid, N. (2015) Salt and drought stresses in safflower: a review. Agronomy for sustainable development, 36, 1-31. Available at http://cat.inist.fr/?aModele=afficheN&cpsidt=29664479.

Kamel, S. M., Afifi, M. M. I., El-shoraky, F., El-Sawy, M. M. (2014) Fulvic acid: a tool for controlling powdery and downy mildews in cucumber plants. International Journal of Phytopathology, 3 (2), 101-108. https://www.researchgate.net/publication/310604804.

Khang, V.T. (2011) Fulvic foliar fertilizer impact on growth of rice and radish at first stage. Omonrice, 18, 144-148. Available at http://www.clrri.org/*ver2*/uploads/noidung/18-18.pdf

Li, M.S., Li, S., Zhang, B. L. C. (2005) Physiological effect of new FA antitranspirant on winter wheat at ear filling stage. Journal of Agricultural sciences in China, 11, 820-825. Available at http://caod.oriprobe.com/articles/9786136.

Lotfi, R., Pessarakli, M., Gharavi-Kouchebagh, P., Khoshvaghti, H. (2015) Physiological responses of *Brassica napus* to fulvic acid under water stress: Chlorophyll a fluorescence and antioxidant enzyme activity. The Crop Journal, 3, 434 - 439. DOI: 10.1016/j.cj.2015.05.006

Lulakis, M. D., Petsas, S. I. (1995) Effect of humic substances from vine-canes mature compost on tomato seedling growth. Bioresource Technology Journal, 54,179–182. DOI: org/10.1016/0960-8524 (95)00129-8

Malan, C. (2015) Review: humic and fulvic acids. A Practical Approach.In Sustainable soil management symposium. Stellenbosch, 5-6 November 2015, Agrilibrium Publisher.

Mündel, H. H., Morrison, R. J., Blackshaw, R. E., Roth, B. (2004) Safflower production on the Canadian prairies. Agricultural Research Stations, Lethbridge, Canada, 2004, 43 p.

Nardi, S., Pizzeghello, D., Muscolo, A., Vianello, A. (2002) Physiological effects of humic substances on higher plants. Soil Biology & Biochemistry, 34, 1527–1536. http://dx.doi.org/10.1016/S0038-0717(02)00174-8.

Oosterhuis, D. M., Robertson, W. C. (2000) The use of plant growth regulators and other additives in cotton production. In: Arkansas cotton research, extension, production and marketing group, The 2000 cotton research meeting. Helena, February 15, 2000, University of Arkansas.

Priya, B. N. V., Mahavishnan, K., Gurumurthy, D. S., Bindumadhavh, A., Ambika, P. U., Navin, K. SH. (2014) Fulvic acid for enhanced nutrient uptake and growth: insights from biochemical and genomic studies. Journal of Crop Improvement, 28,740–757. Available at http://dx.doi.org/10.1080/15427528.2014.923084.

Savci, S. (2012) Investigation of effect of chemical fertilizers on environment. In: Asia-Pacific Chemical, Biological & Environmental Engineering Society, International Conference on Environmental Science and Development. Hong Kong, 5-7 January 2012, Elsevier.

Schmidt, W., Cesco, S., Santi, S., Pinton, R., Varanini, Z. (2005) Water-extractable humic substances as nutrient acquisition signals for root hairs development in Arabidopsis. In: Hartmann, A., Schmid, M., Wenzel, W., Hinnsinger, P. (2004) Rizosphere - Perspectives and Challenges, 1171-1178, Elsevier.

Sharma, M., Gupta, S. K., Mondal, A. K. (2011) Production and trade of major world oil crops. Technological innovations in major world oil crops, 1, 1-15. Available at https://link.springer.com/chapter/10.1007%2F978-1-4614-0356-2_1.

Silva, R. D. A., Santos, J. L., Oliveira, L. S., Soares, M. R. S., Santos, S. M. S. D. (2016) Biostimulants on mineral nutrition and fiber quality of cotton crop. Revista Brasileira de Engenharia Agrícola e Ambiental, 20 (12), 1062-1066. http://dx.doi.org/10.1590/1807-1929/agriambi.v20n12p1062-1066.

Suh, H. Y., Yoo, K. S., Suh, S. G. (2014) Effect of foliar application of fulvic acid on plant growth and fruit quality of tomato (*Lycopersicon esculentum* L.). Horticulture, Environment, and Biotechnology, 55 (6), 455–461. http://dx.doi.org/10.1007/s13580-016-1004-x.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., Polasky, S. (2002) Agricultural sustainability and intensive production practices. Nature, 418, 671-677. DOI: 10.1038/nature01014.

Weiss, E. A. (2000) Oil seed Crops. 2nd ed. Oxford: John Wiley-Blackwell Publisher.

Xudan, X. (1987) The effect of foliar application of fulvic acid on water use, nutrient uptake and yield in wheat. Australian Journal of Agricultural Research, 37, 343-350. https://doi.org/10.1071/AR9860343.

Yamauchi, M., Katayama, S., Todoroki, T., Watanable, T. (1984) Total synthesis of fulvic acid. Journal of the Chemical Society, Chemical Communications. 23, 1565-1576. http://pubs.rsc.org/en/content/articlelanding/1984/c3/c39840001565#!divAbstract.

Yang, S., Zhang, Z., Cong, L., Wang, X., Shi, S. (2013) Effect of fulvic acid on the phosphorus availability in acid soil. Journal of Soil Science and Plant Nutrition, 13 (3), 526-533. Available at http://www.scielo.cl/pdf/jsspn/v13n3/aop4113.pdf

Yazdani, B., Nikbakht, A., Etemadi, N. A. (2014) Physiological effects of different combinations of humic and fulvic acid on gerbera. Communications in Soil Science and Plant Analysis, 45, 1357–1368. http://dx.doi.org/10.1080/00103624.2013.875200.

Yigit, F., Dikilitaş, M. (2008) Effect of humic acid applications on the root-rot diseases caused by *Fusarium spp*. on tomato plants. Plant Pathology, 7 (2), 179-182. DOI: 10.3923/ppj.2008.179.182.

Zahra, M. K., Monib, M., Abbdel-Al, Sh. I., Heggo, A. (1984) Significance of soil inoculation with silicate bacteria. Zentralblatt fur Mikrobiologie, 139, 349-357. https://doi.org/10.1016/S0232-4393(84)80013-X.

Zancani, M., Bertolini, A., Petrussa, E., Krajňájivá. J., Piccolo, A. (2011) Fulvic acid affects proliferation and maturation phases in *Abies cephalonica* embryogenic cells. Journal of Plant Physiology, 168, 1226-1233. http://www.suprahumic.unina.it/home/images/pdf/2011_jpp_zancani.pdf.