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Phytochemical Properties of Roselle (*Hibiscus sabdariffa,* L.) Plants Grown under Bio and Mineral Fertilizers in Different Types of Soil

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Abstract. The aim of this study is to evaluate the phytochemical properties of Roselle plants grown under bio Azotobacterine (*Azotobacter chroococcum*) and phosphorein (*Bacillus polymyxa*) and mineral (N, P and K fertilizers at the rates of 25, 50 and 100% from the doses recommended by Ministry of Agriculture) fertilizers applied in different types of soil. Samples of Roselle plants were obtained from different soils (clay soil at Dar El-Ramad farm, sandy loam and saline loamy sand soil at Demo farm, Faculty of Agriculture) at El-Fayoum governorate conditions. Moreover, some of the phytochemical properties (N, P and K percentage in roselle herb and its uptake, photosynthetic pigments (chlorophyll A, chlorophyll B and carotenoids), anthocyanin pigment and pH value) of roselle plants (*Hibiscus sabdariffa*, L.) under different soils were determined.

The data obtained that, adding bio and mineral (NPK) fertilizers increased the above compositions of roselle plants under different soils of experiment. The maximum increase of these compositions was obtained by the treatment clay soil × 100% NPK + bio fertilizers, followed by clay soil × 50% NPK + bio fertilizers as compared to saline loamy sand soil × non fertilizer treatment, although, the differences between these treatments and mineral fertilizer at the rate of 100% NPK alone were insignificant. Therefore, it is economically and environmentally recommended to inoculate roselle seeds with mixture of Azotobacter + Bacillus and fertilize these inoculated plants with 50% NPK for improve chemical compositions (N, P and K percentage in roselle herb and its uptake, photosynthetic pigments (chlorophyll A, chlorophyll B and carotenoids), anthocyanin pigment and pH value) of roselle plants under clay soil.

Introduction

Roselle*(Hibiscus subdariffa* L.), is a shrub belonging to the family Malvaceae. It is thought to be a native to Asia (India to Malaysia) or Tropical Africa. The plant is widely grown in tropics like Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Plilippines as a home garden crop. In addition to Roselle, in English speaking regions it is called as Rozelle, Sorrel, Red sorrel, Jamanica sorrel, Indian sorrel [1].In Egypt it is called "Karkade" [2]. There are more than 300 species of hibiscus around the world; one of them is Roselle [3].

Nutrient deficiency in soils is one of the major issues for agricultural production around the globe affecting the quantity and quality of crops [4]. For the improvement of crop yields, farmers have commonly used chemical fertilizers for agricultural production causing an adverse effect on autochthonous organisms and deteriorating the quality of agro-ecosystems and aquatic resources[5]. Nitrogen is required in large quantities for plants growth, since it is the basic constituent of proteins and nucleic acids. Nitrogen is provided in the form of synthetic chemical fertilizer. Excessive use of such chemical fertilizers for increasing crop yield caused health hazard and microbial population problems in soil besides beings quite expensive and making the cost of production high[6]. Most of the phosphate fertilizers remain unused and accumulate in the soil as precipitates. Apart from this, the accumulated phosphates (in soil) may also reach water bodies causing eutrophication. Therefore, the use of phosphate solubilizing microbes along with the applied phosphate can provide

a solution to the menace of accumulation of phosphates in the fields and water bodies. *Bacillus* sp. are well-known rhizobacteria which facilitate the plant growth either by solubilization of minerals like phosphorus or production of metabolites such as siderophores and phytohormones, and are excellent colonizers of the roots [7].Bio-as a microbial-based fertilizer composed of different microorganism that has the ability to fix atmospheric N making it available for plant growth which can drastically reduce N- fertilizer input and enhance the yield of wheat. These bacteria can convert the gaseous nitrogen from the air to ammonia at an equivalent rate of 40 kg of N per hectare or higher. It complements the use of chemical fertilizer by 30-50%, an apparent big savings for the farmers. Other results show that Bio-N inoculation, in the presence of 50% the recommended mineral fertilizer, could parallel the yield of fully fertilized corn [8]. Several researchers reported that inoculation of some plants with bio fertilizers (singly or in different combinations with mineral fertilizers) improved plant growth, yield and chemical composition [9-12]. Moreover, several researchers justified the idea that nutrients like N may be taken up through roots and leaves and may spread within the plant[13-16]. Also, several researchers justified the idea that nutrients like phosphorus may be taken up through roots and leaves and may spread within the plant[17-21]. Many investigators agreed that application of phosphate dissolving bacteria; *Bacillus megaterium* increased growth characters [22-26].

The aim of this work was to study the effect of adding N and P fertilizers either as minerals (different rates) or bio fertilizers as soil application on chemical composition of roselle plants.

Material and Methods

A field experiments were conducted during the two successive seasons of 2007and 2008 to study the effect of bio, nitrogen and phosphorus fertilizers on chemical composition of roselle plants (*Hibiscus sabdariffa,* L.) under different types of soil at El-Fayoum governorate conditions. Three types of soil in two experimental stations of the Faculty of Agriculture, namely clay soil at the Dar El-Ramad farm, a saline loamy sand and sandy loam soil at Demo farm. Some chemical and physical characteristics of the experimental stations soils are presented in Table (1).

Table 1. Some physical and chemical properties of the used experimental soils (Dar El-Ramad and Demo farms) through seasons 2007 and 2008.

Soil properties	Demo				Dar El- Ramad	
	2007	2008	2007`	2008	2007	2008
Physical properties						
Coarse sand %	28.69	22.73	30.20	31.05	5.14	6.37
Fine sand %	37.19	31.66	42.33	40.38	21.55	19.40
Silt %	22.16	31.84	15.72	16.04	24.43	27.48
Clay %	11.96	13.77	11.75	12.53	48.88	46.75
Texture class	**Sandy loam**	**Sandy loam**	**Loamy sand**	**Loamy sand**	**Clay**	**Clay**
Chemical properties						
Organic matter %	0.48	0.38	0.41	0.40	1.69	1.43
$CaCO_3$ %	8.61	7.83	7.42	7.69	5.82	5.12
pH (soil paste)	7.82	7.75	7.76	7.81	7.89	7.55
ECe (paste extract), ds/m	3.12	3.64	7.51	7.26	1.58	2.10
Soluble anions, meq/L*						
CO_3^{--}	….	….	….	….	….	….
HCO_3^-	5.40	4.83	4.37	4.96	2.24	3.83
Cl^-	12.35	15.42	38.93	35.43	7.12	10.29
SO_4^{--}	13.45	16.15	31.80	32.21	6.44	6.88

Soluble cations, meq/L*						
Ca^{++}	8.67	10.25	30.75	30.24	6.15	7.43
Mg^{++}	6.89	7.32	17.41	14.39	6.80	8.92
Na^{+}	15.03	18.11	26.15	27.32	2.13	3.80
K^{+}	0.61	0.72	0.79	0.65	0.72	0.85
Available nutrients, ppm**						
N	3.54	3.80	4.44	4.96	21.02	22.16
P	6.87	6.59	6.42	6.42	12.27	12.27
K	54	42	36	24	83	89

- In saturation paste extract
** On dry weight basis

Biofertilizers namely Azotobacterine (*Azotobacter chroococcum)* as nitrogen fixing bacteria and phosphorein (*Bacillus polymyxa*) as a phosphate dissolving bacteria were obtained from Agricultural Research Center, Ministry of Agriculture, Egypt.The seed of roselle cv. Sabahia 17 were obtained from the Research Center of Medicinal and Aromatic Plants, Ministry of Agriculture, Egypt. Seeds were immersed in Arabic gum solution (16%) as a sticking agent, then, the seeds were mixed with the powder of mixed biofertilizers; inoculated seeds were allowed to dry before sowing, according to [27]. Application of N, P and K fertilizers were 100, 50 and 25% for each fertilizer from that recommended doses by the Ministry of Agriculture, 500 kg ammonium sulfate (20.6% N), 150 kg calcium superphosphate (15% P_2O_5) and 50 kg potassium sulfate/fed (48% K_2O). Nitrogen fertilizer was applied in two equal doses, the first dose during sowing and before the first irrigation, and the second dose after one month from sowing. Phosphorus and potassium fertilizers were applied during soil preparation.

Table 2. Mineral fertilization rates

Application of mineral fertilization	N	P	K
	Ammonium sulfate (20.6% N)	Calcium superphosphate (15% P_2O_5)	Potassium sulfate (48% K_2O)
100%	500 kg/fed	150 kg/fed	50 kg/fed
50%	250 kg/fed	75 kg/fed	25 kg/fed
25%	125 kg/fed	37.5 kg/fed	12.5 kg/fed

The experiment included eighteen treatments with three replicates (three different soils × six levels of fertilizers) as following.

1- Non fertilizer 2- Biofertilizers 3- 100% NPK 4- (Biofertilizers + 100% NPK) 5- (Biofertilizers + 50% NPK) 6- (Biofertilizers + 25% NPK)

The experimental field was plowed, rolled and divided into 54 plots, each one of 10.5m^2 area (1/400 fed) and contains 5 rows (3 m width and 3.5 m in length). The seeds inoculated were sown on 1st and 2nd May, in the two experimental seasons. Four seeds were sown per hill (50 cm apart) on one side of the ridge. After one month from sowing the seedlings were thinned to one plant per hill. In this regard, each experimental unit (plot) contains 30 plants (6 plants /row) and 12000 plants/fed. All other agricultural practices were followed as recommended in roselle management.

Plant samples:

Samples of roselle plants (9 plants) were randomly chosen from each treatment (3 plants for each replicate) in the early morning after 140 days from sowing immediately transferred to the laboratory to study the following plant analysis.

Plant analysis:

At the age of 140 days from sowing samples were taken and ground plant materials were wet digested using the H_2SO_4 + H_2O_2 mixture as described by [28] to determine phosphorus and potassium. The digestion solution was prepared by mixing 350 ml H_2O_2 with 0.42 g Se powder and 14 g $Li_2SO_4.H_2O$ in a flat bottomed boiling flask of 1 liter capacity. Four hundred and twenty ml

H_2SO_4 (S.G. 1.84) were carefully added with swirling and cooling. The mixture was then stored at 1°C. Equal portions of 0.2 g of dry ground plant materials were weighted into a 50 ml round bottom long neck reflex flask and digestion, the solution was allowed to cool, diluted to 50 ml, filtered and stored for analysis. The digestions of all samples were analyzed using the following procedures.

1. Phosphorus content in plant materials was determiend by ammonium molybdate, hydroquinon and sodium sulfide and determined using colorimetric method according to [29].

2. Potassium was determined by flame photometrically according to [30].

3. Total nitrogen of plant materials was estimated colorimetrically by using the Orange G dye method as described by [31], as follows:

The dye solution was prepared by dissolving 1 g Orange G dye (87% assay) in one litter of distilled water followed by the addition of 21 g of citric acid (which acts as a buffer to maintain proper pH) and 2.5 ml thyml 10% in alcohol (as microbial inhibitor). Powdered dry plant material (0.2 g) plus 20 ml of dye solution was placed in a test tube and shacked for 15 minutes, then filtered and filtrates were diluted 100 times with distilled water. The absorbance was measured using a spectrophotometer (spectronic 20 Bauch and Lomb) at a wave length of 482 nm.

Calculation was done as follows:

$$\text{Dye absorbed } (\text{gm}/100\text{gm}) = \frac{(a - b)}{a} \times \frac{cfv}{w} \times 100$$

Where:

a = absorbance at 482 nm of dye reagent solution diluted 100 times.

b = absorbance at 482 nm of dye solution after shaking with plant material, filtrated and diluted 100 times.

c = concentration of dye reagent 1 g/100 ml.

f = purity factor of dye reagent solution i.e. 96/100.

v = volume of dye reagent solution used.

w = weight of dry plant material in grams (0.2 g).

4. pH determinations in sepals extract were prepared by boiling samples (3 g dry sepals/L) in distilled water for a period of 10 minutes. The pH was measured using pH meter[32] .

5. Anthocyanin was determined according to the method described by[33] .

6. At age of 140 days flowering stage (in both seasons) samples of fresh leaves were taken for determination of photosynthetic pigments. Pigments concentrations of extraction solution were calculated according to[34] .

Chlorophyll a = (11.63 × E 665)-(2.39 × E 649) = mg/L.

Chlorophyll b = (20.11 × E 649)-(5.18 × E 665) = mg/L.

Carotenoids = (4.695 × E 440)-(0.268 × (chl. a + chl. b) = mg/L.

Where E= optical density at given wave length, and concentration of each pigment was calculated and presented as mg/g fresh leaves.

Statistical Analysis:

A split-split plot design was used. Also, all the collected data were reduced to proper format and units and were statistically analyzed using the procedures outlined by [35].

Results

Effect of bio and mineral fertilization under different soil types on chemical composition.

1-Nitrogen, phosphorus and potassium percentage in roselle herb (leaves and stem):

Data presented in Table (3) indicated the effect of different soil types on N, P&K percentage in roselle herb. Data revealed that soil type had significant effect on N, P&K percentage in roselle herb in the first season but was found a significant increase in the second one. Clay soil gave higher N, P&K percentage in roselle herb compared to sandy loam and saline loamy sand soil in the second season.

A significant increment of N, P&K percentage in roselle herb was obtained by mineral NPK in the first and second seasons compared to non-fertilized plants (Table 3).
In regard with biofertilization, data shown in Table (3) indicated that application of bio fertilizers led to significant increase in N, P&K percentage in roselle herb compared to non-fertilizer treatment in the two experimental seasons.
The combination between mineral NPK and biofertilizers significantly affected N, P&K percentage in roselle herb in the two experimental seasons. All plants which received 100% NPK and inoculated with bacteria strains were higher than those which not received neither NPK nor biofertilization treatments alone. The most effective interaction treatments were 100% NPK + biofertilization followed by 50% NPK + biofertilization and then 25% NPK + biofertilization.

The interaction between soil type and fertilizer treatments (S×F) increased N, P&K percentage in roselle herb above the lowest values at the treatment (saline loamy sand soil × non fertilizer).

Table 3. Effect of bio and mineral fertilization and their interactions on N%, P % and K% in roselle herb under clay, sandy loam and saline loamy sand soils during the two successive seasons 2007 and 2008

Treatments	**N% in roselle herb**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	1.02	0.95	0.83	1.01	093	1.83
Biofertilizers	1.14	1.10	0.98	1.13	1.06	0.96
100% NPK	1.77	1.70	1.65	1.71	1.62	1.57
Bio + 25% NPK	1.27	1.26	1.19	1.19	1.22	1.14
Bio + 50% NPK	1.48	1.42	1.31	1.38	1.38	1.29
Bio + 100% NPK	1.80	1.71	1.28	1.79	1.67	1.34
Mean	1.41	1.36	1.21	1.37	1.31	1.19
L.S.D. at 5%	S=N.S. F= 0.07 S.F=0.04			S= 0.06 F=0.04 S.F=0.02		
Treatments	**P% in roselle herb**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	0.16	0.15	0.13	0.13	0.13	0.11
Biofertilizers	0.19	0.18	0.15	0.16	0.16	0.13
100% NPK	0.29	0.26	0.28	0.27	0.22	0.26
Bio + 25% NPK	0.28	0.22	0.20	0.24	0.19	0.17
Bio + 50% NPK	0.30	0.28	0.26	0.27	0.25	0.24
Bio + 100% NPK	0.33	0.30	0.30	0.30	0.28	0.27
Mean	0.26	0.23	0.22	0.23	0.20	0.20
L.S.D. at 5%	S= N.S. F=0.03 S.F= 0.02			S=0.03 F=0.02 S.F= 0.01		
Treatments	**K% in of roselle herb**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	1.27	0.98	0.76	1.24	0.94	0.71
Biofertilizers	1.42	1.30	1.03	1.40	1.23	0.98
100% NPK	2.56	2.25	2.18	2.43	2.12	2.12
Bio + 25% NPK	1.73	1.42	1.38	1.70	1.39	1.40
Bio + 50% NPK	2.03	2.02	1.95	2.00	1.98	1.88
Bio + 100% NPK	2.56	2.36	2.23	2.42	2.30	2.16
Mean	1.93	1.72	1.59	1.86	1.66	1.54
L.S.D. at 5%	S=019 F=0.07 S.F=0.04			S=0.11 F=0.04 S.F=0.02		

S= Soil **F= Fertilizer**

2-Nitrogen, phosphorus and potassium uptake (g/plot and kg/fed):

A significant increment of N,P&K uptake g/plot and kg/fed by using mineral NPK fertilization was observed in the first and second seasons compared to unfertilized plants (Tables 4&5).
Treating roselle plants with biofertilization, data shown in Tables (4&5) indicated that N, P&K uptake was significantly increased compared to non-fertilizer treatment in the two experimental seasons. The response of N, P&K uptake/plot and fed to combinations of mineral NPK and bio fertilizers were observed in the two experimental seasons (Tables 4&5). The most effective interaction treatments were 100% NPK + biofertilization followed by 50% NPK + biofertilization and then 25% NPK + biofertilization.

Table 4. Effect of bio and mineral fertilization and their interactions on N, Pand K uptake g/plot in roselle herb under clay, sandy loam and saline loamy sand soils during the two successive seasons 2007 and 2008

Treatments	**N uptake g/plot**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	95	83	59	95	76	58
Biofertilizers	141	117	92	139	108	85
100% NPK	252	227	193	245	210	179
Bio + 25% NPK	158	148	119	149	144	111
Bio + 50% NPK	215	184	152	200	174	146
Bio + 100% NPK	267	232	158	264	221	162
Mean	188	165	129	**182**	**155**	**123**
L.S.D. at 5%	S= N.S. F=16		S.F=14	S=33	F=10	S.F=5
Treatments	**P uptake g/plot**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	15.0	13.9	8.9	14.2	11.9	8.8
Biofertilizers	23.1	19.6	14.1	22.1	18.0	13.0
100% NPK	41.8	34.3	33.1	41.5	31.5	31.6
Bio + 25% NPK	35.4	25.8	19.8	34.9	24.7	18.4
Bio + 50% NPK	42.9	36.2	30.0	42.0	33.6	29.3
Bio + 100% NPK	48.3	41.2	36.6	47.4	39.8	35.1
Mean	34.4	28.3	23.7	33.7	26.6	22.7
L.S.D. at 5%	S=N.S.	F=7.4	S.F=3.1	S=10.2	F=4.5	S.F=5.1
Treatments	**K uptake g/plot**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	119	87	54	117	77	50
Biofertilizers	175	139	97	172	126	87
100% NPK	365	301	255	347	274	242
Bio + 25% NPK	216	167	139	212	163	136
Bio + 50% NPK	293	261	226	289	249	212
Bio + 100% NPK	378	321	275	359	305	262
Mean	258	213	174	249	199	165
L.S.D. at 5%	S=60	F=15	S.F=9	S=37	F=8	S.F=4

S= Soil **F= Fertilizer**

Table 5. Effect of bio and mineral fertilization and their interactions on N, P and K uptake kg/fed of roselle plants under clay, sandy loam and saline loamy sand soils during the two successive seasons 2007 and 2008

Treatments	N uptake kg/fed					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	38	33	24	38	30	23
Biofertilizers	57	47	37	55	43	34
100% NPK	101	91	77	98	84	72
Bio + 25% NPK	63	59	48	60	58	44
Bio + 50% NPK	86	74	61	80	70	58
Bio + 100% NPK	107	93	63	106	88	65
Mean	75	66	51	73	62	49
L.S.D. at 5%	S=N.S.	F= 6	S.F=5	S=12	F= 4	S.F=2
Treatments	**P uptake kg/fed**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	6.0	5.2	3.6	5.7	4.8	3.5
Biofertilizers	9.2	7.8	5.6	8.8	7.2	5.2
100% NPK	16.7	13.7	13.2	16.6	12.6	12.7
Bio + 25% NPK	14.2	10.3	7.9	14.0	9.9	7.4
Bio + 50% NPK	17.2	14.5	12.0	16.8	13.4	11.7
Bio + 100% NPK	19.3	16.5	14.6	19.0	15.9	14.1
Mean	13.8	11.3	9.5	**13.5**	**10.6**	**9.1**
L.S.D. at 5%	S=N.S.	F=2.9	S.F=1.2	S=4.0	F=1.8	S.F= 2.0
Treatments	**K uptake kg/fed**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	48	35	22	47	31	20
Biofertilizers	70	56	39	69	50	35
100% NPK	146	120	102	139	110	97
Bio + 25% NPK	86	67	55	85	65	54
Bio + 50% NPK	117	105	90	116	100	85
Bio + 100% NPK	151	128	110	144	122	105
Mean	103	85	70	100	80	66
L.S.D. at 5%	S=24	F=6	S.F=4	S=23	F=4	S.F=2

S= Soil **F= Fertilizer**

3-Photosynthetic pigments content:

Data presented in Tables (6) show the effect of soil type on photosynthetic pigments content. Data revealed that soil type has no significant effect on photosynthetic pigments content except, chlorophyll b in the first season, but has a significantly increase in the second season. The clay soil gave the highest values of chlorophyll a, 0.75, chlorophyll b, 0.40, and carotenoids, 0.45, mg/g compared to sandy loam (0.63, 0.34 and 0.45 mg/g) and saline loamy sand soil (0.61, 0.33 and 0.43 mg/g) in the second one.

Taken the mean values (in the second season) of photosynthetic pigments content under different soil type at clay soil as 100, the relative photosynthetic pigments concentration were as follows:

	Clay	Sandy loam	Saline loamy sand
Chlorophyll a	100	84	81
Chlorophyll b	100	85	83
Carotenoids	100	100	96

These values indicated that clay soil increased photosynthetic pigments concentration compared to sandy loam or saline loamy sand soil.

The photosynthetic pigments i.e. chlorophyll a, b and carotenoids of the roselle fresh leaves were significantly responded to mineral NPK fertilization in comparison to check treatment (non fertilizer) in the two growing seasons as shown in Tables (6). Mineral fertilization with NPK increased contents of chlorophyll a by 47.1 and 52.9%, chlorophyll b by 18.7 and 21.8% and carotenoids by 40.0 and 38.2% in the first and second seasons, respectively more than non-fertilizer treatment.

Also, data listed in Tables (6) revealed that biofertilization significantly promoted photosynthetic pigments content in comparison with non-fertilized plants. Inoculating seed plants with biofertilizer insignificantly increased chlorophyll a, b and carotenoids in the first season, while significant increase was observed (7.8, 6.2 and 5.8%) in the second season. Many investigators obtained the promoting influences of N and P-biofertilization treatments on the content of the three photosynthetic pigments recorded in this work**.** The interaction between soil type and fertilizer treatments (S×F) in Tables (6) caused an increase of pigments content especially, at the treatment clay soil × Bio + 100% NPK above the lowest values at the treatment saline loamy sand × non fertilizer. The increase of chlorophyll a, b and carotenoids were 83, 40 and 90.6% in the first season and 106.3, 44.8 and 84.3% in the second one, respectively.

Table 6. Effect of bio and mineral fertilization and their interactions on plastid pigments of roselle plants under clay, sandy loam and saline loamy sand soils during the two successive seasons 2007 and 2008

Treatments	**Chlorophyll a (mg/g F. W.)**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	0.53	0.49	0.48	0.52	0.48	0.43
Biofertilizers	0.56	0.52	0.50	0.55	0.51	0.79
100% NPK	0.93	0.67	0.66	0.92	0.67	0.65
Bio + 25% NPK	0.66	0.59	0.54	0.65	0.58	0.54
Bio + 50% NPK	0.77	0.71	0.61	0.75	0.70	0.97
Bio + 100% NPK	0.97	0.73	0.61	0.95	0.72	0.70
Mean	0.74	0.62	0.57	0.72	0.61	0.58
L.S.D. at 5%	S=N.S.	F=0. 05	S.F=0.03	S=0.07	F=0.03	S.F=0.02
Treatments	**Chlorophyll b (mg/g F. W.)**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	0.35	0.30	0.29	0.34	0.30	0.28
Biofertilizers	0.37	0.32	0.30	0.37	0.31	0.30
100% NPK	0.41	0.35	0.33	0.41	0.35	0.33
Bio + 25% NPK	0.40	0.33	0.32	0.39	0.32	0.31
Bio + 50% NPK	0.41	0.34	0.33	0.40	0.33	0.33
Bio + 100% NPK	0.41	0.35	0.33	0.40	0.34	0.33
Mean	0.39	0.33	0.32	0.39	0.33	0.31
L.S.D. at 5%	S=0.06	F= 0.03	S.F=0.02	S=0.03	F=0.02	S.F=0.01
Treatments	**Carotenoids (mg/g F. W.)**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	0.36	0.33	0.30	0.33	0.32	0.30
Biofertilizers	0.37	0.36	0.32	0.34	0.35	0.33
100% NPK	0.47	0.43	0.44	0.44	0.41	0.39
Bio + 25% NPK	0.40	0.38	0.39	0.37	0.37	0.35
Bio + 50% NPK	0.57	0.49	0.48	0.54	0.47	0.45
Bio + 100% NPK	0.60	0.58	0.54	0.58	0.57	0.55
Mean	0.46	0.43	0.41	0.43	0.42	0.40
L.S.D. at 5%	S= N.S.	F=0.05	S.F=0.03	S= 0.02	F=0.02	S.F=0.01

S= Soil **F= Fertilizer**

Anthocyanin content:

Data presented in Table (7) show that clay soil gave the highest anthocyanin content in the sepals of rosel1e plants (18.19 mg/g) compared to sandy loam soil (16.17 mg/g) or saline loamy sand soil (14.90 mg/g) in the first season and 17.20, 15.08 and 13.79 mg/g in the second season, respectively.

Taken the mean values (in the first and second seasons) of anthocyanin pigment content in the sepals of roselle plants under different soil types, at clay soil as 100, the relative anthocyanin pigment contents were obtained as follows:

	Clay	Sandy loam	Saline loamy sand
First season	100	88.8	81.9
Second season	100	87.7	80.2

These values indicated that clay soil increased anthocyanin pigment content than sandy loam or saline loamy sand, respectively.

Supplement of 100% NPK fertilization treatment significantly increased anthocyanin pigment content in the sepals of rosel1e plants in comparison to the untreated plants (Table7). The increase was found to be 41.6 and 44.4% in the first and second seasons, respectively.

Data illustrated in Table (7) indicated that treated roselle plants with biofertilization increased anthocyanin pigment content compared to non fertilized plants in the experiment two seasons. The increases reached to 14.1 and 14.7% in the first and second seasons, respectively.

A combination between NPK and biofertilizers had a significant positive effect on anthocyanin content than non fertilizer treatment in both seasons as shown in Table (7). The most effective interaction treatments were 100% NPK + biofertilization followed by 50% NPK + biofertilization than 25% NPK + biofertilization. The increases were found to be 47.2, 40.2 and 21.8% in the first season and by 48.8, 41.8 and 24.3% in the second one, respectively. The differences between 100% NPK alone and Bio + 50% NPK or Bio + 100% NPK treatment were slightly.

The interaction effect between soil type and fertilizer treatments slightly increased anthocyanin content in the first season, while, significant increase (89.2%) was observed by the treatment (clay soil × Bio + 100% NPK) above the lowest value (19.59 mg/g) for the treatment (saline loamy sand soil × non fertilizer) in the second season (Table 7).

pH values

Data presented in Table (7) show the effect of different soil types on pH values in the sepals of roselle plants. Data revealed that clay soil gave the higher pH value of 3.22 compared to sandy loam (3.00) or saline loamy sand soil (2.77) in the first season only.

Data recorded in Table (7) show that pH values in sepals of roselle were significantly increased (that means decrease of the acidity) by applying the treatment 100% NPK in comparison with non fertilized plants. The pH value increase reached to 3.36 in the first season and 3.28 in the second one.

Biofertilization treatment led to an increase in pH value compared to untreated plants (Table 7). The highest increases of pH values were obtained from inoculation of plants by biofertilizers (2.56) over non fertilizer (2.33) treatment which reached to 9.9% in the second season, but it was insignificant in the first one.

In respect with the combined effect of mineral and biofertilization treatments, it could be noticed that combination treatments, in most cases, significantly affected pH values. The most effective interaction treatments were 100% NPK + biofertilization followed by 50% NPK + biofertilization than 25% NPK + biofertilization. The significant increase (3.24 and 3.42) was obtained by the treatment 50% NPK + biofertilization and 100% NPK + biofertilization in the first season while, slight increase was observed by the treatment of 25% NPK + biofertilization in the first season. Also, rate of increase in the second season was 3.41, 3.24 and 2.55 for 100% NPK + biofertilization, 50% NPK + biofertilization and 25% NPK + biofertilization treatments,

respectively.The interaction effect between clay soil × Bio + 100% NPK increased pH values by 57.9% (3.90) and 72.2% (3.79) above the lowest values (2.47 and 2.20) at the treatment saline loamy sand soil × non fertilizer of the first and second seasons, respectively.

Table 7. Effect of bio and mineral fertilization and their interactions on anthocyanin and pH values of roselle plants under clay, sandy loam and saline loamy sand soils during the two successive seasons 2007 and 2008

Treatments	**Anthocyanin content (mg/g)**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	14.27	12.28	10.24	12.63	11.28	9.07
Biofertilizers	16.18	14.19	11.20	15.07	12.86	10.33
100% NPK	19.01	17.04	13.66	17.34	15.64	12.40
Bio + 25% NPK	17.10	14.97	12.53	15.81	13.50	11.11
Bio + 50% NPK	18.65	16.65	14.54	17.31	15.31	13.30
Bio + 100% NPK	20.20	18.20	15.84	18.72	16.66	12.49
Mean	17.57	15.55	13.00	16.15	14.21	11.75
L.S.D. at 5%	S=1.59 F=1.18 S.F=N.S.			S=2.44 F=1.14 S.F=0.66		
Treatments	**pH values**					
	2007			2008		
	Clay	Sandy loam	Saline loam sand	Clay	Sandy loam	Saline loam sand
Non fertilizer	2.49	2.44	2.12	2.12	2.07	2.00
Biofertilizers	2.49	2.46	2.16	2.41	2.16	2.11
100% NPK	3.30	3.29	2.96	3.16	3.05	2.93
Bio + 25% NPK	2.55	2.53	2.20	2.25	2.22	2.13
Bio + 50% NPK	3.25	3.16	2.86	3.15	3.15	2.76
Bio + 100% NPK	3.72	3.23	2.93	3.64	3.30	2.90
Mean	2.97	2.85	2.54	**2.79**	**2.66**	**2.47**
L.S.D. at 5%	S=0.39 F= .42 S.F=.07			S=N.S. F=0.16 S.F=0.10		

S= Soil **F= Fertilizer**

Discussion

The increase of macronutrients i.e. N, P and K in herb of roselle plants may be due to the vigorous vegetative growth and root system area, consequently, increasing nutrients uptake by plant. The positive effect of clay soil on nutrients uptake/plant may be due to its mineral enrichment and higher content of organic materials than other soils. While sandy soil doesn't hold many nutrients and less organic matter. [36] found that clay soil is usually mineral rich and high fertility. However, they have high content of organic matter than saline loamy sand. The chemical compositions were increased in clay soil than saline loamy sand; this might be due to high content of organic matter and availability nutrients. On the other hand, the increase in salt concentration led to an unfavorable effect on nutrient balance.

[37] on roselle plants found that salinity decreased mineral uptakes and anthocyanin pigments. This decrease of chemical composition was mainly attributed to one or more of responses, i.e. toxicity of one or more specific ions present in high dose[38] or combination of both reasons [39]. Salinity is one of the most important environmental factors limiting absorption of nutrients. Salt stress can affect plant survival, biomass and plant morphology and affect the capacity of plant to collect water and nutrients [40].

Phosphorus availability is reduced in saline soils because of ionic strength effects that reduce the activity of phosphorus and because P concentration in soil solution is tightly controlled by absorption processes and by the low solubility of Ca-P minerals [41].

The positive effect of NPK fertilization treatment has significant promoting influences on the chemical constituents with roselle plants in comparison to non fertilized plants. The

enhancement of the aforementioned parameters due to NPK fertilization could be explained in light of the physiological and important roles of each macronutrients which were described by different authors namely, [42,43] as follows:

Nitrogen is essential for protein formation, all simple and conjugated proteins and forms part of nucleic acids (DNA and RNA) are major constituents of plant cell cytoplasm. This macronutrient is present in different organic compounds i.e. amino acids, many enzymes, many energy transfer compounds (ADP and ATP), chlorophylls, pigments, vitamins, alkaloids, as well as, other organic substances of plant cell and protoplasm. Also, the levels of endogenous hormones were found to response to nitrogen, therefore, the content of free auxin and GA-like substances were decreased and growth inhibiting substances were increased due to the deficiency of nitrogen. Moreover, the plant cannot carry on its life processes if it lacks nitrogen to form all aforementioned vital constituents, for examples: N deficiency is accompanied by failure to synthesize normal amounts of chlorophylls, which affect directly the photosynthesis process and this process can produce carbohydrates, but it cannot go on the production of protein, nucleic acids unless N is available.

Phosphorus in cell becomes united with carbon, oxygen, nitrogen hydrogen and other elements to form complex organic molecules. Phosphorus is involved in the processes of phosphorylation, which cause production of higher energy compounds (ADP and ATP) which are responsible for most of the energy changes in the life processes. In addition, phosphorus has fundamental roles in different enzymatic reactions. Moreover, phosphate compounds are essential and important for many physiological processes namely, photosynthesis, interconversion of carbohydrates and related glycolsis, amino acids metabolism, fat metabolism and biological oxidation. Also, phosphorus is a constituent of the coenzymes NAD and NADP which are the very important materials of two physiological processes i.e. photosynthesis and respiration in the plants.

Potassium aids in the uptake of other nutrients and in their movement within the plant. In addition, the presence of potassium and other ions in solution helps to maintain the osmotic concentration necessary to cell turgid, which is essential for adjustment of stomatal movement and water relations. Moreover, potassium is necessary for the metabolism of carbohydrates via its influence on photosynthesis process and plays an important role in translocation of carbohydrate from the plant leaves to the roots. [44] confirmed that K-fertilization stimulates carbohydrate synthesis in roselle plant leaves. Concerning anthocyanins, it is well known that they are directly related to flavonoids, which can be found as free aglycons (anthocyanidins) or, more often, as heterosides (anthocyanosides). They are coloured compounds; their colour varying with the pH, the applied organic compost induced a significant increment in flavons content in roselle sepales expressed as mg g^{-1} DW due to the significant increase in sepales yield.

Nitrogen fixation can be defined as the phenomenon of conversion of free nitrogen into nitrogenous salts to make it available for absorption by plants. The fixation of nitrogen can be either a physical nitrogen fixation or a biological nitrogen fixation [45]. N-fixing bacteria strains produce some amino acids, namely, aspartate, glutamate, serine and glutamate[46], improve water status, augmenting the activity of nitrate reductase, produce siderophores which are responsible for improving iron nutrition of the plant [47].Moreover, N-fixing bacteria, in general, positively affect their host plants through one or more mechanism i.e. N-fixation, producing of organic acids, enhancing the uptake of different nutrients or protecting the roselle against plant pathogens. Plant inoculation with nitrogen-fixer such as Azotobacter affected many foliage parameters. These changes were directly attributed to positive bacterial effects on mineral uptake by the plants, enhancement in uptake of NO_3^-, NH_4^+, PO_4^{--}, K^+ and Fe^{++} by nitrogen fixing bacteria was proposed to cause an increase in foliar dry matter [48]. Effect of biofertilizer on chemical constituents of leaves had been reported by several researchers [14] such beneficial effects of the non- symbiotic N_2-fixing acteria on the morphology and/or physiology of root system which; consequently, promoted uptakes of mineral nutrients nitrogen, phosphorus and potassium.

Phosphate dissolving bacteria leads to an increment of available phosphorus in plant tissue, which affects a large number of enzymatic reactions that depend on phosphorylation [49]. Moreover, inoculating the plants with or without mineral phosphorus fertilizers augments the

concentration of phosphorus in the soil and plant tissue and improves the uptake of minerals and water use efficiency [50].

From the aforementioned discussion of the results regarding the influences of mineral nitrogen, phosphorus and potassium fertilization and inoculation with N-fixing bacteria strains (*Azotobacter chroococcum*) and (*Bacillus polymyxa*) as phosphate dissolving bacteria, it could be concluded that the beneficial and unique roles of NPK fertilization and N and P biofertilization were responsible for stimulation of different physiological and biological processes which reflected on enhancing different chemical constituents of roselle plants. Biofertilizers, which can be defined as preparation containing, live cell of efficient strains of nitrogen fixing organisms or phosphate-solublizing bacteria could be used instead of chemical fertilizers. Moreover, these components increase the availability of nutrients in a form, which can be easily assimilated by plants [51]. Moreover,[52] mentioned that bacteria of Azotobacter could produce Indol-acetic acid and cytokinin, which increase the surface area per unit root of nutrients from soil

Because the percentage of potassium in the stem is higher, to strengthen the stem, and the determination was made in the stem and leaves.

(The pH is the negative logarithm of hydrogen concentration. Decreased pH means increased acidity)

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