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Research Article

Physiological Role of Iron Chelators and/or Arginine for Improving Yield and Active Constituents of Roselle Sepals

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Abstract

Background and Objective: Roselle (*Hibiscus sabdariffa* L.) plants are grown in Africa, South east Asia, Central America, it is known as Karkadeh (Egypt). Many active constituents present in Roselle have potential health benefits and support its medicinal uses. The target of this investigation was to evaluate the physiological function of ferric ethylenediaminetetraacetic acid-iron chelators (Fe-EDTA) or hemin and/or arginine for improving yield and nutritional value of sepals of roselle plant grown under the reclaimed sandy soil. **Materials and Methods:** A field experiment was conducted in 2016 and 2017 summer successive seasons at the experimental farm, National Research Centre, Al Nubaria District El-Beheira Governorate, Egypt. The foliar application separately or in combination with arginine (at 50 g L^{-1}), Fe-EDTA (50 and mg L^{-1}) and/or hemin at (50 and 100 mg L^{-1} of treatments were practiced twice at 45 and 60 days after planting. **Results:** Foliar treatment of Fe-EDTA, hemin and arginine alone or mixture of them with different concentrations, significantly increased all yield parameters, non-photosynthetic pigments as anthocyanin, lycopene and β-carotene, flavonoids, radical scavenging activity as 1,1-diphenyl-2-picrylhydrazyl (DPPH), as well as all sugar fractions, total soluble sugars, total carbohydrates, macro and microelements of roselle plants as compared with control (untreated) plants. It also showed that most treatments decreased significantly in total acidity and sodium (Na) contents of roselle sepals, when compared with untreated plant. **Conclusion:** Foliar application with arginine, Fe-EDTA and/or hemin separately or in a combination of roselle plants could be increase all active constituents as compared with untreated plants grown under new reclaimed sandy soil.

Key words: Roselle, arginine, hemin, anthocyanin, acidity, plant growth, sandy soil

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hibiscus sabdariffa L. (Roselle) be a member of family of Malvaceae. It has different names in different countries. In Egypt it Known with the name of "Karkadeh"[1]. Roselle is originated from West Africa and widely cultivated in Tropical Africa, Sudan, Egypt, Ethiopia[2]. The plants can success on a wide range of soil conditions. It can permit relatively high temperature via the growing and fruiting periods[3]. In this connection, Aziz *et al.*[4] concluded that, roselle is suitable for cultivation in Egypt across the country, where, the southern areas are the most fitting for its planting, and added the newly reform sandy soils are fit for plant growth, which are able to grow healthy under several climatic circumstances.

The purplish sepals (calyx and epicalyx) of *Hibiscus sabdariffa* L. are the most important commercial parts of the plant which is used in human nutrition and beauty product industries as a raw material of natural coloring catalyst[5]. The brilliant red color associated with surprising flavor and other organoleptic attributes make them precious food products[6] such as wine, syrup, jam, ice cream, pies, jelly, hot and cold beverages, snakes, tarts and flavoring agent's preparation[7].

The importance of roselle calyces comes because it contains high active constituents' values, as anthocyanins, polysaccharides and its fractions, flavonoids and organic acids[8,9]. As well as increase nutritional value. So, Roselle considered an important medicinal plant for developing countries due to its easy growth and has multipurpose uses as food, fiber source and all vegetative parts of roselle is used as traditional medicine for the treatment of several diseases[10]. So, many medicinal implementations of different roselle parts have been reported in several countries of the world[11]. Several reports listed proved the traditional health benefits of roselle extract and encourage the ethnomedicinal use of it in preventing hypertension, elevate cardio-vascular health, febricity and liver disturbance, microorganism growth boundary, as well as a diuretic, digestive and calmative[12]. The red diversity of roselle has antioxidant activity and cyclooxygenase inhibitory and inters in pharmaceutical and beauty product industries[13].

Long-time ago, and according to 2030 vision of Egypt, improving plant productivity (quantitative and/or qualitative) is the main purpose in good agriculture practices (GAP). The opinion of GAP has progressed to address the concerns of different researchers, stakeholders, share owners and farmers about food manufacture and security, food integrity, value and the environmental sustainability of agriculture[14]. So, using different agricultural practices, cultural practices, natural fertilization and treatment with natural antioxidants as amino acids, vitamins, natural growth regulators and micronutrients considered the most effective applications for that purpose. Availability of nutrients is a limiting factor affecting plant growth and productivity, nitrogen is one from essential elements for crop productivity. Amino acids could affect directly or indirectly on the different physiological processes in plant cells. It can be involved in enzymes associated with photosynthesis, chelating effect on micronutrient absorption and transportation in plant[15]. Nitrogen is a determinant factor for plant proliferation in several habitats. Nitrogen in large amounts is required to synthesize nucleic acids and proteins. Arginine has the highest N/C ratio, which makes it fit for organic nitrogen store[16].

Arginine metabolism acts a vital role in plant recognition and acclimation to unfavorable environmental conditions[17]. Also, arginine responsible for formation for polyamines (putrescine, spermidine and spermine) as a by-product which is essential for growth, differentiation of plants at normal or stressed conditions[16]. They also added that polyamines are concerned with multiple physiological reactions, it could be expedient for plants to slow down arginine catabolism. Recently Wuddineh *et al.*[18] reported that, the potential benefits of PAs application in improving plant patience to biotic and abiotic environmental stresses as crucial tools for planned plant boost strategies.

Application of EDTA with different concentrations is recommended on plants at functioning stages (flowering and fruiting)[19]. Foliar treatment of minerals nutrient is an effective mean for supplying nutrients for the plant. Iron (Fe) is a stimulator for more than 140 enzymes that catalyze distinctive biochemical reactions[20]. Iron is one from vital plant micronutrients that is considered a key for most metabolic reactions in plant cells, especially for chlorophyll biosynthesis consequently, plant growth and productivity; therefore, its deficiency strongly affect plant growth and development[21] Fe-EDTA as an example of iron chelate compounds which easily available and assimilates for plants. In this connection, Mishra *et al.*[22] reported that foliar treatment with Fe^{2+} as Fe (SO_4) H_2O increased seed and fruit yields of chickpea and kinnow plants, respectively. Most of the Fe-EDTA studies have concerned with their effects on fruit weight and size, firmness, dry matter, sugar concentration, acidity, phenolic compounds and vitamin C[23].

Fruit colors depend on anthocyanins which are involved in several biological roles in plants such as protective effects, antioxidant agents and pathogen invader[24]. So, perceive Fe effect on the anthocyanin metabolism is important for optimizing anthocyanin value in fruits. Plant physiologists and agricultural scientists are focusing on iron and discovering more exciting applications in the development of bio-factories[25].

Several researchers focused on hemin as a source of iron and have hormone-like effects on tomato[26] or act as plant growth regulators in facing salinity stress[27].

So, the target of this investigation was to evaluate the physiological function Fe-EDTA or hemin and/or arginine for improving yield and nutritional value of sepals of roselle plant grown under the reclaimed sandy soil.

MATERIALS AND METHODS

Plant material and growth conditions: A field experiment by the randomized complete block design in split-plot arrangement with 3 replications was conducted in 2016 and 2017 summer seasons at the experimental farm, National Research Centre, Al Nubaria district El-Beheira Governorate, Egypt. The soil of both experimental sites was reclaimed sandy soil where mechanical and chemical analysis according to Chapman and Pratt[28] is reported in Table 1.

Seeds of Roselle *Hibiscus sabdariffa* L. (dark colored) cultivar were obtained from Agricultural Research Centre Giza, Egypt. Roselle seeds were assigned to the main plots while the foliar application concentrations of treatments were practices twice at 45 and 60 days after planting and recorded as such:

- Control - Hemin 50 mg L^{-1}-Hemin 100 mg L^{-1}
- Fe-EDTA 50 mg L^{-1}-Fe-EDTA 100 mg L^{-1}-Arginine 50 mg L^{-1}
- Arginine 50 mg L^{-1}+Hemin 50 mg L^{-1}-Arginine 50 mg L^{-1} +Hemin 100 mg L^{-1}
- Arginine 50 mg L^{-1}+Fe-EDTA 50 mg L^{-1}-Arginine 50 mg L^{-1}+Fe-EDTA 100mg L^{-1}

Roselle (*Hibiscus sabdariffa* L.) seeds were sown on the 7th May in both seasons. The treatments were arranged in the subplots. Each sub-plot area was in rows 3.5 m long and the distance between rows was 20 cm apart. The plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). The recommended agricultural practices of growing roselle seeds were applied and the seeding rate was (140 kg seeds ha^{-1}). Pre-sowing, 360 kg ha^{-1} of calcium super-phosphate (15.5% P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of 180 kg ha^{-1} was applied at 5 equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52% K_2O) was added at 2 equal doses of 120 kg ha^{-1}, before the 1st and 3rd irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days.

At harvest, the following characters were recorded on random samples of 10 girded plants in each plot to estimate the following characters: The number of pods/plants, sepals' yield/plant (g), Fruit yield /plant (g) biological fruit and sepals yield (kg ha^{-1}).

Chemical analysis

The titratable acidity: The titratable acidity of sepals was determined according to AOAC[29].

Phenylalanine ammonia-lyase and tyrosine ammonia-lyase: The extraction and assay of phenylalanine ammonia-lyase (PAL, EC, 4.3.1.1) and tyrosine ammonia-lyase (TAL, EC, 4.3.1), enzyme activities were carried out according to the method adopted by Beaudoin-Egan and Thorpe[30].

Anthocyanin: Anthocyanin was extracted and measured according to Mirecki and Teramura[31].

Table 1: Mechanical, chemical and nutritional analysis of the experimental soil

Parameter	Values
Mechanical analysis	
Sand	
Coarse 2000-200 μ (%)	47.46
Fine 200-20 μ (%)	37.89
Silt 20-0 μ (%)	12.66
Clay<2 μ (%)	4.48
Soil texture	Sandy
Chemical analysis	
pH 1:2.5	7.60
EC dS m^{-1}	0.15
$CaCo_3$	5.4
OM%	0.23
Soluble cations meq L^{-1}	
Na^+	0.97
K^+	0.23
Mg^+	0.92
Ca^{++}	0.9
Soluble anions meq L^{-1}	
CO_3^-	0.0
HCO_3^-	1.05
Cl^-	0.68
SO_4^-	0.69
Nutritional analysis	
Available nutrients	
Macro element ppm	
N	52
P	12.4
K	72
Micro element ppm	
Zn	0.12
Fe	2.11
Mn	0.8
Cu	0.08

β- carotene and lycopene: The β-carotene and lycopene were determined according to the method of Nagata and Yamashita[32].

Flavonoids: The total flavonoid content was determined following the spectrophotometric method of Dewanto *et al.*[33].

DPPH radical scavenging capacity assay: The free radical scavenging activity was determined as 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to Liyana-Pathiranan and Shahidi[34].

The extracted soluble sugars (Sugar fractions): It was analyzed using HPLC according to the method of Oefner *et al.*[35], standard of galacturonic acid (Gal.), glucose (Glu.), rhamnose (Rham.), mannitol (Mannit.), Arabinan (Arab.) and Xylose (Xyl.) were used.

Total carbohydrates: It determined using the colorimetric method described by Herbert *et al.*[36].

Macro and microelement contents: Macro and microelement contents of the yielded sepals were determined according to Chapmen and Pratt[28], using a Spekol spectropolarimeter (VEB Carl Zeiss, Jena, Germany), flame photometer and atomic absorption spectrophotometer.

Statistical analysis: All data collected were analyzed with analysis of variance (ANOVA) Procedures using the Co-Stat Statistical Software Package. Differences between means were compared by LSD at 5% level of significance by Gomez and Gomez[37].

RESULTS

Changes in yielded fruits and sepals: Foliar treatment of Fe-EDTA, hemin and arginine alone or mixture of arginine+Fe-EDTA or arginine+hemin with different concentrations, increased number of pods/plant, sepals yield/plant (g), fruits yield/plant (g), biological fruit yield and sepals yield (kg ha^{-1}) of roselle plants significantly as compared with control (untreated plants) (Table 2). Increasing concentration of hemin or Fe-EDTA increased gradually with significant differences (at high concentration) all studied yield components of roselle sepals as compared with untreated plants. Also, foliar treatments with mixture of Arg+Fe-EDTTA or Arg+hemin with different concentrations were more potent than Arg, Fe-EDTA or hemin alone. Data clearly show that Arg+100 mg L^{-1} hemin was more effective than Arg+50 mg L^{-1} hemin as well as, Arg+100 mg Fe-EFTA were more effective than Arg+50 Fe-EDTA. The maximum yield was obtained with the Fe-EDTA 100+Arginine 50 treatment.

Changes in total acidity of roselle sepals: Figure 1 presents the effect of different concentrations of hemin, Fe-EDTA and/or arginine treatments on total acidity and total anthocyanin of roselle sepals. Results showed that either hemin or Fe-EDTA treatment with different concentrations decreased significantly total acidity of roselle sepals, meanwhile, arginine treatment with 50 mg L^{-1} treatment increased significantly total acidity as compared with untreated control. The results also showed that the interaction between arginine with different concentrations of hemin or Fe-EDTA decreased total acidity significantly except 50 mg Fe-EDTA+50 mg L^{-1} arginine which records non-significant increases as compared with control plant. In the meantime, the results showed gradual significant increases in the total acidity in response to adding arginine to different concentrations of either hemin or Fe-EDTA as compared with corresponding treatments with hemin but these increments still significantly less than application of arginine alone.

Changes in the phenylalanine ammonia-lyase (pal) and tyrosine ammonia-lyase (tal): Results in Table 3 cleared the activities of PAL and TAL enzymes in roselle leaves increased significantly in response to all treated plants with either

Table 2: Effect of hemin, Fe-EDTA and/or arginine on yield components of roselle plant grown under reclaimed sandy soil

Treatment	No. of pods/plant	Sepals yield/plant (g)	Fruit yield/plant (g)	Biological fruit yield (kg ha^{-1})	Biological sepals yield (kg ha^{-1})
Control	8.33±0.882	1.30±0.013	11.49±1.079	387.58±27.695	47.26±1.117
Hemin50	11.00±0.577	2.26±0.009	17.76±1.255	448.42±32.218	57.07±4.766
Hemin 100	13.00±0.577	4.86±0.023	25.80±0.609	601.82±15.630	113.38±3.341
Fe-EDTA 50	13.67±1.856	4.46±0.032	24.27±3.161	576.98±81.130	106.03±21.168
Fe-EDTA 100	15.00±2.309	4.83±0.002	25.75±3.593	702.46±92.221	131.76±18.536
Arginine 50	11.33±0.882	4.50±0.033	21.89±0.790	532.63±20.268	109.49±22.374
Hemin 50+Arginine 50	15.00±3.000	5.29±0.018	22.82±4.724	664.84±21.254	141.17±35.436
Hemin 100+Arginine 50	20.00±1.000	7.02±0.049	36.49±1.943	803.11±49.875	154.51±21.955
Fe-EDTA 50+Arginine 50	15.33±0.577	6.03±0.042	28.40±1.037	564.10±26.627	130.78±17.411
Fe-EDTA 100+Arginine 50	22.00±0.667	9.42±0.033	38.13±1.969	838.13±50.549	207.24±4.234
LSD at 0.05	4.26	0.85	2.06	2.06	2.67

Each value represents the mean of 3 replicates±standard error

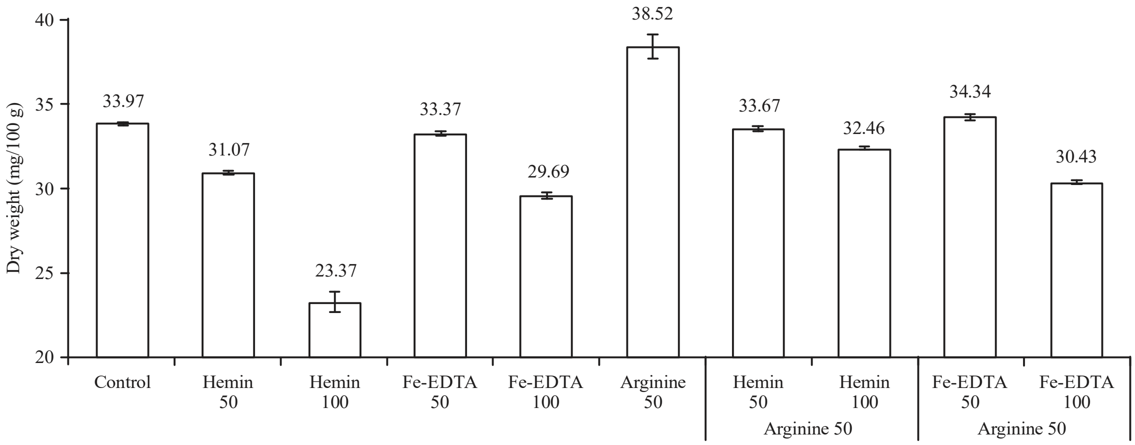

Fig. 1: Effect of hemin, Fe-EDTA and/or arginine on total acidity (mg/100g air-dry weight) on sepals of roselle plant grown under reclaimed sandy soil

Each value represents the mean of 3 replicates±standard error (LSD at 5% = 0.51)

Table 3: Effect of hemin, Fe-EDTA and/or arginine on PAL and TAL enzyme activities on leaves of roselle plant grown under the reclaimed sandy soil

Treatments (mg L^{-1})	PAL activity (µg t-cinnamic acid g^{-1} f. wt. min^{-1})	TAL activity (µg p-coumaric acid g^{-1} f. wt. min^{-1})
Control	1.440±0.0462	4.605±0.141
Hemin 50	2.145±0.0029	5.905±0.026
Hemin 100	2.575±0.0433	6.385±0.020
Fe-EDTA 50	2.081±0.0173	5.380±0.017
Fe-EDTA 100	2.365±0.0260	6.085±0.038
Arginine 50	2.465±0.0260	6.344±0.046
Hemin 50+Arginine 50	2.765±0.0491	5.295±0.032
Hemin 100+Arginine 50	2.901±0.0346	6.232±0.012
Fe-EDTA 50+Arginine 50	2.730±0.0115	6.485±0.017
Fe-EDTA 100+Arginine 50	2.712±0.0289	6.735±0.009
LSD at 0.05	0.270	0.390

PAL: phenylalanine ammonia-lyase, TAL: Tyrosine ammonia-lyase, each value represents the mean of three replicates±standard error

hemin, Fe-EDTA and/or arginine increased as compared with control plant. The highest activities are recorded at treatment with hemin (100 mg L^{-1}) + arginine (50 mg L^{-1}) and Fe- EDTA (100 mg L^{-1})+arginine (50 mg L^{-1}) at PAL and TAL with percent of increase reached to 101.45 and 46.25%, respectively compared to untreated plants.

Changes in anthocyanins, lycopene, b-carotene and total flavonoid of roselle sepals: With respect to all tested flavonoid pigments (anthocyanins, Lycopene, B- Carotene and total flavonoids) contents, different treatment of hemin, Fe-EDTA and/or arginine increased them significantly at roselle sepals as compared with the untreated plant (Table 4). Data clearly showed that Fe-EDTA (50 mg L^{-1}) followed by Hemin (100 mg L^{-1}) alone or in combination with arginine were more effective than other concentrations which caused significant increases in anthocyanin. Moreover, data showed that the combined effects of hemin (100 mg L^{-1}) or Fe-EDTA with arginine were more effective than each of them alone at most studied parameters.

Changes in antioxidant activity in yielded roselle sepals: Data in Fig. 2, showed that foliar application of the different concentrations of hemin, Fe-EDTA and/or arginine treatments induced significant increases in the antioxidant activity (as DPPH- radical concentrations (50 or 100 mg L^{-1}) alone or in combination with arginine recorded the higher values as compared with all other treatments. The highest value was obtained at 100 mg L^{-1} Fe EDTA alone.

Changes in sugar fractions, TSS and total carbohydrates in roselle sepals: Data in Table 5 recorded the values of sugar fractions in roselle sepals affected by different concentrations of hemin, Fe-EDTA alone or in combination with arginine grown under the reclaimed sandy soil. Results showed that, except Mannitol (Man), all other tested sugars (Gal, Glu, Rha, Man, Arab, TSS and T Carb) increased as the results of all treatments as compared with untreated plants. The combination of arginine and different concentrations of hemin or Fe-EDTA surpassed other single treatments in all mentioned tested sugars.

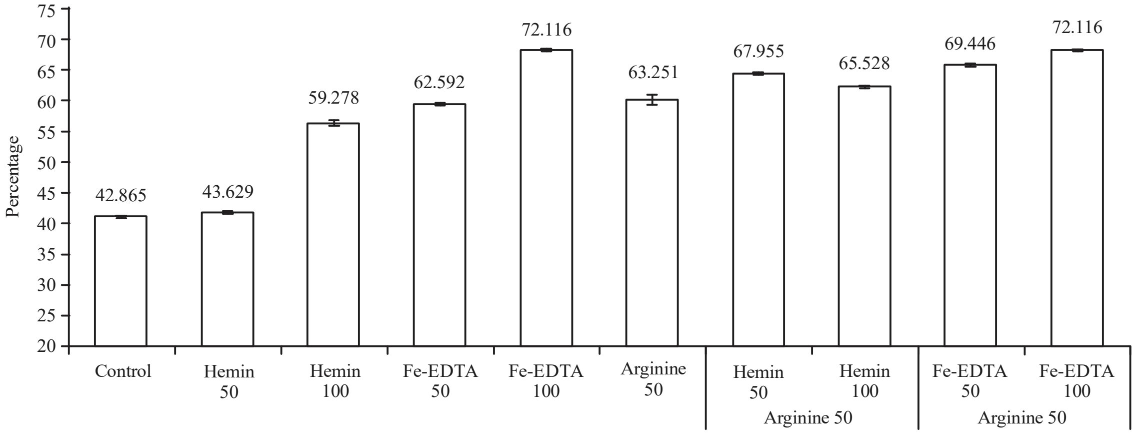

Fig. 2: Effect of Hemin, Fe-EDTA and/or arginine on radical scavenging capacity as 1,1-diphenyl-2-picrylhydrazyl (DPPH%) on sepals of Roselle plant grown under reclaimed sandy soil

Each value represents the mean of 3 replicates±standard error (LSD at 5% = 3.17)

Table 4: Effect of hemin, Fe-EDTA and/or arginine on anthocyanin, lycopene, β-carotene and flavonoids (mg/100g air-dry weight) on sepals of roselle plant grown under reclaimed sandy soil

	Air dry weight (mg/100 g)			
Treatments (mg L^{-1})	Anthocyanin	Lycopene	β-carotene	Flavonoids
Control	325.85±4.705	0.106±0.0017	0.34±0.0003	2949.60±25.81
Hemin 50	391.50±2.021	0.127±0.0017	0.52±0.0006	3477.90±64.70
Hemin 100	562.08±20.785	0.137±0.0012	0.62±0.0017	3858.90±35.30
Fe-EDTA 50	656.10±34.064	0.223±0.0007	0.68±0.0009	3466.10±250.30
Fe-EDTA 100	533.04±61.199	0.154±0.0015	0.74±0.0012	3678.70±33.94
Arginine 50	434.00±8.660	0.233±0.0009	0.64±0.0029	3181.50±52.42
Hemin 50+Arginine 50	592.20±30.022	0.178±0.0012	0.66±0.0006	3171.40±134.91
Hemin 100+Arginine 50	720.00±15.011	0.272±0.0032	0.69±0.0015	3342.60±80.90
Fe-EDTA 50+Arginine 50	626.50±3.753	0.209±0.0017	0.61±0.0032	3510.80±40.42
Fe-EDTA 100+Arginine 50	732.00±1.764	0.336±0.0009	0.58±0.0020	3670.60±66.55
LSD at 0.05	80.33	0.005	0.004	30.27

Each value represents the mean of 3 replicates±standard error

Table 5: Effect of hemin, Fe-EDTA and/or arginine on sugar fractions galactose, glucose, rhamnose, mannitol, arabinan and xylose (g /100 g air-dry weight), total soluble sugars (TSS) and total carbohydrates (TCA) of roselle sepals grown under reclaimed sandy soil

	Dry weight (g/100 g)						Air-dry weight (g/100 g)	
Treatments (mg L^{-1})	Galactose	Glucose	Rhamnose	Mannitol	Arabinan	Xylose	Total soluble sugar (TSS)	Total carbohydrates (TCA)
Control	35.52	5.35	0.35	0.75	1.15	0.65	46.09±0.069	61.94±0.242
Hemin 50	36.98	6.35	1.52	1.68	1.34	0.86	51.05±0.121	62.31±0.027
Hemin 100	38.47	6.34	1.68	1.78	1.35	0.75	52.69±0.115	63.50±0.043
Fe-EDTA 50	37.24	7.35	0.69	0.75	1.32	0.38	50.09±0.127	62.45±0.173
Fe-EDTA 100	40.65	7.85	1.35	1.68	1.38	0.74	55.97±0.067	64.37±0.301
Arginine 50	39.87	7.68	0.5	0.75	1.26	0.65	53.03±0.173	63.14±0.031
H 50+Arginine 50	41.52	8.65	1.45	1.35	1.38	1.85	58.52±0.115	67.37±0.163
H 100+Arginine 50	45.35	7.35	1.75	2.14	1.74	1.35	62.12±0.133	69.89±0.010
Fe-EDTA 50+Arginine 50	43.85	8.68	1.65	1.95	1.35	1.75	62.55±0.133	68.66±0.235
Fe-EDTA 100+Arginine 50	49.52	8.35	1.68	2.47	1.65	2.01	68.28±0.064	70.79±0.083
LSD at 0.05							0.36	0.51

Each value represents the mean of 3 replicates±standard error

With respect to man, hemin at both concentrations followed by Fe-EDTA at 100 induced significant increases as compared with control plants. While Fe-EDTA at 50 and arginine induced non-significant variation as compared with control plants. Data also showed that arginine alone has no significant effect on Xyl content. While the combination of different concentrations of either hemin or Fe-EDTA with arginine induced significant increases compared to untreated plant.

Table 6: Effect of hemin, Fe-EDTA and/or arginine on macro and microelements of roselle sepals grown under reclaimed sandy soil

Treatments (mg L^{-1})	N (%)	P (%)	K (%)	Ca (%)	Na (%)	Fe (%)	Zn (%)	Mg (%)
Control	1.759±0.012	0.032±0.0003	0.120±0.0015	0.537±0.0015	0.151±0.0003	50.00±1.528	1.263±0.009	0.097±0.001
Hemin 50	1.835±0.001	0.069±0.0010	0.334±0.0022	0.614±0.0003	0.107±0.0012	77.00±0.333	1.463±0.010	1.343±0.003
Hemin 100	1.952±0.002	0.102±0.0003	0.302±0.0025	0.698±0.0013	0.138±0.0006	94.00±0.577	1.660±0.022	1.397±0.012
Fe-EDTA 50	2.381±0.001	0.081±0.0012	0.277±0.0015	0.650±0.0027	0.121±0.0009	82.67±1.155	1.440±0.010	1.177±0.012
Fe-EDTA 100	2.684±0.002	0.151±0.0009	0.314±0.0009	0.554±0.0012	0.097±0.0010	106.33±0.882	1.653±0.010	1.330±0.009
Arginine 50	2.137±0.005	0.068±0.0015	0.303±0.0017	0.626±0.0013	0.105±0.0007	57.67±2.667	1.360±0.020	1.057±0.010
Hemin 50+Arginine 50	1.957±0.002	0.072±0.0007	0.278±0.0012	0.578±0.0003	0.117±0.0003	92.33±0.333	1.543±0.019	1.433±0.009
Hemin 100+Arginine 50	2.075±0.002	0.136±0.0012	0.311±0.0015	0.650±0.0003	0.114±0.0007	115.67±0.882	1.743±0.003	1.633±0.010
Fe-EDTA 50+Arginine 50	2.014±0.001	0.058±0.0006	0.350±0.0020	0.675±0.0017	0.117±0.0012	97.67±1.453	1.633±0.009	1.273±0.007
Fe-EDTA 100+Arginine 50	2.161±0.031	0.069±0.0015	0.384±0.0030	0.723±0.0007	0.137±0.0012	118.00±1.333	1.753±0.003	1.320±0.007
LSD at 0.05	0.032	0.003	0.005	0.002	0.003	3.51	0.04	0.024

Each value represents the mean of 3 replicates±standard error

The most effective treatment was 100 mg L^{-1} DTA+50 mg L^{-1} arginine in increasing Xylose sugar content as compared with all other treatments.

Changes in mineral compositions of roselle sepals: Application of various concentrations of hemin, Fe-EDTA and/or arginine treatments induced significant increases in macro (N, P, K, Ca and Mg) and micro (Fe and Zn) elements, while Na ion contents decreased in roselle sepals as compared to those of control plants (Table 6). Generally, Fe- EDTA was the most effective treatment alone or in combination with arginine in increasing the most studied minerals and decreasing Na contents of sepals as compared with other treatments. It is worthy to mention here that, hemin concentrations alone or in combination with arginine surpassed other treatments significantly in increasing magnesium contents as compared with other treatments.

DISCUSSION

Application of different concentrations of hemin or Fe-EDTA increased gradually with significant differences all studied yield components, biological fruit yield and biological sepals yield (kg/fed) of roselle plants as compared with control. These increments in roselle yield may be attributed to promotive effect of these compounds in increasing number of fruits/plants, sepals/plant and fruit yield/plant as shown in Table 2. The same results were obtained as spraying plants with hemin increased plant height, fresh and dry weight of plants m^{-2}, succulence, leaf area and yield of barley, sesame and roselle plants, respectively[27,38,39]. These increments positively influenced by increasing the hemin concentration and may be due to the regulatory role of hemin as plant growth regulators.

The increase in yield attributes of roselle plants as a result of Fe-EDTA may be attributed to the vital role of iron in enhancing different growth parameters, photosynthetic pigments and total carbohydrate (Table 5). These results are in accordance with those obtained by Hassanein *et al.*[40] on roselle plants[41] on wheat plants[39] on roselle plants. They concluded that iron has different promotive and cofactor for several plant functions. In addition, the stimulatory effect of hemin and Fe-EDTA on yield components of roselle sepals might be as a result to their role in enhancing synthesis of metabolites, increasing absorption of water, increasing photosynthetic rate and increasing the photosynthetic output and accumulation of carbohydrates, soluble sugars, minerals and soluble nitrogen in plant cells, the increased levels of these metabolized was transported from source to sink (Different yield components)[39].

Regarding the stimulatory role of arginine on yield attributes, it may be due to its role as nitrogen-containing compounds which reflected in increasing growth, consequently increasing yield components. These results were positively related with those obtained by El-Bassiouny *et al.*[42] and Ghoname *et al.*[43] they proved that the increases in growth-promoting substances in response to arginine is normal result due to its role in increasing the biosynthesis and assimilation of the endogenous growth promoters and inhibiting their inactivation. Generally, these increments in growth criteria could be attributed to the stimulatory effect of applied treatments on cell division, development, enlargement and differentiation. In this regard, Taha *et al.*[44] on barley plant[45] on mung bean plants,[46] on strawberries fruits suggested that, the induced effect of arginine treatment may be attributed to their role in increasing photo-assimilate and enhancing their translocation to the developing pods which parallel to improve qualitative or quantitative characteristics in studied plants.

Results show that either hemin or Fe-EDTA treatment with different concentrations decreased significantly total acidity of roselle sepals as compared with untreated plants. The decrease in titratable acidity values used as an indication of fruit ripening. These results are documented by Anthon *et al.*[47] who showed marked decreases in acidity with fruit maturity of tomato plants. So, we could attribute the stimulatory effect of hemin, Fe-EDTA and/or arginine in reducing acidity values to the promoting role of these compounds for enhancing growth and yield of roselle sepals (Table 2). In this connection Hassanein *et al.*[40] concluded that, the lowest acidity value is detected in the sepals of roselle plants in the presence of Fe-EDTA may be due to the shift of sugars and several catabolic intermediates to the developing seeds where they involved into the biosynthesis of oils and ions uptake and may be accompanied to their effects on membrane carbohydrates[48]. In addition, several authors reported that, concentration of arginine used in their investigations increased the fruit properties in terms of stimulating the accumulation of some antioxidants like ascorbic acid, titratable acidity, therefore, arginine probably improves qualitative characteristics of crop such as tomato[49], cherry[50] and strawberry[46].

Our results reveal the increase in the activities of PAL and TAL enzymes in roselle leaves significantly in response to all treated plants with either hemin, Fe-EDTA and/or arginine as compared with control plant (Table 3). PAL and TAL are the main enzymes in managing anthocyanin production from phenylalanine, tyrosine and other phenols compounds. In addition, PAL and TAL enzymes catalyze in plants via the conversion of L-phenylalanine and/or tyrosine through betalain into secondary metabolites as anthocyanins, flavonoids and other phenolic derivatives[40,51]. These enzymes can act a vital role in controlling stress conditions[52, 53]. In this study, treated plants promote the activation of PAL and TAL enzymes, these activations may be attributed to the stimulatory roles of these compounds to some metabolites as sugar fractions, TSS, mineral ions (Table 5 and 6) which act as co-factors in accelerating enzyme responsible on active constituent production as anthocyanin and flavonoids (Table 4). These results are in accordance with those obtained by Sadak *et al.*[39] they proved that the promotive effect of iron (either in Fe- EDTA or in hemin) on anthocyanin contents may be via their role as a constitutive of enzymes as PAL and TAL of roselle plant. Also, arginine acts as stimulator to non-photosynthetic output (anthocyanin and flavonoids) of roselle plant through enhancing the biosynthesis process and incorporation of bioactive forms[54].

The master pigments that responsible on the coloration of numerous plant tissues and organs are anthocyanin and other flavonoids. Anthocyanin and other flavonoids values of roselle sepals promoted as a result of plant treatments with different concentrations of hemin, Fe-EDTA and/or arginine (Table 4). These promotive effects could be attributed to the close relation between PAL and TAL enzymes (Table 3) which parallel to stimulation the production of anthocyanin in plants. Also, stimulatory roles of the used treatments in increasing that activities concerning anthocyanin and other pigment production, especially photosynthetic pigments contents (data not shown) with strong correlation with extractable sugar and TSS contents and mineral ion which use as a cofactor for production of active constituents in plants (Table 5a, b and 6). The same results were obtained by Hassanein *et al.*[40] suggested that there has been a close circumstance between the PAL and TAL activities and anthocyanins and phenol contents of roselle plants,[55] who recorded sugar accumulation during development, it is appealing to alter that sugars trigger the expression of anthocyanin genes, through sugar modulated regulatory genes. The role of arginine in establishment assimilates accumulation in treated plants recorded by several researchers. Sadak *et al.*[54] reported that, there is a close correlation between arginine and photosynthetic output of plant (total carbohydrates) through enhancing the biosynthesis and incorporation a fully bioactive form. The promotive effect of iron (either in Fe- EDTA or in hemin) on anthocyanin contents of roselle sepals maybe through their role as a constituent of enzymes on different biological processes as photosynthesis, chlorophyll biosynthesis, respiration, nitrogen fixation, absorption mechanisms[39].

With respect to flavonoids, which are secondary metabolites of phenolic nature. The results show a stimulatory effect of hemin, Fe-EDTA and/or arginine on the values of all flavonoid pigments (Lycopene, β-carotene) and total flavonoid in roselle sepals (Table 4). These stimulatory effects may be due to the role of these compounds in enhancement of metabolism, biosynthesis and assimilation of secondary metabolites as extractable sugars and TSS. These results are in accordance with those obtained by Solfanell *et al.*[55]. They exhibit that the flavonoid and anthocyanin biosynthetic pathways are strongly up-regulated after adding different sugars especially (sucrose). Also, proteins (as enzymes) are involved in metabolism and the biosynthesis of secondary metabolites including flavonoids, terpenoids and alkaloids and phenolic compounds[56,57]. In this connection, the flavonoids metabolism pathway is determined by a number of enzymes, such as anthocyanidin synthase (ANS) and the phenolic acids branched pathway controlled through two parallel precursor pathways (phenylpropanoid-derived and tyrosine-derived pathways)[58]. Recently, Li *et al.*[59] reported that flavonoids and non-flavonoids (such as phenolic acids) are manufactured as a result of synthesis of anthocyanins from leucoanthocyanidin in the flavonoids metabolic pathway.

Also, Nahed *et al.*[60] reported that putrescine (one product of arginine) treatments increased the total phenols of gladiolus plants. Different levels of arginine application inducing qualitative characteristics such as sugar, anthocyanin and flavonoid (as phenol)[46]on strawberry plants. Hemin-elevated the levels of antioxidants, activities of antioxidative enzymes and resulting in improved pigments accumulation, photosynthetic attributes of rice seedlings[61]. Moreover, Sadak *et al.*[62] on roselle shoots and on grapefruits[57] recorded that, presence of Fe-EDTA stimulated the marked increases in the total phenol contents as compared with control plants. In plants, most flavonoids are linked to sugars (glycosides), although they are sometimes found as aglycones[63].

Plants have both enzymatic and non-enzymatic antioxidant defense systems to tolerate free radicals produced compounds, especially those grown in newly reclaimed sandy soils. The non-enzymatic systems consist of a number of active metabolites as carotenoids, flavonoids and other osmolytes. Antioxidant activity of these metabolites revealed against DPPH under different treatments of roselle sepals (different concentrations of hemin, Fe-EDTA and/or arginine). The stimulatory effect of such treatments reflected in the increases of DPPH% (radical scavenging capacity) of all treated sepals as compared with control plants. In this connection, Meda *et al.*[64] and Kasote *et al.*[65] concluded that, phytochemical studies of various extracts of medicinal folk plants proved the presence of metabolically active secondary metabolites such as alkaloids, carotenoids, flavonoids, phenols, saponins, tannins, etc. most of these chemicals possess scientifically proven free radical scavenging activity.

These promoted results may be attributed to the role of either arginine, hemin or Fe-EDTA in enhancement of the secondary active metabolites as anthocyanin, Lycopene, β-carotene and all flavonoid contents of sepals at this study (Table 4). In this regard, carotenoids as one group from the common lipid-soluble phytonutrients synthesized from phytoene, which scavenge peroxyl radicals generated during the process of lipid peroxidation of membranes and play a vital role in the conservation of cellular membranes and lipoproteins against free radical scavenging activity[66]. Lycopene also is the most dominant antioxidant naturally found in many fruits and vegetables. Lycopene demonstrates the strongest singlet oxygen quenching ability as compared to β-carotene[67.]

β-carotene is a naturally occurring orange-colored carotenoid, copiously found in most plants[68]. The free radical scavenging activity of flavonoid fractions from *Cleome gynandra* against DPPH, was proven[69]. Antioxidant activity of phenolic and flavonoid fractions of various extracts of *Cleome gynandra* was detected against DPPH[64]. Recently, anthocyanidin is one component of flavonoids with antioxidant capability. They are valuable in the reducing of lipid oxidation because their activity as ion-chelating. Flavonoids stabilize the ROS by conduct with them to become a flavonoid radical. This is occurred as a result of high reactive hydroxyl group of the flavonoids[63].

Our results are concomitant with those recorded by Akashi *et al.*[70] who reported that arginine residues in a polypeptide are very sensitive to oxidation by free radicals and may cause high reactivity by increasing compatible solutes which act as radical scavengers. Also, L-arginine as necessary amino acid, available from various foods sources and has several valuable functions in living organisms and implicated in oxidative stress resulting in highest rate of DPPH scavenging activity[71].

According to the iron effect, Fe+2/ascorbate showed high scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and was more effective in decreasing the phosphatidylcholine liposome peroxidation more than hemin[72]. Hemin pretreatment prevented toxic response which proved by the reduction of lipid peroxidation[73]. The iron chelators recorded a concentration-dependent increase in their radical scavenging activities, so, all the iron chelators were induced significant activities with significant values in DPPH[74]. Also, Hemin -elevated the levels of antioxidants, activities of antioxidative enzymes and resulting in improved pigments accumulation, photosynthetic attributes and seedling growth of rice[61]. Results recorded the high values of sugar fractions (Gal, Glu, Rha, Man, Arab, TSS and T carb) in roselle sepals affected by different concentrations of hemin, Fe-EDTA alone or in combination with arginine as compared with control plants (Tables 5 and b). These increments in sugar fractions, TSS consequently total carbohydrates could be results to stimulatory role of that treatment compounds in inducing all active metabolites under such conditions. In this connection, Müller and Franz[8] isolated specific sugar fractions (rhaminose, arabinose, galacturonic acid (24%) and glucose) from roselle flower. They suggested that these sugars which found in aqueous extract are effective in several pharmaceutical actions as lowering blood pressure, relaxation, inhibition the motility of taenia and inhibit bacterial growth. These findings approved those obtained by Hassanein *et al.*[40] and EL-Bassiouny *et al.*[75] who reported various promotive iron effects associated with foliar application of Fe EDTA which increased the growth parameters, chlorophyll which reflected in TSS and total carbohydrate contents significantly when compared to untreated roselle sepals. Also, Watanabe *et al.*[76] observed the promotion effect of ALA (precursor of Hemin) on the growth photosynthetic rate, CO2 fixation and carbohydrates of grapevines plants.

In this regard, López-Millán *et al.*[77] reported that glucose concentrations decreased with iron deficiency, whereas fructose concentrations increased 2-fold with no markable changes in sucrose. The enhancing effects of Fe treatments on the content of TSS or total carbohydrates in mature fruits were recorded[78]. Also, at insufficient- Fe plants, the activity of ribulose-1,5-bisphosphate carboxylase (RuBPC) and the content of Chl, carotenoids decrease, hence leaf CO2 exchange rate and Chl photosynthetic efficiency were decreased[79]. They also added that iron excess might result in higher sugar content than Fe deficiency. Moreover, Trejgell *et al.*[20] concluded that adding Fe-EDDHA to the medium as Fe source increased significantly the value of chlorophyll accompanied with increase in the carbohydrate levels in the leaves of *Carlina onopordifolia*. Recently, Shi *et al.*[57] reported that Fe is the main constituent of chlorophyll (Chl) synthesis, mitochondrial and photosynthetic electron transport chains, parallel with carbohydrate formation and regulation of Calvin cycle enzymes such as stromal fructose-1,6-bisphosphatase (FBPase).

Concerning the promotive effects of arginine on the production of sugar fraction, TSS and total carbohydrates, El-Tantawy[80] concluded that amino acid (arginine) application increased chlorophyll contents of leaves with concomitant increase in carbohydrate synthesis and consequently increment in plant production of tomato plants. The same trend was obtained by Ghoname *et al.*[43] who proved that, application of arginine at different concentration induced growth parameters, yield, endogenous growth regulators (IAA, GA3, ABA) as well as nutritive components in the yielded fruits such as ascorbic acid, anthocyanins, phenolic compounds, carbohydrate contents on hot pepper (*Capsicum annuum* L. cv. Albasso). In addition, Hosseini *et al.*[81] concluded that arginine induced membrane stabilization via soluble sugars which act as ROS scavengers' compatible solutes as total soluble sugar and proline either in both stressed and unstressed plants. Arginine assimilation accompanied by the availableness of carbohydrates, sugar starvation stimulated enzyme activities of arginase and urease and arginine decarboxylase (ADC), leads to polyamine synthesis[16]. Also, arginine as high nitrogen source stimulate various biological aspects in plants as growth promoters through metabolic activities via increasing the efficiency of water uptake, the photosynthetic pigments and higher levels of carbohydrates[82]. Moreover, exogenous application of arginine, hemin and Fe EDTA increased significantly total carbohydrate contents of sesame than that of treatments without arginine[38]. Different levels of arginine application increased quantitative characteristics, total soluble solid, inducing sugar, anthocyanin, phenol and qualitative characteristics of strawberry fruits[46].

Roselle sepals contain high nutritional values of macro (N, P, K, Ca and Mg) and micro (Fe and Zn) elements, these contents enhanced as a result of a plant treated with various concentrations of hemin, Fe-EDTA and/or arginine (Table 6). These increments were parallel to significant decrease in Na content at all treated plants. This stimulatory effect may be attributed to the stimulatory effects of these compounds to increase availability of mineral in sepals through increasing mineral uptake from soil. All these elements have major functions in plant life, assimilators for several plant assimilates. These results agreed with those obtained by Hassanein *et al.*[40] on Roselle. In this connection, several authors postulated that some fertilizer as nitrogen, phosphate, potassium, zinc and iron are very important factors that can influence anthocyanin biosynthesis[24].

Concerning the stimulatory role of iron, like one from a micronutrient that plays an important role in agriculture to face plants requirement for all major functions started with growth, chlorophyll biosynthesis, development, plant proteins and enzymes to energy transfer and inorganic cation and anion concentrations[25]. They also added that iron deficiency or excess can alter the homeostasis of a plant's cell and result in declining of photosynthetic rate, respiration and increased accumulation of Na^+ and Ca^{++} ions which end with an excessive formation of ROS. In this connection, several iron transporters or chelating are able to transport iron along with Zn and other metals. Mn and iron are parallel in their concentrations under iron deficiency in the roots and shoots of plants and Mn can be transported by the plant through iron transporters[83]. In addition, iron deficiency resulted in lower sulfur and Cu concentrations in the roots and shoots of plant varieties and this is parallel with the iron requirements for their assimilation[84].

Hemin has identified as novel roles in regulatory processes such as transcription, translation, protein translocation and ion-channel function[85]. So, barley treated with hemin gave the highest values of Ca, Mg, K and Fe compared to control. These increments were gradually parallel with a decrease in Na of shoots with increasing hemin concentration. This may be a result of osmoregulation process of hemin as growth regulators. Also, the increase in Mg according to hemin treatment is considered a normal result because hemin is a precursor of chlorophyll which has Mg as central atom[27].

It is worthy to mention here that, hemin concentrations alone or in combination with arginine surpassed other treatments significantly in increasing magnesium contents as compared with other treatments. These results may be due to the promotive role of arginine as a nitrogen source or as precursor of polyamine components responsible for osmoregulation processes in plant cells. The same results were obtained by those obtained by Bakry *et al.*[38] who concluded that, exogenous application of different concentrations of hemin as well as Fe EDTA in presence of arginine on sesame induced significant increase in all tested parameters as compared with corresponding treatments without arginine. In this regard, arginine has vital roles in several biological features in plants, growth stimulants. Arginine is playing critical roles in several metabolic pathways of plant growth via promoting the capability of water uptake, assimilation and protecting the photosynthetic pigments consequently markedly higher levels of carbohydrates. Moreover, the promotive effect of amino acid may be owing to their acceptable nitrogen source needed for increased growth rate of shoots[82]. Several authors recorded that, arginine at all concentration used reduced significantly Na content of different plants, while K and P highly significantly increased while, Ca, Mg and N slightly affected[45,39,86].

So, this study clearly proved that, application of Fe-EDTA, Hemin and/or Arginine at different concentrations were recommended in roselle plants to promote their active constituents, regulatory osmolytes in sepals grown under reclaimed sandy soils.

CONCLUSION

Foliar treatment of roselle plants with Fe-EDTA, hemin and arginine alone or mixture of them with different concentrations, significantly increased all yield parameters, PAL, TAL, non-photosynthetic pigments as anthocyanin, lycopene and β-carotene, flavonoids, DPPH, as well as all sugar fractions, TSS, Total carbohydrates, macro and microelements of roselle sepals as compared with control (untreated) plants. While total acidity and Na contents decreased significantly due to such treatments as compared with untreated plants. These results reflect the stimulatory effects of these compounds in homeostasis through increasing active constituents, non-enzymatic antioxidants and regulatory osmolytes. The maximum stimulatory effects were obtained with the Fe-EDTA 100+Arginine 50 treatment.

SIGNIFICANCE STATEMENT

This study discovered the role of either Fe-EDTA, Hemin and/or Arginine as promoters for most active constituents, nonenzymatic antioxidants and regulatory osmolytes in sepals of roselle plants grown under sandy soils, that can beneficial for explore and discuss the relation among all studied parameters. This study will help researcher to explain the role of studied compounds on Roselle plants and their stimulatory mode of action.

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