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Effect of Cobalt and Nickel on Plant Growth, Yield and Flavonoids Content *of Hibiscus sabdariffa* L.

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Abstract: The anthocyanins have a long history as a part of human diet, and their other flavonoids are receiving, renewed attention for their positive health attributes. The principal commercially availability anthocyanins food colorant sources is roselle (*Hibiscus sabdariffa* L). Thus, the aim of this investigation was to study the effect Co (0, 20, and 40 mg/kg^{-1} soil)and Ni (0, 25 and 50 kg^{-1} soil) and their combination on growth, flower yield, macro and micronutrients contents and the quantity of anthocyanins and flavons of roselle calyces. The obtained results showed that the application of Co and Ni at 20 + 25 mg/kg^{-1}soil gave the highest effect on increasing plant height, No. of branches as well as fresh and dry weight of roselle calyces. Mineral content is an essential component of nutritive values of roselle leaves and calyces. The low Co doses (20 mg/ kg soil) posses a synergistic effect on the status of Ni, Mn, Zn and Cu but it's gave adverse effect on Fe. Increasing Ni up to 50 mg/kg soil posses promotive effect on the status of Co, Mn, Zn, and Cu in leaves and calyces of roselle plant The addition of Co and Ni at 20 + 25 kg^{-1} soil was the most effective on increasing the status of N, P, K, Co, Ni, Mn, Zn and Cu on leaves and calyces of roselle plant. However, there were significantly correlations of some studied micronutrients and the quantity of anthocyanins and flavons and the highest contents were obtained with applying Co and Ni at the lowest level (20 + 25 mg/kg^{-1} soil).

Key words: Roselle (*Hibiscus sabdariffa* L), anthocyanins, flavons, Co, Ni., macro-micronutrients

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L) family Malvaceae, known commonly as "karkade" is cultivated in the tropical and subtropical countries. It is considered as one of the important medicinal plant. The part used is the dried, fleshly calyces which had a large quantities of organic acids (oxalic, malic, citric and tartaric acid) having therapeutic and diuretic properties. It affects on organisms, shows on abundant diuerisis accompanied by slightly diaphoretic action, activation and neutralization of hepatic secretions, activation of gastric secretions, and intestinal contractions which permit of rapid digestion decrease in hyper-viscosity of the blood and in arterial pressure, hence its efficiency in arteriosclerasis is found. Its sporific action has a favourable effect on the functions of the stomach, possesses a high intenstinal antiseptic action, and can be used to compact various infectious intenstinal diseases (Rovesti, 1936). The drug can also be used in cases of bacterial infections as it kills various micro-organisms (Sharaf, 1962). Roselle calyces contain two types of anthocyanins; hibiscin and gossyptin that used in conjuction with a natural base for colouring syrups and liquors. The anthocyanins pigments of *Hibiscus sabdariffa* L., flowers are suitable for use as natural food colouring agents (Sanyo, 1981). The flower buds of *H. sabdariffa* are used in refreshing infusion, decrease blood pressure, and cause relaxation of rat uteri, inhibition of taenia mortality and bacterial growth (Muller and Franz, 1992).

Roselle is cultivated in Egypt throughout the country from north to south, although the southern regions are more suitable for its cultivation. However, the new reclaimed soils are suitable for such plants, which are able to grow under different climatic conditions.

Cobalt is an essential element for the synthesis of vitamin B_{12}, which is required for human and animal nutrition (Young, 1983 and Smith, 1991). Unlike other heavy metals, cobalt is saver for human consumption up to 8 mg can be consumed on a daily basis without healt hazard (Young, 1983). The discovery in 1975 that Ni is a component of the enzyme urease (Dixon *et al*., 1980) which is represented in a wide range of plant species (Welch, 1981). It is known that certain inorganic trace elements such as vanadium, Zinc chromium,

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copper, iron, potassium, sodium and nickel play an important role in the malignance of normologycemia by activing the beta-cells of the pancreas. Thus the elemental composition in leaves of *Murraya koeningii, Mentha piperita, Bcimum Sanctum,* and *Aegle marmelos* widely used in treatment of diabetes-related metabolic disorders (Narendhirakannan *et al*., 2005).

Apati *et al*. (2003) showed a relationship between some element concentrations and the presence and quantity of flavonoids of *Solidago canadensis* . Blazovics *et al*. (2003) showed that metallic ion analysis showed significantly high concentrations of Al, As, Ba, Cr, Cu, Fe, Mn, Ni, and Ti in the drug of Chinese Beiqishen tea. The tea in fusion contained some non-desirable trace elements and caffeine in addition to polyphenols and tannis in high concentrations. Therefore, the consumption of the tea may involve risks. Maryam Mirza *et al*. (2004) stated that trace elements (Cu, Zn, Mn, Fe, Co, Ni, Cd, Pb, Cr, Ag, Na and K) in indigenous medicinal diuretic plants (*Cymbopogon citrates, Raphanus sativus and Zea mays)* have possible role in human health and disease. Pan Zuewu *et al*. (2004) reported that the addition of microelements (BO_3^{---} - MoO_4^{--} , Co^{++}, Cu^{++}, Fe^{++} and Zn^{++} have important roles on the biosynthesis of comptothecin and growth of suspension cultures of *comptotheca acuminate*.

Therapeutical relevance, presumably related to the combined effect of organic and inorganic compounds, like flavonoids and metal ions, has received great attention in recent years (Szentmihhalyi *et al*., 1998). Flavonoids, especially quercetin and derivatinesm, inhibit the enzyme neutral endopeptidase, which is responsible for the the interaction of the atrial natriuetic peptide and thus regulate the formation of urine via the excretion of sodium ions. Thus it could be interpreted as basis of enhanced urinary flow therapy (Budzianowski *et al*. 1990; Melzig and Major, 2000).

Thus, the aim of this investigation was to study the effect of Co (0, 20, and 40 mg/kg^{-1} soil)and Ni (0, 25 and 50 mg/kg^{-1} soil) and their combination on growth, flower yield, macro and micronutrients contents and the quantity of anthocyanins and flavons of roselle calyces.

MATERIALS AND METHODS

This investigation was carried out during two seasons of 2005 and 2006 under greenhouse of National Research Centre, Dokki, Cairo, Egypt.

Soil analysis:

Physical and chemical properties of the soil used in the experiment are shown in Table (1). Particles size distribution along with soil moisture of the soil samples were determined as described by Blackmore (1972). Soil organic matter, $CaCO_3$, EC, pH as well as soluble cations and anions were determined according to Black *et al* (1982). Determination of soluble and available micronutrients was run after Jackson (1973). Total, available and soluble Co and Ni were determined according to Cottenie *et al* (1982).

Seeds of roselle (*Hibiscus sabdariffa* L.) were obtained from Medicinal and Aromatic plants, Research, Ministry of Agriculture. The design of the experiment was complete randomized block with three replicates. The seeds of roselle were sown on May 15th in 30 cm diameter porous pots filled with 12 kg soil.

Three weeks after sowing, the seedling were thinned to one seedling per pot, and directly irrigated only once with cobalt or nickel sulphate solutions of different concentration as follows:

1. Control
2. Cobalt 20 mg/ kg soil.
3. Cobalt 40 mg/ kg soil.
4. Nickle 25 mg/ kg soil.
5. Nickle 50 mg/ kg soil.
6. Cobalt (20 mg/ kg soil) + nickel (25 mg/ kg soil).
7. Cobalt (20 mg/ kg soil) + nickel (50 mg/ kg soil).
8. Cobalt (40 mg/ kg soil) + nickel (25 mg/ kg soil).
9. Cobalt (40 mg/ kg soil) + nickel (50 mg/ kg soil).

Basic dressing was applied to all plants and consisted of nitrogen (ammonium sulphate 20.5 %N), phosphorus (superphosphate 15.5 % P_2O_5) and potassium (potassium sulphate 48 % K_2O) at the ratio 2:2:1. The quantities of NPK 4 g/pot were added twice, the first after a month from emergence and the second at the beginning of the flowering. The samples from plants were taken at fruiting stage.

The data were recorded as follows: plant height (cm), number of branches and fruits /plant, fresh and dry weights of calyces and epicalyces g/pant. The data were statistically analyzed according to Snedecor and Cochron (1980).

Table 1: Physical and chemical properties as well as nutrient content of used soil.

Physical properties							
Particle size distribution %				Soil Moisture constant %			
Sand	Silt	Clay	Texture	Saturation	FC	WP	AW
13.40	25.00	61.60	Clay	75.30	42.60	12.30	30.30
Chemical properties							
pH	EC_e dS/m			$CaCO_3$ %			OM %
8.40	3.10			3.27			0.87
Soluble cations (meq/l)				Soluble anions (meq/l)			
Ca^{++}	Mg^{++}	K^+	Na^+	$CO_3^=$	HCO_3^-	Cl^-	$SO_4^=$
7.50	5.00	0.60	22.70	0.00	9.20	21.60	5.00
Nutrients content							
N%	P%	K%	Fe ppm	Mn ppm	ZN ppm	Cu ppm	
13.20	3.80	0.48	45.40	22.82	19.46	13.60	
Nickel (ppm)				Cobalt (ppm)			
Soluble	Available	Total		Soluble	Available		Total
0.56	3.74	13.50		0.49	4.43		15.00

Soil pH was measured in 1:2.5 soil-water suspension, EC was measured as dSm^{-1} in extract soil paste
FC: Field capacity
WP: Willting point
AW: Available water

Chemical analysis:

Representation samples of leaves, calyces and epicalyces of flowers were dried under shading, then dried again at 70 °C until constant was recorded. Anthocyanin was colorimetrically determined in dried calyces and epicalyces of flowers according to the method described by Fahmy (1970). Flavones content in calyces were estimated after De Losse (1970). Macronutrients (N, P and K)as well as micronutrients (Fe, Mn, Zn, Co and Ni) contents were determined according to Jackson (1973).

RESULTS AND DISCUSSIONS

Plant growth and yields:

Data presented in Table (2) show that addition of Co and Ni level singly or in combination with each other significantly increased all the growth parameter i.e. plant height , No. of branches and fruit per plant and fresh and dry weight of calyces of roselle plant as compared with control treatment. The low level of Co (20 mg/kg soil) caused significant increase in plant height, No. of branches and fruits per plant, as well as fresh and dry weights of roselle calyces as compared with the high level (40 mg /kg soil). These observations are consistent with previous reports according to Atta-Aly *et al* (1991) who stated that responses associated with low Co level may be attributed to catalsae and peroxidase enzymes activities which were found to decrease with low levels of Co. In contrary to higher Co ones. These enzymes are known to induce plant respiration (Flanagan and Owens, 1985), so superior resulting in successive consumption for products of photosynthesis and consequently reduction in plant growth. In this concection Liala Helmy and Nadia Gad (2002) stated that plant growth of parsley i.e. plant height, number of leaves per plant as well as fresh and dry weight of leaves and root were significantly increased with low levels of Co (25 mg/kg soil).

Increasing Ni from 25 to 50 mg/kg soil significantly increased plant height from 83.5 to 98.6 cm, No. of branches from 8 to 10, No. of fruits per plant from 16 to 18 fruits as well as fresh and dry weights of calyces from 23.50 and 5.12 to 30.00 and 6.59 g/ plant respectively. This may be due to the effect of Ni as an essential element not only for nitrogen metabolism but also for protein synthesis in higher plants (Brown *et al*, 1990). Confirm the obtained results Yossef *et al* (1998) who reported that Ni deficiency resulted in marked disruption of N metabolism, malate and amino acids in barley while application of Ni at 30 mg/kg soil enhanced dry matter. Moreover, Khan *et al* (2000) found that addition of 0.05 mg Ni / liter to nutrient solution gave the best results in terms of qualitative and quantitative characteristics of spinach.

Most of the basic researcher conducted on Ni, as an essential micronutrients (Brown *et al*, 1987), showed that Ni involved nitrogen metabolism and its related enzymes in higher plants. (Brown *et al*, 1990) stated that Ni is important for improving yield quality and safety of leaf crops for human consumption as reduces leaf content of nitrate and urea. Moreover, Laila Helmy *et al*. (2002) showed that the low level of Ni (40 mg/kg soil) improved not only coriander leaf yield and quality (i.e. leaf area, mineral content, and oil yield) but also the leaves were safer for human consumption since their nitrate and ammonium content were reduced.

The application of Co and Ni at low level (20 + 25 mg /kg soil) gave the highest effect on increasing plant height, as well as fresh and dry weight of calyces as compared with the level of Co20+ Ni 50 mg/kg soil, while the No. of branches and fruits per plant were higher when Co20+ Ni 50 used as compared with

Table 2: Morphological parameters of roselle plants as affected by cobalt and nickel. (mean of two seasons)

Treatments	Plant height (cm)	No. of branches (per plant)	No. of fruit (Per plant)	Calyces weight	
				Fresh (g/plant)	Dry (g/plant)
Control	85.5	5	9	15.6	3.42
Co 20 (mg/kg soil)	104.0	10	19	32.9	7.25
Co 40 (mg/kg soil)	91.8	7	13	25.3	5.56
Ni 25 (mg/kg soil)	83.5	8	16	23.5	5.12
Ni 50 (mg/kg soil)	98.6	10	18	30.0	6.59
Co 20 + Ni 25 (mg/kg soil)	114.8	11	20	37.8	8.24
Co 20 + Ni 50 (mg/kg soil)	106.3	12	24	33.4	7.27
Co 40 + Ni 25 (mg/kg soil)	100.0	10	18	33.1	7.21
Co 40 + Ni 50 (mg/kg soil)	95.3	9	17	27.0	5.89
LSD 5%	0.4	1	1	0.6	0.13

Co20+ Ni 25mg/ kg soil. Data also show that all the growth parameter under study were significantly higher when the level of Co 40 + Ni 25 was used than using the level of Co 20 + Ni 25 and Co20 + Ni 50 and Co40 + Ni 50 respectively.

Anthocyanine and flavons contents:

The concentrations of anthocyanine and flavons as affected by Co and Ni levels are given in Table (3). Results indicate that anthocyanine and flavons contents of roselle calyces were significantly increased by the addition of Co and Ni levels singly or in combination with each other as compared with control treatment.

The addition of Co at the low level (20 mg/kg soil) significantly increased the anthocyanin and flavons content as compared with the addition of the highest Co level (40mg/ kg soil). On the contrary Ni level of 50 mg/kg soil significantly increased the anthocyanins and flavons content as compared with Ni at the level of 25mg /kg soil.

Moreover, the combined treatment of Co and Ni at the low levels (Co 20 + Ni 25 mg /kg soil) was the most significantly effective treatment for increasing anthocyanin and flavons content which gave 31.70 and 26.20 mg/g dry weight, respectively as compared with the other combined treatments (Co20 + Ni50 , Co 40+ Ni25, and Co 40 + Ni 50 mg/kg soil). These results agree with those reported by Apati *et al* (2003) who found that a relationship between some element concentration and the presence and quantity of flavonoids of *Solidaga Canadensis*. Maryan Mirza *et al* (2004) stated that trace elements (Cu, Zn, Mn, Fe, Co, Ni, Cd, Pb, Cr, Ag, Na and K) in indigenous medicinal diuretic plants (*Cymbopgon citrates, Raphanus satevus and Zea mays*) have possible role in human health and disease. Also, the addition of microelements (BO_3^{---}, MoO_4^{--}, Co^{++}, Fe^{++} and Zn^{++} have important roles on the biosynthesis of comptothecin and growth of suspension cultures of *Comptotheca acieminate*.

Table 3: Anthocyanin and flavons content (mg/g dry weight) of roselle calyces as affected by cobalt and nickel. (mean of two seasons)

Treatments	Anthocyanin (mg/g dry weight)	Flavons (mg/g dry weight)
Control	16.20	14.12
Co 20 (mg/kg soil)	29.11	25.00
Co 40 (mg/kg soil)	23.14	21.16
Ni 25 (mg/kg soil)	20.96	19.19
Ni 50 (mg/kg soil)	24.87	22.47
Co 20 + Ni 25 (mg/kg soil)	31.70	26.20
Co 20 + Ni 50 (mg/kg soil)	28.20	20.24
Co 40 + Ni 25 (mg/kg soil)	25.30	18.67
Co 40 + Ni 50 (mg/kg soil)	21.50	15.85
LSD 5%	0.28	0.25

Macronutrients Contents:

Mineral content is an essential component of the nutritive values of roselle leaves and calyces. Data presented in Table (4) show that Co and Ni addition singly or in combination with each other increased the N, P and K concentration in leaves and calyces of roselle plants as compared with control. The application of Co at the low level (20 mg/ kg soil) significantly increased the status of macronutrients (N, P, and K) in leaves and calyces as compared with the higher level of Co (40 mg /kg soil). Moreover the low level of Co combined with the low level of Ni (20+ 25 mg/ kg soil) was the most effective treatment for increasing N, P, and K content in leaves and calyces of roselle plant as compared with other combined treatment and control. (Laila Helmy and Nadia Gad, 2002).

Table 4: Macronutrients content (%) in leaves and calyces of roselle plant as affected by cobalt and nickle. (mean of two seasons)

Treatments	Leaves (Macronutrients (%))			Calyces (Macronutrients (%))		
	N	P	K	N	P	K
Control	2.09	0.39	1.78	1.56	0.19	1.08
Co 20 (mg/kg soil)	2.79	0.49	1.93	1.94	0.29	1.26
Co 40 (mg/kg soil)	2.65	0.41	1.89	1.88	0.24	1.22
Ni 25 (mg/kg soil)	2.66	0.40	1.89	1.87	0.41	1.18
Ni 50 (mg/kg soil)	2.71	0.47	1.96	1.91	0.44	1.24
Co 20 + Ni 25 (mg/kg soil)	2.86	0.64	2.04	2.03	0.47	1.30
Co 20 + Ni 50 (mg/kg soil)	2.81	0.56	1.99	1.98	0.43	1.25
Co 40 + Ni 25 (mg/kg soil)	2.75	0.50	1.87	1.91	0.29	1.19
Co 40 + Ni 50 (mg/kg soil)	2.61	0.42	1.80	1.78	0.24	1.14
LSD 5%	0.11	0.04	0.04	0.12	0.04	0.09

Micronutrients Contents:

Data presented in Table (5) reveal that all the used levels of Co and Ni alone or in combination together significantly increased the content of Co, Ni, Mn, Zn and Cu in roselle leaves and calyces when compared with control treatment. The low Co doses (20 mg/ kg soil) posses a synergistic effect on the status of Ni, Mn, Zn and Cu but it's gave adverse effect on Fe. Increasing Ni up to 50 mg/kg soil posses promotive effect on the status of Co, Mn, Zn, and Cu in leaves and calyces of roselle plant

The highest content of Mn, Zn and Cu in leaves and calyces were attained when Co 20 + Ni 25 was used as compared with the other treatments. The obtained results are in accordance with Bisht (1991) who showed certain antagonistic relationship between cobalt and iron.

In conclusion, there were correlations of Co , Ni , Macro and Micronutrients and the quantity of anthocyanins and flavons in roselle calyces ,and the highest contents were obtained with applying Co and Ni sulphate at the lowest level (20 + 25 kg^{-1} soil). Data noticed that Co and/or Ni alone and combination between them at low rate have a great promotive effect on all the studied parameters. Also, Co and Ni contents (< 8 ppm) in calyces were under the safety human health.

Table 5: Micronutrients content (ppm) in leaves and roselle calyces as affected by cobalt and nickel.

Treatments	Leaves						Calyces					
	Co	Ni	Fe	Mn	Zn	Cu	Co	Ni	Fe	Mn	Zn	Cu
Control	1.69	0.33	162	68.1	51.6	42.2	0.75	0.33	173	52.5	40.9	31.8
Co 20 (mg/kg soil)	8.56	3.64	152	88.2	64.5	55.6	3.22	0.98	166	75.5	52.6	44.0
Co 40 (mg/kg soil)	18.4	1.88	149	83.6	57.0	49.9	5.05	0.86	160	67.8	45.5	39.6
Ni 25 (mg/kg soil)	2.49	10.90	162	76.5	56.4	47.5	1.98	2.02	174	60.6	44.2	36.5
Ni 50 (mg/kg soil)	5.07	21.50	162	65.7	65.7	52.8	3.76	4.56	178	68.0	53.0	41.0
Co 20 + Ni 25 (mg/kg soil)	9.04	6.62	155	72.0	72.0	61.5	4.57	4.20	169	77.7	59.7	50.6
Co 20 + Ni 50 (mg/kg soil)	18.50	14.00	148	68.6	68.6	56.4	6.02	5.91	163	72.5	55.5	46.1
Co 40 + Ni 25 (mg/kg soil)	21.0	14.01	140	65.5	65.5	50.3	7.87	6.52	155	72.3	50.4	43.0
Co 40 + Ni 50 (mg/kg soil)	22.6	11.60	132	57.2	57.2	45.0	8.00	7.60	147	66.9	46.0	37.8
LSD 5%	0.1	0.08	3.00	0.53	0.53	0.82	0.06	0.12	3	0.93	0.54	6.08

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