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Chelated Iron and Magnesium Boost Productivity and Anthocyanins Content in Calyces of *Hibiscus sabdariffa* L.

Ibrahim, O.H.M.

Department of Ornamental Plants and Landscape Gardening, Faculty of Agriculture, Assiut University
E-mail: omer.ibrahim@aun.edu.eg

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Abstract:

The present experiment was carried out to define the efficiency of foliar applications (3 times) with chelated magnesium (0, 200, 400 and 600 ppm) and iron (0, 150, 300 and 450 ppm) for improving growth, productivity and anthocyanins content in calyces of roselle. Results showed that calyces production and their contents of anthocyanins were significantly increased with increasing either Mg or Fe concentration. The combination of Mg at 600 ppm and Fe at 450 was the most effective combined treatment in stimulating number of leaves and flowers, flower weight per plant, calyces dry weight per plant, seed dry weight per plant, weight of 1000 seeds and calyces content of anthocyanins. However, calyx fresh weight attained the highest values when the medium concentration of Mg (400 ppm) combined with Fe at the highest concentration (450 ppm) was employed.

Keywords: *Roselle, foliar fertilization, pigments, vegetative growth*

Introduction

Hibiscus sabdariffa L. is a summer annual crop that belongs to family Malvaceae and commonly known as roselle. It is indigenous to tropic Africa (Kirby, 1963) and widely cultivated in Upper Egypt governorates. Roselle is cultivated mainly for its fleshy red calyces (sepals) which is the commercial plant part. Calyces are famous for the preparation of hot or cold drinks and as a source of anthocyanins which is natural food coloring pigments (Diab, 1968). In addition to anthocyanins, calyces contain gossyperin and glycol side hibiscin, which have been reported to be hypotensive lowering blood pressure and stimulating intestinal peristalsis (Da-Costa-Rocha *et al.*, 2014). Roselle seeds are also important as a source of fixed oil with antimicrobial activities (Hussin *et al.,* 1991 and Fasoyiro *et al*., 2005). In folklore medicine, roselle is a remedy for cancer, abscesses, cough and fever in addition to many other diseases (Duke, 1979).

Despite the fact that roselle plant is adopted to a wide range of soil conditions, economic production requires high sepals yield and quality and therefore the soil should be well supplied with essential minerals (Adanlawo and Ajibade, 2006). Under the arid and semiarid conditions in Egypt, it has been recommended by several authors to supply nutrients through foliar application to allow quick compensation of nutrient deficiency particularly when it is difficult for roots to uptake necessary nutrients. Also, in foliar application lesser rate of nutrients are employed avoiding the toxicity of excessive elements and preventing nutrients fixation in case of soil fertilization (Malakouti and Tehrani, 1999 and El-Fouly *et al*., 2002).

Of the nutrients strongly related to pigmentation and many other yield traits, iron and magnesium are employed in the current investigation. Magnesium is involved in protein synthesis and associated with chlorophyll pigments, for it is an important cofactor of serval enzymes involved in photosynthetic carbon fixation and metabolism (Guo *et al*., 2016). Foliar applications of magnesium have also been reported to overcome the yellowing and browning of leaves associated with magnesium deficiency (Walker and Fisher, 1957) and may therefore also affect calyces color in roselle. Recently, Nissim-Levi *et al.* (2007) noticed an increase in anthocyanins accumulation in aster flowers due to the application of higher magnesium levels which probably help increase pigments stability. In this concern, several previous studies have reported the presence of stable complexes of anthocyanins with magnesium (Kondo *et al*., 1992, Takeda *et al*., 1994 and Takeda, 2006).

Elicitation of anthocyanin production in roselle and many other plant species has been recently reported due to the application of iron by Dahmardeh *et al*. (2017) who recorded a significant increase in anthocyanins content due to the elevated concentrations of foliar iron fertilization. Gomaa *et al*. (2018) also reported that Fe foliar spray increased the growth and yield characteristics including total anthocyanins content of roselle plants. Similar results were also demonstrated by Ghatas and Mohamed (2018) on *Cymbopgon citruts*. Iron is a metal components of various enzymes and plays an important role as a functional, structural, or regulatory cofactor. Therefore, it is important in photosynthesis, in enzyme systems and respiration of plants (Tariq *et al*., 2004 and Marschner, 2012). Although anthocyanins structure doesn't contain iron, enhancement in anthocyanin production has been reported by the addition of the alternative iron source FeED-DHA. This increase in anthocyanin content could be ascribed to the greater availability of iron or through iron’s ability to inhibit anthocyanin degradation (Fang *et al*., 1999). The effects of Fe on the biosynthesis of anthocyanins starting with phenylalanine as a precursor, as well as the accumulation of individual anthocyanins have been little studied as stated by Shi *et al*. (2017).

Considering the role of both magnesium and iron in enhancing growth and anthocyanins content, the current investigation was initiated to clarify the response of roselle plants to the foliar application of Fe and/or magnesium at different concentrations.

Materials and Methods

The present study was carried out during the two successive seasons of 2016/2017 and 2017/2018 at the Floriculture Experimental Farm, Faculty of Agriculture, Assiut University, Egypt. The soil of the experimental field was analyzed according to the methods described by Jackson (1973) which revealed that the soil type was clayey loam and its properties include: pH 8.71, EC (dS/m) 1.03, organic matter 0.97%, total $CaCO_3$ 1.97%, total N 0.70%, total P 0.21% and total K 0.41%.

The experiment was 4 x 4 factorial laid out in a split-plot design with three replications. Magnesium (Mg) and Iron (Fe) employed in the current study were used as foliar sprays in the form of EDTA chelate (13%) produced by Nature SA, Greece. Magnesium was used at 4 concentrations (0, 200, 400 and 600 ppm) which were assigned to the main-plots. The sub-plots comprehended iron at four concentrations (0, 150, 300 and 450 ppm). The experimental site was prepared as recommended before sowing the seeds. Plot area was 2 x 1.5 m comprising 3 rows. In mid-April, seeds were sown in holes at a distance of 30 cm in each row and then thinned after germination (one seedling/ hole). Foliar sprays of the two nutrient elements were applied three times starting one month after planting and repeated twice at one month interval for both seasons. Control plants were sprayed with distilled water. All agricultural practices were performed as recommended during both seasons.

At the end of the growing season, data were recorded on plant height, number of branches and leaves per plant, number and weight of flowers, calyces fresh and dry weights, seed dry weight and weight of 1000 seeds. Besides, total anthocyanin content was determined in air-dried calyces according to the pH differential method described by Lee *et al.* (2005). The absorbance was recorded at 510 and 700 nm in two buffers; the first at pH 1.0 (potassium chloride, 0.025M) and the second at pH 4.5 (sodium acetate, 0.4 M). The concentration of anthocyanins was calculated as mg cyanidin 3-glucoside equivalents/g dry weight using the formula:

Total anthocyanin (mg/g DW) = (A × MW × DF × 1000) / (□ × 1)

Where A = $(A_{510\ nm} - A_{700\ nm})$ pH 1.0 − $(A_{510\ nm} - A_{700\ nm})$ pH 4.5; MW (molecular weight) = 449.2 g/mol; DF = dilution factor; 1 = cuvette path length in cm; ε = 26900 L/mol.cm which is the molar extinction coefficient for cyanidin 3-*O*-β-D-glucoside. 1000= factor to convert g to mg. The measurements were performed using a UV-visible spectrophotometer (Optizen Pop, Mecasys - Korea).

Data were subjected to statistical analysis using "F" Test (Snedecor and Cochran, 1989) and L.S.D. value for comparison according to Steel and Torrie (1982). Statistical analysis was performed using Statistix 8.1 program.

Results and Discussion

Vegetative growth

Elevated Mg concentrations caused significant augmentation in plant height and leaf number as shown in Table 1. The highest Mg concentration (600 ppm) resulted in significantly taller plants and higher leaf number than the other treatments or the control. Although Mg at 600 ppm was the most effective treatment in stimulating shoot elongation and leaf formation, it produced the least branch number. However, Mg at 200 ppm showed a pronounced increase in branch number during both seasons. The role of magnesium in enhancing vegetative growth has been previously demonstrated by Ibrahim *et al.* (2016a) on parsley plant. They reported significant effect of Mg foliar application on the vegetative growth parameters where the effect became more pronounced as the Mg concentration was increased. The results are in line with the findings of Upadhyay and Patra (2011). Similarly, Nakhumicha Muriithi *et al*. (2009) recorded an increase in tuberose leaf area and leaf dry weight in response to the application of Mg. Abou El-Nour and Shaaban (2012) also detected a significant increase in wheat plant height in response to the foliar the application of magnesium sulphate at 5000 ppm. Other authors have emphasized the enhancement influence of Mg on number of branches and leaves such as Waskela *et al*. (2013) on guava and Venkataramana (2012) on black pepper.

The promotive effect of Mg on vegetative growth may be ascribed to its contribution in photosynthesis as a carrier of phosphorus and its role in nutrient uptake, sugar synthesis and starch translocation (Abou El-Nour and Shaaban, 2012 and Marschner, 2012).

Vegetative characteristics also showed significant enhancement in response to the increase in Fe concertation reaching the peak in plant height and branch number when the medium concertation (300 ppm) was sprayed. However, roselle plants sprayed with Fe at the highest concentration (450 ppm) possessed more leaves than the other concentrations. In accordance with these results Ibrahim *et al*. (2016a) reported that foliar fertilization of parsley plants with Fe at different concentrations revealed significant effects on growth parameters. Increasing the concentration of Fe resulted in significantly better results. The role of iron in improving plant growth and quality was reported by Ibrahim *et al*. (2016b) who confirmed the significant improvement of safflower plant growth and productivity in response to the foliar application of iron at 300 ppm. Similar results were reported by Said-Al Ahl and Omer (2009) regarding the significant effect of the foliar application of iron at 200 ppm on growth of coriander plants. Also, Ghatas and Mohamed (2018) and Gomaa *et al*. (2018) reported considerable enhancement in vegetative growth of the plants sprayed with micronutrients including Fe.

Table 1. Effect of Fe and Mg concentrations on vegetative growth characters of roselle plant during 2016/2017 and 2017/2018 seasons.

Mg (ppm)	Fe (ppm)	Plant height (cm)		Branch number/plant		Leaf number/plant	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	Control	101.85	104.28	3.22	3.28	123.95	124.65
	150	121.54	124.50	3.46	3.15	168.50	170.83
	300	125.00	125.83	4.25	4.29	181.50	182.83
	450	122.50	123.40	3.48	3.54	184.12	186.71
Mean		117.72	119.50	3.60	3.56	164.52	166.26
200	Control	105.30	106.77	4.00	4.07	147.15	149.72
	150	113.00	113.67	4.45	4.52	176.50	178.83
	300	124.06	125.69	5.55	5.63	202.30	207.43
	450	128.42	129.22	5.50	5.59	209.50	211.83
Mean		117.69	118.84	4.88	4.95	183.86	186.95
400	Control	115.77	115.33	4.39	4.41	149.50	151.17
	150	123.92	126.25	3.75	3.58	184.75	186.92
	300	132.40	134.47	4.65	4.64	187.49	188.83
	450	128.60	129.53	4.25	4.27	216.60	217.53
Mean		125.17	126.40	4.26	4.23	184.58	186.11
600	Control	118.00	119.33	3.15	3.28	152.50	154.17
	150	129.00	130.67	3.49	3.60	238.35	239.45
	300	129.50	131.50	3.50	3.60	253.00	254.67
	450	131.25	132.58	4.42	4.51	299.00	300.67
Mean		126.94	128.52	3.64	3.75	235.71	237.24
Means of Fe	Control	110.23	111.43	3.69	3.76	143.28	144.93
	150	121.86	123.77	3.79	3.71	192.03	194.01
	300	127.74	129.37	4.49	4.54	206.07	208.44
	450	127.69	128.68	4.41	4.48	227.31	229.19
LSD 0.05	Mg	0.62	2.02	0.93	0.36	4.16	3.25
	Fe	1.41	2.21	0.60	0.16	5.30	4.88
	Interaction	2.82	4.41	NS*	0.32	10.60	9.75

* NS denotes non-significant differences using ANOVA

To understand the role iron in augmenting plant vegetative growth, it is important to take into consideration that iron is a metal component of various enzymes in addition to being a cofactor in many biological functions including photosynthesis, respiration and in enzyme systems of plants (Tariq *et al*., 2004 and Marschner, 2012). This beneficial effect might be due to interaction of nutrients and their role in the synthesis of IAA, metabolism of auxin and formation of chlorophyll synthesis as reported by Rathore and Tomar (1990).

When both elements were combined together, they significantly boosted vegetative growth where the combination between the highest concentrations of both Mg and Fe produced the tallest plants with the highest number of leaves. Whereas, number of branches exhibited the best behavior in those plants sprayed with the lowest Mg concentration combined with Fe at the medium or the highest concentration. In accordance with these results, Ibrahim *et al.* (2016a) proved that the best interaction between Fe and Mg was found in parsley plants sprayed with Fe at 200 or 400 ppm combined with Mg at 200 ppm. The authors ascribed the promotive effects of both Mg and Fe to the fact that plants receiving Fe and/or Mg might have been helped in terms of vigorous root growth, formation of chlorophyll, resulting in higher photosynthesis and protein which resulted in better growth.

Flowering characteristics

To elucidate the response of roselle plants to the foliar application of Mg and Fe, data on flowering characteristics are presented in Tables 2 and 3. A positive relationship was observed between the raise in Mg concentration and the incident increment in flowering characteristics. Flower number and weight showed higher values when Mg was applied at the highest concentration (600 ppm), meanwhile the medium concertation (400 ppm) was superior in case of fresh and dry weights of calyces. Similar results were obtained by Nakhumicha Muriithi *et al*. (2009) on tuberose and Harris *et al*. (2018) on chilli, who recorded enhancement effects on flower characteristics in response to the application of Mg.

Flower number and weight showed considerable responses to Mg foliar application in a similar pattern of the vegetative characteristics implying the strong influence of the vegetative growth vigor on the flowering performance. Harris *et al.* (2018) suggested an explanation based on the assumption that the higher number of flowers might be due to the adequate supply of Mg which results in increased leaf formation that leads to increase in photosynthesis activity, consequently more photosynthetic products leading to more carbohydrate accumulation which closely correlated with dense flowers and their pigments content. Street and Opik (1976) concluded that leaf number per plant affects the profusion of flowering and the processes involved in the transformation of a vegetative apex into a reproductive apex and these factors control the development of functional flowers. Marschner (2012) demonstrated that Mg plays two very essential roles in the plant which are found in the important processes of photosynthesis and carbohydrate metabolism. The role of Mg at certain concertation is closely correlated with chlorophyll synthesis, it is a constituent of chlorophyll molecule and without it, photosynthesis would not occur. Magnesium application exerted promotive influence on plant height and the production of branches that in turn increases the number of nodes and then the number of flowers. The promotive effect of Mg on vegetative growth may be ascribed to its role in nutrient uptake, sugar synthesis and starch translocation (Marschner, 2012).

Table 2. Effect of Fe and Mg concentrations on flower characters of roselle during 2016/2017 and 2017/2018 seasons.

Mg (ppm)	Fe (ppm)	Flower number/plant		Flower weight (g)/plant		Calyx FW* (g)/plant	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	Control	21.67	22.89	206.11	208.37	84.28	86.02
	150	36.00	36.50	244.75	248.68	124.53	125.36
	300	39.10	39.73	268.00	270.67	130.50	135.83
	450	58.27	41.38	282.88	284.22	138.21	145.33
Mean		38.76	35.12	250.44	252.98	119.38	123.14
200	Control	36.80	37.41	213.25	216.08	104.50	107.17
	150	48.30	48.10	261.25	262.42	137.50	140.50
	300	49.17	43.35	287.43	292.10	150.49	155.33
	450	55.59	42.59	348.80	350.36	155.70	158.33
Mean		47.46	42.86	277.68	280.24	137.05	140.33
400	Control	37.25	38.08	216.95	218.98	106.65	108.55
	150	50.66	51.56	251.30	257.10	149.25	152.08
	300	53.65	54.35	313.45	321.15	160.90	166.97
	450	50.05	51.28	354.95	359.98	164.50	168.83
Mean		47.90	48.82	284.16	289.30	145.33	149.11
600	Control	40.50	41.17	220.95	226.98	104.90	106.63
	150	52.45	52.88	280.00	283.33	153.29	160.10
	300	52.85	52.95	326.25	332.08	151.85	157.28
	450	58.45	58.48	360.50	363.50	152.45	157.82
Mean		51.06	51.37	296.93	301.48	140.62	145.46
Means of Fe	Control	34.05	34.89	214.32	217.61	100.08	102.09
	150	46.85	47.26	259.33	262.88	141.14	144.51
	300	48.69	47.60	298.78	304.00	148.43	153.85
	450	55.59	48.43	336.78	339.52	152.71	157.58
LSD 0.05	Mg	1.57	2.21	3.66	6.28	3.80	2.90
	Fe	0.74	1.21	4.49	4.10	2.97	2.94
	Interaction	1.49	2.43	8.97	8.21	5.93	5.87

* FW denotes fresh weight

The same trend aforementioned for Mg was noticed when Fe was applied where flowering characteristics showed significant gradual increase as the concertation of Fe was raised. Spraying roselle plants with the highest concertation of Fe (450 ppm) led to the best results regarding number and weight of flowers as well as fresh and dry weights of calyces (Tables 2 and 3). In case of calyx dry weight, the medium Fe concentration (300 ppm) showed non-significant differences with the highest concentration during the second season. These results are consistent with the findings of Dahmardeh *et al*. (2017) and Gomaa *et al.* (2018) who recorded significant increase in fresh and dry weights of roselle calyces in response to the iron fertilization. As previously mentioned by Tariq *et al*. (2004) and Marschner (2012), iron has an important role in photosynthesis and in enzyme systems and respiration of plants affecting all plant growth stages including the flowering stage.

The interaction between Mg and Fe concentrations exerted significant effects on flowering characteristics. The highest concentrations of both elements combined together resulted in the highest values regarding number and weight of flowers as well as calyx dry weight. However, calyx fresh weight attained the highest values when sprayed with the medium concentration of Mg combined with Fe at the highest concentration. It was also noticed that the same combined treatment was competitive and showed high values in fresh weight of flowers and calyces.

Table 3. Effect of Fe and Mg concentrations on dry weight of calyces and seed characteristics of roselle plant during 2016/2017 and 2017/2018 seasons.

Mg (ppm)	Fe (ppm)	Calyx DW* (g)/plant		Seed DW (g)/plant		Weight of 1000 seeds (g)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	Control	15.79	17.97	3.33	3.51	10.83	10.34
	150	17.13	18.02	4.40	4.61	13.65	13.82
	300	17.73	17.20	7.73	7.72	13.95	14.32
	450	17.53	15.73	7.77	8.01	15.56	15.85
Mean		17.05	17.23	5.81	5.96	13.50	13.58
200	Control	17.30	17.93	3.49	3.72	13.94	14.81
	150	21.46	21.29	4.41	4.81	13.97	14.52
	300	19.40	20.47	7.13	7.34	15.06	15.45
	450	21.81	22.04	8.03	7.94	18.12	18.04
Mean		19.99	20.43	5.76	5.95	15.27	15.71
400	Control	21.07	21.39	3.43	3.38	14.48	13.49
	150	22.35	23.12	3.95	3.78	16.05	15.35
	300	22.80	23.43	7.37	7.12	16.87	16.62
	450	24.30	24.43	8.50	8.17	17.41	18.47
Mean		22.63	23.09	5.81	5.61	16.20	15.98
600	Control	18.40	19.97	4.22	4.07	15.08	15.36
	150	19.40	20.47	4.96	4.32	15.05	14.68
	300	24.30	25.43	9.00	7.33	16.63	14.88
	450	22.65	23.88	10.50	9.83	22.11	22.04
Mean		21.19	22.44	7.17	6.39	17.22	16.74
Means of Fe	Control	18.14	19.31	3.62	3.67	13.58	13.50
	150	20.08	20.72	4.43	4.38	14.68	14.59
	300	21.06	21.63	7.81	7.38	15.63	15.32
	450	21.57	21.52	8.70	8.49	18.30	18.60
LSD 0.05	Mg	0.65	1.05	0.07	NS**	0.44	0.96
	Fe	0.32	0.59	0.07	0.30	0.29	0.52
	Interaction	0.64	1.18	0.13	0.59	0.59	1.03

* NS denotes non-significant differences using ANOVA, ** DW denotes dry weight.

Seed weight

Considering the importance of roselle seeds possessing valued active components for pharmacological uses, dry weight of seeds and weight of 1000 seeds were recorded and the data are presented in Table 3. A gradual increase in dry weight of seeds and weight of 1000 seeds was noticed with increasing Mg concentration. Thus, the concertation of 600 ppm exhibited superior results comparing to the other concentrations or the control. These results agree with those revealed by Harris *et al.* (2018) who demonstrated that Mg played an important role in increasing the number of fruits in chilli plants. Also, Babaeian *et al*. (2012) on barley and Venkataramana (2012) on black pepper proved the contribution of Mg in increasing yield.

The strong and direct relationship between the plant performance during both the vegetative and the flowering stages with seed yield is clear. As previously mentioned, foliar application of Mg at elevated concentrations resulted in consistent enhancement in vegetative growth and flowering of roselle which led ultimately to high seed yield. This interpretation was suggested as well by Harris *et al.* (2018) who revealed that the increase in the number of fruits might be due to the contribution of $MgSO_4$ in improving vegetative growth and flowering. A similar suggestion was also mentioned by Ram and Bose (2000) indicating that adequate quantity of micronutrients at the correct time is needed for greater growth which in turn leads to higher yield. Hence, the higher yield was attributed to the better flowering and higher fruit-set.

The effect of iron on both traits was also significant. When Fe concertation was increased, a consistent increment in dry weight of seeds and weight of 1000 seeds were observed. The highest values of both traits were recorded in the plants sprayed with Fe at the highest concentration (450 ppm) in both seasons (Table 3). Increment in seed weight due to the foliar application of iron has been previously pointed out by Gomaa *et al.* (2018) who reported a remarkable effect of Fe fertilization on seed yield per roselle plant. Similar results were obtained by Goos and Johnson (2000) on soybean. Likewise, Goos *et al.* (2004) and Wiersma (2005) recommended the application of FeEDDHA at high rates in order to correct chlorosis and increase seed yield.

The role of iron in the growth and reproduction of the plants is well documented (Tato *et al.*, 2013) including its important role in cell metabolism. Therefore, Fe deficiency causes malfunction in several important physiological processes which adversely affects plant growth and leaf chlorosis reducing photosynthetic efficiency. Moreover, Fe deficiency influences root, shoot, and leaf growth and decreases fruit yield (Bertamini and Nedunchezhian, 2005, Álvarez-Fernández *et al.*, 2011 and Tato *et al.*, 2013).

Data presented in Table 3 also indicate that the combined treatments between Mg and Fe concentrations significantly varied in terms of dry weight of seeds and weight of 1000 seeds. The combination between the highest concentrations of both elements produced the best results in both traits.

Total anthocyanins content

One of the most important traits strongly affects the quality and therefore the price of roselle calyces is their content of anthocyanins. Data illustrated in Figs 1 exhibit significant improvements in anthocyanins content due to the foliar application of magnesium comparing to the control. In comparison with low Mg concentration, both the medium and the highest concentrations (400 and 600 ppm, respectively) exerted better results without significant differences between them in both seasons. Nissim-Levi *et al.* (2007) noticed an increase in anthocyanin accumulation in aster flowers due to higher magnesium levels. They attributed these results to the role of magnesium application in increasing stability of plant pigments. In this concern, several previous studies have reported the presence of stable complexes of anthocyanins with magnesium (Kondo *et al*., 1992, Takeda *et al*., 1994 and Takeda, 2006). Nissim-Levi *et al*. (2007) tested the effect of $Mg(NO_3)_2$ treatment on a variety of plants and pigmented plant organs and have shown that the effect on pigmentation is broad. Shaked-Sachray *et al*., (2002) recorded an increase in anthocyanin concentration of aster flowers treated with magnesium On the other hand, Nakhumicha Muriithi *et al.* (2009) disclaimed the increased accumulation of Mg in tuberose tissues in response to supplying magnesium, and may not lead to accumulation of anthocyanins in the florets.

Due to the nature of anthocyanins owing enormous variation in location, timing, and inducibility, it is tricky to generate a unified explanation for the presence of these pigments (Hatier and Gould, 2009). To find out an acceptable explanation for the increment of anthocyanins content in response to the high concentrations of Mg, we should take into account the previously discussed vegetative, flowering and seed yield traits. All these characteristics positively responded to the raise in Mg concentration which is reasonably justifiable in view of the importance of magnesium to plants for protein synthesis and chlorophyll pigments, acting mainly as a cofactor of several enzymes required for photosynthetic carbon fixation and metabolism (Guo *et al*., 2016). Anthocyanin-magnesium complexes, if formed, may increase the half-life time of the pigments, and/or inhibit their catabolism, and accordingly improve flower pigmentation (Shaul *et al*., 1999). In the same context, Nissim-Levi *et al*. (2007) hypothesized that the effect of Mg on pigmentation is due to the direct accumulation of Mg^{2+} ions in the colored plant organs.

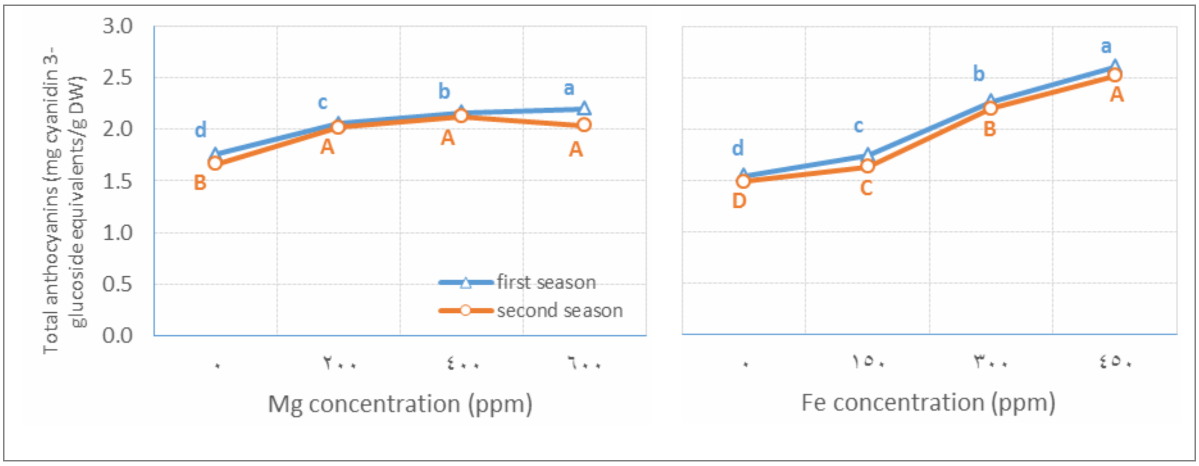

Fig. 1. The variations in total anthocyanins content in calyces of roselle plants under the foliar applications with Mg or Fe at different concentrations during 2016/2017 and 2017/2018 seasons. Different letters indicate significant differences using LSD at $p \leq 0.05$, small and capital letters are assigned for first and second seasons, respectively.

The raise in iron concentration led to a consistent increase in calyx content of anthocyanins as the best results were attained when the highest Fe concentration (450 ppm) was applied (Fig. 1). Elicitation of anthocyanin production in roselle and many other plant species has been reported due to the application of iron by several authors such as Dahmardeh *et al*. (2017) who recorded significant increase in anthocyanins content due to the elevated concentrations of foliar iron fertilization. Gomaa *et al*. (2018) also reported that foliar spray of iron increased the growth and yield characteristics including total anthocyanins content of roselle plants especially highest concentration in both seasons. Similar results were also demonstrated by Ghatas and Mohamed (2018) on *Cymbopgon citruts*.

Effect of iron on anthocyanins content is consistent with that on the vegetative, flowering and seed yield characteristics. Being a metal components of various enzymes or as functional, structural, or regulatory cofactors, Fe is important in photosynthesis and in enzyme systems and respiration of plants (Tariq *et al*., 2004 and Marschner, 2012). Although anthocyanins structure doesn't contain iron, enhancement in anthocyanin production has been reported by the addition of the alternative iron source FeED-DHA. This could be interpreted based on the greater availability of iron in a high light environment or through iron's ability to inhibit anthocyanin degradation (Fang *et al*. 1999). The effects of Fe on the biosynthesis of anthocyanins starting with phenylalanine as a precursor as well as the accumulation of individual anthocyanins have been little studied as stated by Shi *et al*. (2017).

Statistically, there were significant differences among the combined treatments between Mg and Fe at various concentrations in terms of anthocyanins content as illustrated in Fig 2. The combination between the highest Fe concertation and any of the three concentrations assigned for Mg showed significant superiority over the other treatments.

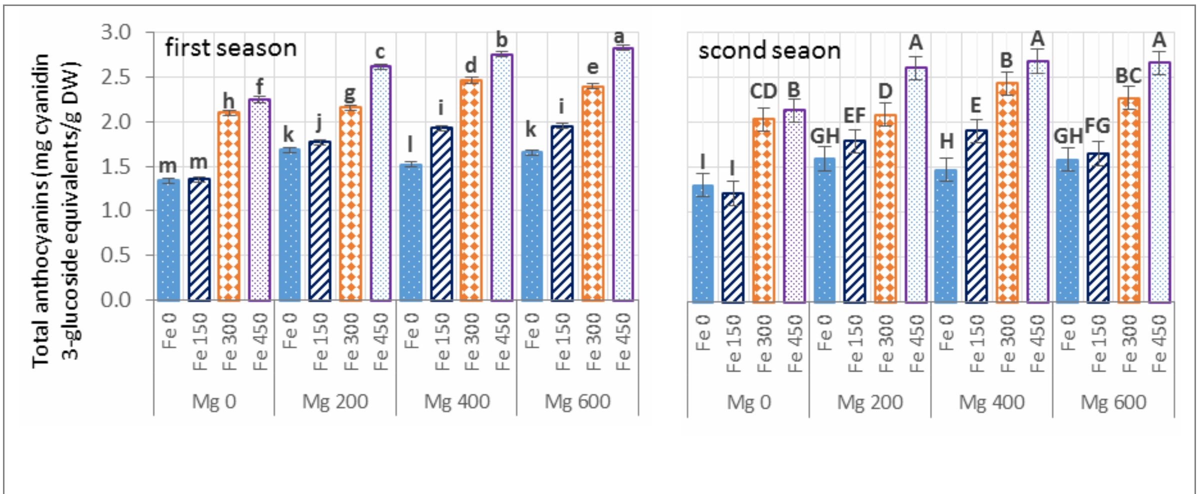

Fig. 2. The variations in total anthocyanins content in calyces of roselle plants in response to the combined treatments between different concentrations of Mg and Fe foliar applications during 2016/2017 and 2017/2018 seasons. Vertical bars above mean denote LSD values ($p \leq 0.05$), Different small and capital letters indicate significant differences in the first and second seasons, respectively.

Conclusions

Despite the close relation between anthocyanin concentration with the Mg application and also the roles of Mg in plant metabolism encouraging in one way or another the accumulation of these pigments, there has been limited number of studies addressing the significance of Mg for the quality of roselle plant comparing to other major nutrients. Hence, the results revealed by the current study is considered important which indicated that improvement of roselle growth and productivity as well as calyces content of anthocyanins is attainable by the foliar application of Mg at 600 ppm combined with Fe at 450 ppm.

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الماغنسيوم والحديد المخلبي يحسن إنتاجية الكركديه ومحتوى الكؤوس من الأنثوثيانينات

عمر حسني محمد إبراهيم

قسم نباتات الزينة وتنسيق الحدائق، كلية الزراعة ، جامعة أسيوط

الملخص

أجريت هذه الدراسة لتحديد فاعلية الرش الورقى (ثلاث مرات) بكل من الماغنسيوم المخلبي (صفر، ٢٠٠، ٤٠٠ و٦٠٠ جزء في المليون) والحديد المخلبي (صفر، ١٥٠ ، ٣٠٠ و ٤٥٠ جزء في المليون) في تحسين النمو وإنتاج الكؤوس ومحتواها من الأنثوثيانينات لنبات الكركديه. وأظهرت النتائج أن الإنتاج والأنثوثيانينات قد زادت معنوياً بزيادة تركيز أي من الماغنسيوم والحديد. كما دلت النتائج على أن المعاملة المشتركة (ماغنسيوم بتركيز ٦٠٠ جزء في المليون + حديد بتركيز ٤٥٠ جزء في المليون) أظهرت أفضل المواصفات الخضرية (عدد كل من الفروع والأوراق) والزهرية (عدد الأزهار، وزن الأزهار لكل نبات، والوزن الجاف للكؤوس لكل نبات) والبذرية (الوزن الجاف للبذور لكل نبات ووزن الألف بذرة) ، بالإضافة إلى محتوى الكؤوس من الأنثوثيانينات. فى حين أن الوزن الطازج للكؤوس قد حقق أفضل النتائج عند الرش بالتركيز المتوسط من الماغنسيوم (٤٠٠ جزء في المليون) بالاشتراك مع الحديد بأعلى تركيز.