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Effect of Bio-and Chemical Fertilizers on Growth, Sepals Yield and Chemical Composition of *Hibiscus sabdariffa* at New Reclaimed Soil of South Valley Area

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ABSTRACT

The objective of this study was to evaluate the effect of biofertilizers in combination with different rates of chemical fertilizers on the growth characters, yield component and chemical constituents of *Hibiscus sabdariffa* (Roselle) plants under reclaimed land conditions of Qena. The biofertilizers were used as seeds inoculation with *Azospirillum* sp. (nitrogen fixing bacteria, N.F.B.) and *Bacillus polymyxa* (phosphate dissolving bacteria, PDB) and their mixture. The obtained results revealed that the inoculation with the mixture of biofertilizers combined with 50 or 100% chemical fertilizers improved, in most cases, growth characters and increased sepal yield or at least did not differ significantly from the control (full recommended dose of NPK alone). So, applying 50% of the recommended dose of NPK plus mixture of biofertilizer can save half of the quantity of chemical fertilizer, decrees the production cost and obtain high quality product.

Key words: Roselle, biofertilizers, calyx, medicinal plant, vitamine C

INTRODUCTION

Hibiscus sabdariffa L. (Roselle) is a subtropical medicinal plant belongs to the family Malvaceae. The fruit calyxes of this plant are used for preparing refreshing beverage and jellies of brilliant red color with pleasant acidic taste. In addition the drink has a laxative effect due to organic acids present in the calyxes. The calyx extraction has a great therapeutic action for curing heart and nerve diseases and high blood pressure (Hassan, 2009). It presents antimicrobial activities due to its phenolic compounds. It contains protein, fibers, calcium, iron, carotens and vitamin C (Fasoyiro *et al*., 2005).

Biofertilizers are the formulation of living microorganisms, which are able to fix atmospheric nitrogen and convert insoluble phosphorus to be valuable for the plants (Mahfouz and Sharaf-Eldin, 2007).

Recently, the production of chemical-free medicinal and aromatic plants has been the focus of interest of many researchers and producers in order to ensure the high quality and safety, not only for human, but also for the environment. Investigation took place for using biofertilizers as an alternative to chemical fertilizers or at least to minimize the levels of these chemicals in order to protect the environment from pollution, decrease the production cost and produce chemical-free

product. The vegetation growth parameters as well as sepal yield of roselle plant were increased when seeds were inoculated with *Rhizobium* and *Azotobacter* (Harridy and Amara, 1998; Hassan, 2009) or nitrobin which a bio-source of nitrogen (Shaalan *et al*., 2001).

Inoculation of seeds with *Azotobacter* and *Azospirillum* in the presence of chemical fertilizers resulted in improving both growth and yield of anise (Gomaa and Abo-Aly, 2001), *Foeniculum vulgare* (Kandeel *et al.,* 2001; Mahfouz and Sharaf-Eldin, 2007) and *Nigella sativa* (Shaalan, 2005).

Biofertilizers (N fixing and P dissolving bacteria) had an effective role in partial replacement of N and P mineral fertilization. This role was revealed by Ibrahim (2002), Badran and Safwat (2004) on fennel, Soliman (2002) on anise, Safwat and Badran (2002) on cumin and Badran *et al.* (2002) on black cumin.

The aim of this investigation was to study the effect of some bio-and chemical fertilizers on the growth characters, yield component and chemical constituents of roselle plants under reclaimed land condition of Qena.

MATERIALS AND METHODS

The experiment was conducted at the Experimental Farm of Ornamental Plants. Faculty of Agriculture, South Valley University, Qena, Egypt, which located at latitude 26 11 25 N" and longitude 32 44 42" E, during the two successive seasons of 2008 and 2009.

Physical and chemical analyses of soil were done in the Soil Analysis Laboratory of the Soil and Water Department, Faculty of Agriculture, South Valley University. The soil of the experimental field was salty loam and its characteristics are presented in Table 1.

Seeds of roselle (Dark Red cultivar) were obtained from the Medicinal and Aromatic plants Department, Horticulture research Institute, Ministry of Agriculture, Egypt. Seeds were coated with 10% Arabic gum as an adhesive and rolled into the biofertilizers treatments. The biofertilizers used as inoculums for seed treatments were *Azospirillum* (nitrogen fixing bacteria, N.F.B.) and

Table 1: Some physical and chemical characteristics of the used soil

Characteristics	Value
Physical properties	
Sand (%)	64.91
Silt (%)	13.49
Clay (%)	21.6
Soil texture	Sandyclayloam
Saturation (%)	32.0
Chemical properties	
pH	8.05
EC_e (d Sm^{-1})	3.68
CaCO (%)	6.9
Ca^{++}(meq L^{-1})	13.76
Mg^{++} (meq L^{-1})	11.79
K^+ (meq L^{-1})	0.37
Na^+ (meq L^{-1})	12.97
CO_3^{2-}+HCO^{3-} (meq L^{-1})	13.76
SO_4 (meq L^{-1})	7.71
Cl^- (meq L^{-1})	15.26

Bacillus polymyxa (phosphate dissolving bacteria, PDB) and their mixture. All strains were locally isolated from soil rhizosphere of different plants during a previous study (Badawy *et al.*, 2003). Seeds were sown directly on 1 May 2008 and 2009 for the two seasons.

The experiment was carried out in a complete randomized blocks design with thirteen treatments and three replications. There were five holes per each ridges and four ridges for each treatment/replicate with distance 50 cm between ridges and 50 cm between holes, five seeds were placed in each hole. After one month and half, the plants were thinned at one plant per each hill.

The recommended dose of NPK chemical fertilizers used in this experiment according to the Ministry of Agriculture, Egypt., as control treatment, was 225 kg fed^{-1} ammonium nitrate 33% N, 150 kg fed^{-1} super phosphate 15.5% P_2O_2 and (75 kg potassium sulphate 48% K_2O) which was applied for all experiment treatments. The doses of nitrogen and phosphorus chemical fertilization were manipulated at various levels (0, 25, 50 and 100%) in different combinations with biofertilizers as shown in Table 2.

Phosphorus fertilizer was added during soil preparation prior to sowing, while the amount of nitrogen chemical fertilization was added in two equal batches, the first one was added after thinning with all amount of potassium and after 45 days from the first addition, the second amount of nitrogen was added.

Morphological estimation: The following measurements were recorded at the beginning of flowering stage using five plants for each treatment per replication : number of branches, fresh weight of vegetative growth and roots. Dry weight of vegetative growth and roots which recorded after drying the sample in an oven at 70°C for 72 h, while plant height, number of fruits per plant and calyx dry weight per plant were recorded after the maturity of fruits.

Chemical estimation: Leaf and calyx nitrogen percentage was determined by the micro-Kjeldahl method as recommended by Bremner and Mulvany (1982). Available phosphorus was determined by the method of Jackson (1967). Anthocyanin content were determined by the method of Tibor and Francis (1968). Vitamin C was determined as described by Jacobs (1951). Sepals' acidity was determined according to the method of Chemists (1970).

Table 2: Details of recommended doses of bio- and chemical fertilizers for the different treatments

Treatment	Recomendation
Control	Full recommended dose of phosphorus, nitrogen and potassium(NPK)
PDB	Inoculation with phosphate dissolving bacteria (*Bacillus polymyxa*) without the chemical fertilizers of nitrogen and phosphor
PDB+25%	PDB+25% of recommended dose of NPK
PDB+50%	PDB+50% of recommended dose of NPK
PDB+100%	PDB+100% of recommended dose of NPK
NFB	Inoculation with Nitrogen fixing bacteria (*Azospirillum lipoferum*)
NFB+25%	NFB+25% of recommended dose of NPK
NFB+50%	NFB+50% of recommended dose of NPK
NFB+100%	NFB+100% of recommended dose of NPK
NFB+PDB	Inoculation with mixture of Nitrogen fixing bacteria and phosphate dissolving bacteria
NFB+PDB+25%	(NFB+PDB)+25% of recommended dose of NPK
NFB+PDB+50%	(NFB+PDB)+50% of recommended dose of NPK
NFB+PDB+100%	(NFB+PDB)+100% of recommended dose of NPK

PDB: Phosphate dissolving bacteria and NFB: Nitrogen fixing bacteria

Statistical analysis: The data were statistically analyzed as Means and was compared using LSD at 5% according to Steel and Torrie (1982).

RESULTS

Vegetative growth characteristics

Plant height: Data presented in Table 3 revealed that the addition of NPK recommended dose with mixed biofertilizers increased significantly plant height in both seasons compared to control (NPK recommended dose only). Also highly significant increase was recorded in the case of the same chemical fertilizers dose with NFB in the second seasons.It was found also that the differences between 100% chemical NPK (control) and 50% NPK plus all biofertilizer treatments were not significant in both seasons.

Number of branches/plant: In both seasons, it seems that all biofertilizer treatments with full dose of NPK significantly increased the number of branches/plant compared to 100% chemical fertilizers alone (control), while PDB biofertilizer treatment plus 100% NPK recorded significant increase in the first season only as shown in Table 3.

The increases in number of branches per plant in the first season were 32.2, 24.7 and 31.1% for inoculation treatment with PDB, NFB and their mixture plus 100% NPK for first season respectively, while the increases in the second season were 16.6 and 41.3% for inoculation with NFB and NFB+PDB plus 100% chemical fertilizers, respectively.

Fresh and dry weight of shoots and roots of Roselle plants: Data in Table 4 showed that fresh and dry weight of vegetative growth for plants treated with different biofertilizer treatments plus 50 or 100% chemical fertilization did not differ significantly compared with the control in both seasons, except for fresh weight of plants treated with PDB plus 50 and 100% NPK in the first season. Significant increases in vegetative dry weight were recorded in the case of inoculation

Table 3: Effect of bio-and chemical fertilizers on plant height and numbers of branches of *Hibiscus sabdariffa* L. plants during first and second seasons

	Plant height (cm)		No. of branches/plant	
Treatments	Season 1	Season 2	Season 1	Season 2
Control (100%)	102.72	110.56	31.00	22.02
PDB	70.80	93.00	12.67	11.86
PDB+25%	89.00	97.22	22.00	16.11
PDB+50%	109.32	105.22	28.00	17.22
PDB+100%	113.50	108.78	41.00	23.11
NFB	94.40	109.00	15.00	11.00
NFB+25%	106.00	100.77	25.00	21.22
NFB+50%	107.17	113.44	28.50	23.11
NFB+100%	110.00	128.11	38.67	25.67
NFB+PDB	91.30	104.89	14.00	11.11
NFB+PDB 25%	109.67	107.89	19.33	18.56
NFB+PDB 50%	110.50	114.11	30.83	21.78
NFB+PDB 100%	123.17	120.67	40.67	31.11
L.S.D at 0.05	9.37**	5.73**	5.90**	3.68**

** Significant at p = 0.01

Table 4: Effect of bio-and chemical fertilizers on fresh and dry weight of vegetative growth and roots fresh and dry weight of *Hibiscus sabdariffa* L. during first and second seasons

	Fresh weight of vegetative growth (g/plant)		Dry weight of vegetative growth (g/plant)		Roots fresh weight (g/plant)		Roots dry weight (g/plant)	
Treatments	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Control (100%)	460.83	421.44	124.33	97.86	31.11	23.11	9.97	7.17
PDB	282.00	275.11	64.00	58.49	14.74	16.55	4.29	4.82
PDB+25%	301.67	286.11	71.67	68.50	19.49	18.89	4.80	5.14
PDB+50%	348.33	429.53	110.83	93.74	19.81	20.33	9.80	7.27
PDB+100%	447.50	451.29	115.83	109.67	38.68	31.00	10.37	8.31
NFB	340.00	347.22	80.10	71.29	16.52	18.00	4.51	4.91
NFB+25%	381.00	391.77	82.50	79.70	19.32	18.67	5.71	5.51
NFB+50%	418.50	395.11	113.32	83.20	29.21	21.44	9.05	7.64
NFB+100%	490.97	429.78	128.33	97.84	37.42	31.56	11.50	8.27
NFB+PDB	330.00	255.67	78.40	57.02	16.02	16.51	4.72	4.87
NFB+PDB 25%	363.33	314.87	84.25	76.89	19.85	17.22	5.85	5.08
NFB+PDB 50%	453.00	388.68	123.83	101.31	26.10	20.00	9.35	7.02
NFB+PDB 100%	483.83	434.11	134.83	107.54	39.79	30.87	11.57	8.66
L.S.D at 0.05	89.93**	33.58**	20.21**	6.53**	3.45**	4.09**	1.03**	1.46**

** Significant at 0.01

treatment with PDB and the mixture of NFB and PDB with 100% chemical fertilizers in the second season. All biofertilizer treatments without NPK reduced thr fresh and dry weight of the vegetative growth.

Data presented in Table 4 showed that the roots fresh weight significantly increase as a result of the application all biofertilization treatments plus 100% chemical fertilization in the two seasons compared to 100% chemical fertilizers alone. Results of roots dry weight showed that significant increases in roots dry weight as a result of the application of all biofertilizer treatments plus 100% chemical fertilizer recommended dose in both seasons except of PDB and NFB treatments in the second season and PDB treatment in the first season.

Data also revealed that no significant differences were found in roots dry weight between applications of biofertilization treatments plus 50% of chemical NPK dose compared to 100% chemical fertilizers (control).

The yield parameters

Number of calyx/plants: The maximum number of calyx in the first season was obtained in the plants treated with 100% NPK chemical fertilizers plus NFB biofertilizers or plus mixture of NFB and PDB with compared to 100% NPK chemical fertilizers alone in the first season as shown in Table 5.

In the second season the highest calyx number per plant was recorded in the case of inoculation treatment with the mixture of nitrogen fixing bacteria and phosphate dissolving bacteria plus 100% and 50% chemical fertilizers as compared with 100% chemical fertilizers alone.

Sepals dry weight and yield: Data recorded in Table 5 showed that there were significant increases in sepals dry weight and yield as a result of inoculation with mixture biofertilization treatments plus 100% chemical fertilizers compared to the control in both seasons. Also, addition 50% of chemical NPK dose to mixture of biofertilizers treatment gave almost the same yield obtained by application 100% chemical fertilizers alone.

Table 5: Effect of bio-and chemical fertilizers on number of calyx/plants, calyx dry weight and yield/fed. of Roselle plants during first and second seasons

	Calyx number /plants		Sepals dry weight (g/plant)		Expected sepals yield[1] (Kg fed^{-1})	
Treatments	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Control (100%)	37.33	23.36	35.67	35.71	606.39	607.07
PDB	19.00	10.00	17.63	17.44	299.71	296.48
PDB+25%	27.50	14.55	26.53	20.33	451.01	345.61
PDB+50%	36.17	18.11	36.08	25.64	613.36	435.88
PDB+100%	40.17	24.22	38.33	32.31	651.61	549.27
NFB	20.00	14.00	21.45	21.74	364.65	369.58
NFB+25%	24.17	15.00	26.83	25.19	456.11	428.23
NFB+50%	39.00	19.22	36.17	29.17	614.89	495.89
NFB+100%	42.67	24.88	39.33	30.72	668.61	522.24
NFB+PDB	19.00	12.33	18.50	19.34	314.50	328.78
NFB+PDB 25%	27.17	22.11	26.23	25.16	445.91	427.72
NFB+PDB 50%	34.65	29.67	37.50	35.58	637.50	604.86
NFB+PDB 100%	42.50	38.00	42.62	39.50	724.54	671.50
L.S.D 0.05	3.99**	4.60**	3.98**	3.28**	21.5**	15.27**

** Significant at p = 0.01, [1]From approximately 17000 plants/feddan

With respect to sepals yield, data presented that the highest yield was obtained in case of application of full dose of chemical fertilizers plus BDP, NFB and mixed biofertilizers treatments in the first season. Data also obtained that, application of 50% chemical fertilizers plus mixed biofertilizers recorded significant increase in sepals yield comparing with 100% chemical fertilizers alone. In the second season, application of full dos of chemical fertilizers plus mixed biofertilizers treatment increased significantly sepals yield comparing to chemical fertilizers alone.

The increment in the dry sepal yield for the mixture of biofertilizers plus 100% NPK treatment were 19.48 and 10.61% in the first and second seasons, respectively.

Chemical constituents

Nitrogen percentage (N%): Data in Table 6 indicated that in both seasons the addition of 100% chemical fertilizers in combination with all biofertilizers increased significantly N% in the leaves compared to control treatment (100% chemical fertilizers). Also, data showed that addition of 50% chemical fertihzers dose gave the same effect on N% in case of combination with NFB biofertilizer or mixed biofertilizer.

With regard to N% in sepals, data revealed that the highest N% was obtained in case of application of full dose of chemical fertilizers plus both of NFB or mixed biofertilizers treatments.

Phosphor percentage (P%): Concerning the effect of bio-fertilizers in combination with the different levels of NPK chemical fertilizers on P percentage of roselle plants, data in Table 7 showed that the highest significant increase in P percentage were found, during the two seasons with the biofertilizer treatments of NFB and mixture of NFB+PDB plus 100% chemical fertilizers. Data also showed that sepals P% was not affected by the application of the different biofertilizer treatments with full dose of chemical fertilizer.

Table 6: Effect of bio- and chemical fertilizers on N% of leaves and sepals of roselle plants during first and second seasons

	Leaf N%		Sepals N%	
Treatments	Season 1	Season 2	Season 1	Season 2
Control (100%)	1.24	1.03	2.14	1.94
PDB	0.67	0.60	1.24	1.31
PDB+25%	0.69	0.65	1.81	1.52
PDB+50%	1.33	0.99	1.86	1.62
PDB+100%	1.43	1.34	2.05	2.03
NFB	0.70	0.64	1.33	1.19
NFB+25%	0.60	0.59	1.34	1.30
NFB+50%	1.50	1.32	1.96	1.95
NFB+100%	1.71	1.40	2.41	2.30
NFB+PDB	0.91	0.76	1.65	1.41
NFB+PDB 25%	0.91	0.81	1.72	1.44
NFB+PDB 50%	1.51	1.26	2.08	1.81
NFB+PDB 100%	1.90	1.87	2.47	2.34
L.S.D at 0.05	0.16**	0.10**	0.25**	0.15**

** Significant at p = 0.01

Table 7: Effect of bio-and chemical fertilizers on P% on *Hibiscus sabdariffa* L. leaves and sepals during first and second seasons

	Plant P%		Sepals P%	
Treatments	Season 1	Season 2	Season 1	Season 2
Control (100%)	0.58	0.47	0.87	0.78
PDB	0.38	0.34	0.29	0.26
PDB+25%	0.45	0.42	0.36	0.31
PDB+50%	0.55	0.48	0.38	0.33
PDB+100%	0.65	0.58	0.85	0.79
NFB	0.47	0.43	0.26	0.22
NFB+25%	0.53	0.44	0.30	0.29
NFB+50%	0.53	0.48	0.62	0.62
NFB+100%	0.69	0.66	0.85	0.81
NFB+PDB	0.37	0.31	2.26	0.23
NFB+PDB 25%	0.49	0.45	0.32	0.28
NFB+PDB 50%	0.62	0.51	0.61	0.50
NFB+PDB 100%	0.67	0.67	0.89	0.82
L.S.D at 0.05	0.09**	0.05**	0.09**	0.05**

** Significant at p= 0.01

Moreover, it can be seen in Table 6 that the differences between the treatments of 50% NPK+bio-fertilizers compared with 100% NPK chemical fertilizers treatments were not significant with respect to the phosphorus percentage and its total content in the leaves.

Anthocyanin, Vitamin C and sepals acidity: Data in Table 8 revealed that there were no significant differences on anthocyanin and vitamine C content of the sepals as a result of the biofertilizers treatments compared to 100% NPK chemical fertilizers treatments alone.

Also, the data as shown in Table 8 revealed that a significant increase was recorded in the acidity of roselle sepals. This high acidity was found in case of applying biofertilizers (Mixture) plus 100% chemical NPK fertilization and also with using NFB plus 100% NPK.

Table 8: Anthocyanin, Vitamin C content (mg g^{-1} dry weight) and Acidity in calyces of roselle plant as affected by different bio and chemical fertilizers

	Anthocyanin content (mg g^{-1} dry weight)		Vitamin C content (mg g^{-1} dry weight)		Sepals Acidity%	
Treatments	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Control (100%)	89.37	82.70	25.30	25.80	0.48	0.40
PDB	55.30	49.60	22.20	19.90	0.31	0.30
PDB+25%	59.53	52.80	25.70	22.40	0.41	0.44
PDB+50%	67.53	64.91	28.90	25.30	0.50	0.46
PDB+100%	91.10	85.00	29.20	27.20	0.59	0.50
NFB	58.43	52.50	27.10	25.30	0.45	0.46
NFB+25%	62.16	59.80	18.50	17.80	0.55	0.50
NFB+50%	82.90	82.00	19.80	19.40	0.52	0.53
NFB+100%	67.66	98.10	27.90	25.70	0.59	0.57
NFB+PDB	62.60	54.50	19.50	17.10	0.54	0.50
NFB+PDB 25%	64.16	56.70	24.10	22.00	0.55	0.53
NFB+PDB 50%	96.40	79.70	27.70	22.80	0.59	0.53
NFB+PDB 100%	102.06	100.03	27.80	25.90	0.59	0.58
L.S.D at 0.05	25.95^{NS}	22.09^{NS}	4.22^{NS}	7.20^{NS}	0.09**	0.15*

NS,*, ** Non significant, significant at p = 0.05 and 0.01, respectively

DISCUSSION

The obtained results show that biofertilizers treatments promoted the growth characters and increased the yield component of roselle plants. These results are in agreement with those of Mohamed and Ahmed (2003) on fennel, Shaalan (2005) on *Nigella sativa*, Yuonis *et al.* (2004) on *Ammi visnaga*, Heikal (2005) on *Thymus vulgaris*, Abd El-Latif (2006) on *Salvia officinalis*, Swaefy Hend *et al.* (2007) on peppermint and Hassan (2009) on Roselle.

These results could be attributed to the effect of the non-symbiotic N_2-fixing bacteria (*Azospirillum)* producing adequate amounts of IAA, gibberellins, cytokinins and B group vitamins that promote rooting capacity, root length and enhanced the root hair branching with an eventual increase on the uptake of nutrients from the soil (Rodriguez and Fraga, 1999; Revilas *et al.*, 2000).

Phosphate solubilizing bacteria (*Bacillus polymyxa*) release organic and inorganic acids which reduce soil pH leading to change of phosphorus and other nutrients to available forms ready for uptake by plants (Singh and Kapoor, 1999).

Therefore, the percentages of N and P elements in the leaves were increased and this increment led to promote the growth and yield of roselle plants. Similar results have been reported by Kandeel *et al.* (2001), Mahfouz and Sharaf-Eldin (2007) on *foeniculum*, Hassan (2009) on roselle and Mostafa and Abo-Baker (2010) on sunflower.

The obtained results showed that using bacteria inoculation combined with 50 or 100% chemical fertilizers improved, in most cases, the growth characters and increased sepal yield or at least did not differ significantly from the control (full recommended dose of NPK fertilizers alone).

It is well known that the chemical fertilizers promote plant growth through the role of nitrogen in protein synthesis and increasing the meristmatic activity. In addition, mineral P is an essential component of the energy compounds (ATP and ADP) and phosphoproteins. Similar results have been reported when roselle plants were treated with NPK fertilizers (Harridy and Amara, 1998).

Adding of 50% of the recommended dose of chemical fertilizers to the biofertilizers improved the plant growth and increased the sepal yield. This improvement may be due to the direct effect of chemical fertilizers or indirect through the microbial propagation activation. These results are in accordance with those obtained by Shaalan *et al.* (2001) on roselle plants, Shaalan (2005) on *Nigella sativa* plants and Hassan (2009) on roselle.

CONCLUSION

It could be concluded that biofertilizers promoted the growth and increased the sepal yield of roselle plants compared to the chemical fertilization alone. Applying 50% of the recommended dose of NPK plus mixture of biofertilizers, decrease the production cost and obtain high quality product.

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