World Applied Sciences Journal 25 (8): 1231-1238, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.25.08.13378

Effect of Cobalt Supplement on Stevia Rebaudiana Bert. Growth and Yield

Nadia Gad and Hala Kandil

Department of Plant Nutrition, National Research Centre Dokki, Egypt

Submitted: Sep 13, 2013; **Accepted:** Oct 21, 2013; **Published:** Oct 30, 2013

Abstract: A field experiment was conducted at Research and Production Station, National Research Centre, El-Nubaria Site, Beheara Governorate, Delta Egypt under drip irrigation system during the successive season, 2011. Experiment was carried out to evaluate the response of stevia herb yield and sweeteners content to cobalt supplement. Stevia seedlings were irrigated with cobalt once at concentrations: 0.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 ppm.

The obtained results are summarized in the following:

- Cobalt has a significant promotive effect on all growth and yield parameters as well as nutritional and chemical content compared with control.
- Cobalt significantly increase stevia leaves content of sweetners such as glycosids, steviosides and rebaudiosides compared with untreated plants.
- Cobalt at 12.5 ppm recorded the greatest figures.
- Cobalt at 12.5 ppm increased glycoside to 34.11 and 37.0%, steviosides to 27.6 and 31.6%, rebaudiosides to 44.5 and 45.1% respectively in two cuts.
- Increasing cobalt in plant media more than 12.5 ppm the promotive effect decreased.

Key words: Cobalt · Stevia · Rebaudiosides · Sweetners · Glycosides · Steviosides

INTRODUCTION

Stevia (Stevia rebaudiana Bertoni) is a sweet herb (medical plant) native of Paraguay. It has been widely cultivated in the world for the sweet diterpene glycosides that is mainly contained in its leaves.

Roy *et al.* [1] reported that the leaves of stevia are free from carbohydrates and calories and hence, it can be used safely by diabetic patients. It has now been recognized that stevia has many uses for human beings especially when it acts as a sugar substitute for those persons with blood sugar problems.

The fresh leaves itself 30 - 45 times [2]. Stevioside is a glycoside and it has sweetening power 100-400 temes than sucrose [3,4]. Stevioside concentrations usually range from 3 - 10 % of leaf dry weight; rebaudloside- A (400 times sweeter than sugar) is less concentrated ranging from 1 - 3 % [5]. Brandle [6] stated that the principal importance of stevia is due to the possibility of substituting advantages over saccharine: (1) It is not toxic but, on the contrary, it is healthful, as shown by long experience. (2) It is a sweetening agent of great power. (3) It can be employed directly in its natural state, (Pulverized leaves). (4) It is much cheaper than saccharine.

Stevia is an endemic herb non-caloric nature. Stevia leaves and their compounds are used in many therapeutic applications such as diabetes, obesity and plague retardant, hypoglycemic, indigestion, dental health, yeast infection, oral health, skin toning and healing burns and wounds. Stevia is also used a sweetening agent in products like biscuit, Jams, chocolates and ice-cream [7]. Taking the sweetening powder of the stevia sugar into consideration: these 400 kg stevia sugar are equivalent to about 80000 sweetening units. Note that one Fadden of "sugar can" produces about 5.000 sweetening units and one Fadden of "sugar beet" produces about 3.500 sweetening units. A sweetening unit is equivalent to the sweetness of one kilogram of sucrose. The stevia plant was introduced to agriculture of Egypt in order to produce a natural sweetener than can cover some of the lack of

Corresponding Author: Nadia Gad, Department of Plant Nutrition, National Research Centre Dokki, Egypt.

sugar production in Egypt [8]. Stevia cultivation in different places of the world; it is expected that in the Egyptian agricultural environment; one Fadden of stevia may produce up to 400 kg of stevia sugar, annually [6].

In the recent years stevia is used as a natural low-calorie sweetener as an alternative to chemical sweeteners as well as a dietary supplement in the developed and developing countries [9].

Mineral elements, which either stimulates growth or those essential only for certain plant species, or under special condition, are usually defined as beneficial elements. Cobalt is considered one of those elements, although there is no evidence that cobalt bas any direct role in the metabolism of higher plants. Cobalt is essential element for the synthesis of vitamin B_{12}, which is required for human and animal nutrition [10]. Vinay *et al.* [11] reported that both fresh and dry yield of clusterbean increased with application of 2 mg cobalt. Markova [12] showed that soil application of 0.7 kg cobalt/ hectare transplanting increased both total soluble solids and total soluble sugars compared with untreated tomato plants. Laila Helmy and Nadia Gad [13] pointed that the addition of 25 or 50 mg cobalt per kg soil had a significant beneficial effect on parsley leaves yield as well as the status of N, P, K, Ca, Mg, Zn, Cu and Mn in parsley leaves compared with control. Aziz, Eman *et al.* [14] revealed that cobalt at 15 ppm gave the greatest fresh and dry herb yield, the highest essential oil yield as well as improve the nutritional status of peppermint plants grown in newly reclaimed soil (Nubaria, Delta Egypt). Aziz, Eman and Nadia Gad [15] stated that cobalt at 22.5 ppm gave the highest values of fresh and dry herb yield and the greatest essential oil yield of lemongrass leaves. Nadia Gad and Aziz, Eman [16] added that applying cobalt at 22.5 ppm gave synergistic effect on the endogenous hormones, chemical constituents and minerals composition of lemongrass. Confirm, Nadia Gad [17] cobalt gave a positive effect due to several induced effects in hormonal synthesis and metabolic activity and attributed to catalase and peroxidase activities which enhancement all growth parameters of tomatoes.

Nadia Gad and Hala Kandil [18] found that cobalt treatments significantly increased coriander herb yield, minerals composition (except Fe), chemical constituents as well as essential oils components compared with control plants. Cobalt at 12.5 ppm resulted the maximum figures in each three harvests during two studied seasons.

Nadia Gad [19] reported that, cobalt concentrations significantly improve all groundnut growth and yield quantity and q uality compared with control. Cobalt at 8 ppm gave the highest figures of all nodulation, growth, yield parameters as well as minerals composition, endogenous hormones and chemical constituents especially oil%. Increasing cobalt level more than 8 ppm reduces the promotive effect. Aziz, Eman *et al.* [20] found that cobalt at 15 ppm gave the greatest values of sweet Basil fresh and dry herb yield (66.20 and 13.19 ton ha^{-1}). Increasing cobalt from 0.0 to 22.5 significantly increased the essential oil yield from 38.39 to 181.48 L ha^{-1}. While the highest level of cobalt (30 ppm) reduced the positive effect. Nadia Gad *et al.* [21] stated that applying cobalt at 15 ppm gave a significant increase in endogenous hormones such as auxins, gibberllins, cytokinems and abscisic acid compared with control. Cobalt at 15 ppm gave synergistic effect on total soluble solids, total protein and total phenoles as well as nutritional status of Sweet Basil plants compared with control. Higher concentrations more than 15 ppm reduced the promotive effect.

This investigation, therefore, made an attempt to study the "Effect of cobalt supplement on the growth and productivity of stevia rabaudiana Bertoni. Egypt being a developing country, suffer from a gap between sugar production (1.757 million tons) and its consumption (2.6 million tons) represents a serious problem, since it was estimated to be 0.843 million tons [22]. Nowadays attention is concentrated upon using stevia in food industries, in order to close the sugar gap between production and consumption.

The aim of present experiment t o study the role of cobalt on stevia growth, leaves yield quantity and quality. Cobalt may be help stevia plants in minimizing the gap between production and consumption especially it gave generously in the newly reclaimed soils.

MATERIALS AND METHODS

Soil Analysis: Physical and chemical properties of Nubaria Soil were determined and particle size distributions along with soil moisture were determined as described by Blackmore [23]. Soil pH, EC, cations and anions, organic matter, $CaCO_3$, total nitrogen and available P, K, Fe, Mn, Zn, Cu were run according to Black *et al.* [24]. Determination of soluble, available and total cobalt was determined according to method described by Cottenie *et al.* [25]. Some physical and chemical properties of Nubaria soil are shown in Table 1.

Plant Material and Experimental Analysis Design: A field experiment was conducted at Research and Production Station, National Research Centre, El-Nubaria

Table 1: Some physical and chemical properties of Nubaria soil.

		Particle size distribution		
Field capacity (%)	Soil Texture	Clay (%)	Silt (%)	Sand (%)
21.2	Sandy loam	3.5	26.7	69.8

Chemical properties

Cobalt (ppm)						
Total	Available	Soluble	O.M[c] (%)	$CaCO_3$ (%)	pH[a] (1:2.5)	Ec[b] dsm^{-1}
7.66	1.67	0.34	0.02	3.21	7.8	0.13

Soluble anions (meq L^{-1})				Soluble cations (meq L^{-1})			
$SO_4^{=}$	Cl^-	HCO_3^-	CO_3^-	Na^+	K^+	Mg^{++}	Ca^{++}
1.93	0.8	1.60	-	1.09	0.24	1.10	2.0

Available micronutrients (ppm)				Available (mg/100g)		
Cu	Zn	Mn	Fe	K	P	Total N (mg/100g)
3.01	1.78	2.12	7.77	11.0	13.4	16.5

a) Soil pH was measured in 1 : 2.5 soil-water suspension.

b) EC Was Measured as dSm-1in soil paste.

c) Organic Matter

location, Beheara Governorate, Delta Egypt under drip irrigation system during the successive season at 9 February 2011.

Experiment was carried out to evaluate the response of stevia herb yield and sweeteners content to cobalt supplement. The experiment contains 6 plots and the area was 15 m^2 (5*3 m) containing 3 rows each planted with 10 plants. Seedling of stevia rebaudiana bertoni var St (1) (one month age) was obtained from El- Sabhyia Research Station Abis, Alexandria and Agriculture Research Centre. Seedlings of stevia were transplanting to 6 plots in El-Nubaria site. Calcium superphosphate (15.5 %) at the rate 100 kg P_2O_5/fed; ammonium nitrate NH_4NO_3 (20.5 % N) at the rate of 50 unit N/fed; Wady El-Nile compost 15 m3/fed and potassium sulphate (48% K_2O) at the rate of 30 unit K_2O/fed were added during soil preparation. After each harvest added 15 unit N/fed and 10 unit K_2O/fed.

All required agricultural managements for plants growth and production were carried out as recommended by Ministry of Agriculture during the study season. After one from transplanting seedlings was irrigated with cobalt sulphate once at concentrations: 0.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 ppm cobalt. After two months, according to (Chang and Cook, [26]) from transplanting, plants were harvested (9, April, 2011). Plants were harvest again after two months (9, Jun, 2011) by cutting the plants at 10 cm from the ground.

Measurement of plant vegetative growth: At each harvesting time, plant height, leaves number per plant, leaves area as well as fresh and dry weights. In two cuts, total fresh weight per plant, total dry weight per plant as well as fresh herb (ton/fed) at each cuts were recorded according to FAO [27] and Gabal *et al.* [28].

Measurement of total chlorophyll content: Before plant harvest, chlorophyll was determined in the third leaves using chlorophyllmeter spad 502 according to (Wood *et al*. [29]).

Measurement of Nutritional Status: At each harvesting time, macronutrients (N, P and K); micronutrients (Fe, Mn, Zn and Cu) as well as cobalt were determined according to Cottenie [25].

Measurement of Chemical Constituents: At each harvesting time, total soluble soids, volatile oils, L-Ascorbic acid and carotenoids were determined according to A.O.A.C [30].

Measurement of Sweeteners Content: At Each Harvesting Time:

- Glucosides were determined according to Kohda *et al.* [31].
- Stevioside and Rebaudioside were determined according to Liu and Li [32]. The methanol and water/

methanol extract analyzed by high- performance liquid chromatography (HPLC) as described by Nishiyama *et al*. [33].

Statistical Analysis: All data were subjected to statistical analysis according to procedure outline by SAS [34]. Computer program and means were compared by LSD method according to Snedecor and Cochran [35].

RESULTS AND DISCUSSION

Vegetative growth: Data presented in Table 2, show that the addition of different cobalt doses (from 2.5 to 17.5 ppm) to the plant media has a significantly promotive effect on all growth parameters compared with untreated plants. Cobalt at 12.5 ppm gave the greatest all growth parameters such as plant hight, leaves number of stevia plants in two cuts. Increasing cobalt more than 12.5 ppm decrease cobalt promotive effect. These observations are consistent with previous reports obtained by Nadia Gad [17] who stated that cobalt at 7.5 ppm resulted the maximum growth and yield of tomato as compared with the higher ones. She reported that responses associated with low cobalt levels are attributed to catalase and peroxidase activities, which were found to decrease with low levels of cobalt and increase with the higher ones. These enzymes are known to induce respiration. Also, low cobalt level (7.5 ppm) significantly increased endogenous hormones i.e. Auxins and Gibberellins synthesis and metabolic activity. While, the higher cobalt doses were found increase the activity of these enzymes and decrease the hormonal content and hence increasing the catabolism rather than anabolism.

Herb Yield Characteristics: Data presented in Table 3, show also that the fresh and dry herb yield of stevia (g/plant and ton/ha) in two studied cuts increased gradually by increasing cobalt doses from 0.0 to 12.5 ppm. Cobalt at 12.5 ppm gave the greatest figures of fresh herb and biomass. Increasing cobalt addition in plant media above 12.5 ppm, reduced the promotive effect of all yield parameters. These results are in harmony with those obtained by Aziz, Eman and Nadia Gad [20] stated that cobalt at 15 ppm gave the greatest values of fresh and dry herb of sweet basil as well as oil yield and essential oil content while increasing cobalt levels in plant media reduced the promotive effect.

Nutritional Status in Plants: Macronutrients (N, P and K): Results presented in Table 4, show the effect of the different doses of cobalt (from 2.5 to 17.5 ppm) on macronutrients (N, P and K) in stevia leaves for two cuts. Data reveal that all cobalt concentrations significantly increased the content of N, P and K compared with untreated plants. The highest values of N, P and K content obtained by using cobalt dose at 12.5 ppm as compared with other supplemented levels. While increasing cobalt addition in plant media more than 12.5 ppm significantly decreased the promotive effect.

These results are in harmony with those obtained by Aziz, Eman *et al*. [14], who found that cobalt had a significantly promotive effect on macronutrients (N, P and K) in peppermint herbs while increasing cobalt level above 15 ppm significantly reduced the promotive effect.

Micronutrients: Manganese, Zn and Cu: Cobalt had a significantly positive effect to the content of Mn, Zn and Cu in stevia leaves significantly increasing as cobalt concentrations in plant media increased. These results are agreement with those obtained by Nadia Gad and Hala Kandil [18] who reported that cobalt increased the content of Mn, Zn and Cu of coriander compared with control.

Iron content: Results presented in Table 4, indicate that, increasing cobalt levels in the plant media resulted in a progressive depression effect on iron content in stevia leaves for two cuts. This may be explained on the basis of obtained results by Bisht [36], who showed certain antagonistic relationships between the two elements (Fe and Co). Confirm these results Nadia Gad *et al.* [21], who found that Fe content in sweet Basil significantly reduced as cobalt concentrations increasing in plant media.

Cobalt Content: Increasing cobalt doses in stevia leaves media from 2.5 to 17.5 ppm significantly increased the content of cobalt in stevia leaves for two cuts compared with control (Table, 4). These results clearly indicated that cobalt content goes along with the concentration of supplemented cobalt. These results are in good agreement with those obtained by Nadia Gad and Nagwa Hassan [37]. Young [10] reported that the daily cobalt requirement for human nutrition could reach 8 ppm depending on cobalt levels without health hazard. Level of 6.89 ppm in cobalt treatment (12.5 ppm) is below the safety human health.

Table 2: Effect of cobalt on the growth of stevia plant.

	Plant hight (cm)		Leaves no./ plant		Leaves area (cm^2)	
Cobalt treatments (ppm)	Cut "1"	Cut "2"	Cut "1"	Cut "2"	Cut "1"	Cut "2"
Control	25.6	27.0	11	11	144	147
7.5	29.3	30.4	12	13	185	224
10.0	32.5	33.6	14	14	212	243
12.5	36.0	37.3	16	17	253	294
15.0	33.7	35.0	15	16	236	275
17.5	30.8	32.7	13	14	218	339
LSD 5%	0.23	0.38	0.12	0.15	3.14	4.18

Table 3: Effect of cobalt on stevia herb biomass.

Cobalt Treatments (ppm)	Fresh weight (g/plant)		Dry weight (g/plant)		Total fresh Weight (g/plant)	Total dry Weight (g/plant)	Fresh herb 1 cut (ton/fed)	Fresh herb 2 cut (ton/fed)
	1 Cut	2 Cut	1 Cut	2 Cut				
Control	182.8	185.5	45.71	46.35	368.3	92.06	1.462	1.484
7.5	190.3	194.3	47.59	48.56	384.6	96.15	1.524	1.554
10.0	197.6	203.6	49.33	50.87	401.2	100.20	1.581	1.629
12.5	204.5	211.1	51.22	52.74	415.6	103.96	1.638	1.689
15.0	198.7	206.9	49.65	51.69	405.6	101.28	1.589	1.655
17.5	194.4	201.3	48.40	50.23	395.7	98.63	1.555	1.610
LSD 5%	0.67	0.54	0.13	0.12	3.65	1.25	0.006	0.008

Table 4: Effect of cobalt on minerals composition of stavia leaves.

	Macronutrients (%)			Micronutrients (ppm)				
Cobalt Treatment (ppm)	N	P	K	Mn	Zn	Cu	Fe	Cobalt (ppm)
First cut								
Control	1.63	0.26	1.25	41.6	32.7	26.2	133	0.65
7.5	1.72	0.30	1.29	43.2	35.0	29.0	131	1.01
10.0	1.81	0.35	1.35	45.7	37.6	32.4	128	2.77
12.5	2.04	0.38	1.40	48.4	39.5	35.0	126	5.69
15.0	1.90	0.34	1.37	46.1	38.0	33.3	123	7.88
17.5	1.83	0.31	1.33	43.6	36.2	30.9	121	9.11
Second cut								
Control	1.68	0.29	1.28	44.0	34.5	27.8	137	0.68
7.5	1.79	0.34	1.33	45.8	37.0	31.0	135	1.08
10.0	1.86	0.39	1.38	48.1	39.5	34.1	132	2.83
12.5	2.13	0.42	1.43	50.5	41.0	36.9	130	5.82
15.0	1.96	0.38	1.40	48.7	38.1	34.3	126	7.91
17.5	1.88	0.35	1.36	46.0	36.9	32.8	124	9.20
LSD 5%	0.01	0.004	0.006	0.02	0.03	0.02	0.26	0.05

Chemical Constituents: The amounts of total soluble solids (TSS) %, total proteins %, volatile oils % as well as vitamin "C" as L-Ascorbic acid and vitamin "A" as carotenoids (mg/ 100 g fresh tissue) in stevia leaves as affected by different levels of cobalt are given in Table 5. Results indicate that cobalt significantly increased all the mentioned parameters compared with control. Cobalt at 12.5 ppm had the highest values. Data also show that, increasing cobalt levels in plant media more than 12.5 ppm resulted reduce the promotive effect. These results are in harmony with those obtained by Nadia Gad and Hala Kandil [38], who reported that cobalt significantly increase all mentioned chemical constituents in sweet potato roots such as total soluble solids, proteins, total soluble sugars, mono sugars, starch along with L-Ascorbic acid and carotenoids compared with control.

The results in Table 5, show also the relative calculated values as percentage from control. It is evident that cobalt at 12.5 ppm increased the contents of: total soluble solids 20.8 - 21.4 %, proteins 25.1 - 26.8 %, volatail oils 22.1 - 22.7 %, carotenoids 8.56 - 8.91 % an L-Ascorbic acid 10.3 - 10.8 % respectively for two cuts.

Table 5: Effect of cobalt on chemical constituents of stavia leaves.

Cobalt Treatment (ppm)	Total soluble solids	Proteins	Volatile oils	Carotenoids	L-Ascorbic acid	Chlorophyll (spad)
	------------------(%)------------------			---mg/100g fresh tissue---		
First cut						
Control	13.89	10.19	10.28	18.39	15.57	46.38
7.5	14.76	10.75	10.68	19.11	18.81	46.38
10.0	15.41	11.31	11.22	19.56	16.22	46.34
12.5	16.78	12.75	12.55	20.29	16.85	46.31
15.0	15.62	11.88	12.29	19.89	16.67	46.27
17.5	14.89	11.44	12.08	19.81	15.79	46.27
Second cut						
Control	13.91	10.50	10.36	18.43	15.62	46.40
7.5	14.85	11.19	10.81	19.34	15.87	46.39
10.0	15.66	11.63	11.43	19.71	16.38	46.36
12.5	16.89	13.31	12.71	20.42	16.98	46.46
15.0	15.80	12.25	12.54	19.96	16.75	46.46
17.5	15.14	11.75	12.18	19.91	15.87	46.46
LSD 5%	0.03	0.02	0.01	0.04	0.03	NS

Table 6: Effect of cobalt on stevia leaves content of sweeteners.

Cobalt treatment (ppm)	Sweeteners in stevia leaves (%)		
	Glycosides	Steviosides	Rebaudiosides
First cut			
Control	17.0	50.0	19.1
7.5	18.5	53.6	22.5
10.0	19.4	58.6	24.8
12.5	22.8	63.8	27.6
15.0	22.7	59.1	25.0
17.5	22.1	56.7	22.7
Second cut			
Control	17.3	50.0	19.5
7.5	19.1	54.4	23.0
10.0	20.6	59.5	25.8
12.5	23.7	65.8	28.3
15.0	23.1	58.2	26.1
17.5	22.8	57.0	24.3
LSD 5%	0.13	0.15	0.11

Carotenoids are now recognized antioxidant and are essential to human growth, normal physiological functions and health of the skin as well as muscus membranes. Moreover, vitamin C as L- Ascorbic acid is an antioxidant and is necessary to several metabolic processes (Griffiths and Lunce, [39]). Cobalt significantly increased L-Ascorbic acid. L-Ascorbic acid is the major antioxidant in plant cells is involved in photoprotection matal and xenobiotic detoxification, the cell cycle, cell wall growth and cell expansion. It acts as co-enzyme in metabolic changes and involved in photosynthesis and respiration processes [40]. Data also show that increasing cobalt doses in plant media above 12.5 ppm decreased all the mentioned parameters compared with control. These results are true for the two cuts. The obtained results show that cobalt has a positive role in the studied physiological parameters of stevia leaves.

Colorophyll Content: Table 5, show data concerning the effect of applied cobalt on the green pigments in stevia leaves under condition of different addition of cobalt in the used media. Data indicate that cobalt, almost no responses being ecountreated with leaves. These results are a good agreement with those obtained by Laila Helmy and Nadia Gad [13] who stated that, parsley plants treated with cobalt levels at 25 and 50 mg.kg^{-1} soil behaved in a similar fashion to that control plants.

Sweeteners Content in Stevia Leaves: Results presented in Table 6, show the effect of different cobalt doses on sweeteners in stevia leaves. Data reveal that all cobalt treatments gave a significant beneficial effect on stevia leaves sweeteners such as glycosides, steviosides and rebaudiosides compared with untreated plants for two cuts.

The highest values of sweeteners are obtained by using cobalt at 12.5 ppm (Table 6). Increasing cobalt level more than 12.5 ppm in plant media significantly reduced the beneficial effect. These data are in harmony with those obtained by Nadia Gad and Hala Kandil [38] who stated that all cobalt concentrations significantly increased mono sugars and total soluble sugars in sweet potato roots compared with untreated plants. The highest values of these parameters are obtained by Cobalt at 10 ppm. Increasing cobalt in plant media above 10 ppm significantly decreased the promotive effect.

The results in Table (6) show also the relative calculated values as percentage from control. It is evident that cobalt at 12.5 ppm increased the contents of: glycosides 34.1 - 37.0 %, Steviosides 19.6 - 19.8 % and reboudiosides 23.6 -26.5 % respectively in two cuts.

CONCLUSION

Cobalt is promising element in the newly reclaimed soils and has a significant positive effect of stevia growth, herb yield, nutrients status, chemical constituents, chlorophyll content as well as sweeteners content.

REFERENCES

1. Roy, T.K., N. Bhowmick, S. Pal and R. Deh, 2005. Proceeding of the UGC Sponsored National Conference cum workshop on Scientific Growing Socio- Economic value and proper utilization of medicinal and aromatic plants for the Rural People. Department of Botany, Bejoy Narayan Mahavidyalaya, West Bengal, February, 35: 25-27.
2. Mowrey, D., 1992. Life with stevia: How sweet it is. Op. Cit, pp: 1-9.
3. Gujaral, R. 2004. Entrepreneure, pp: 10-12.
4. Duke, J., 1993. Stevia rebaudiana, CRC handbook of alternative cash crops. CRC press, Boca Raton, FL. pp: 422-424.
5. Cramer, B. and R. Ikan, 1986. Sweet glycosides from the stevia plant chemistry in Britain, 22: 915.
6. Brandle, J.S.A., 1998. Stevia rebaudiana: Its agricultural, biological and chemical properties (Review). Can. J. Plant Sci., 78: 527-536.
7. Maiti, R.K. and S.S. Purohit, 2008. Stevia, a miracle plant for human health. Agrobios (India), pp: 71.
8. Alaam, A.L., 2007. Sugar crops council: Future view. The proceeding of Thirtyeight Annual Conference. Egyptian Sugar Expertese Society hawamdia. Egypt.
9. Dube, K.G., 2011. Asiatic Journal of Biotechnology Resources, 2(5): 508-521.
10. Young, S.R., 1983. Recent advances of cobalt in human nutrition. Victoria B.C. Canada. Micronutrients News. 3: 59-62.
11. Vinay, S., H.B. Singh and V. Singh, 1996. Indian J. Plant Physiol. 39: 219-221.
12. Markova, A., 2001. Hort. Brasileira, 3: 53-56.
13. Liala, M.H. and N. Gad, 2002. J. Agric. Sci. Ain Shams Univ. Cairo, Egypt, 10(3): 779-802.
14. Aziz, E.E., N. Gad, S.M. Khaled, 2011. Australian Journal of Basic and Applied Sciences, 5(11): 628-633.
15. Aziz, E.E. and N. Gad, 2011. J. Appl. Sci. Research, 7(11): 1732-1736.
16. Nadia, Gad and E.E. Aziz, 2011. J. Appl. Sci. Research, 7(12): 1778-1784.
17. Nadia, Gad, 2005. J. Appl. Sci., 20(4): 260- 270.
18. Nadia, Gad and H. Kandil, 2012. Journal of Applied Sciences Research, 8(10): 5184-5189.
19. Nadia Gad, 2012. Role and importance of Cobalt nutrition on groundunt (Arachis hypogaea).World Applied Sciences Journal, 20(3): 359-367.
20. Aziz, E.E., N. Gad; L.K. Bekboyeva and M. Surif, 2013. American- Eurasian J. Agric. And Environ. Sci., (In Press).
21. Nadia, Gad, E.E. Aziz, L.K. Bekboyeva and M. Surif, 2013. American- Eurasian J. Agric. And Environ. Sci. 13(1): 16-21.
22. FAO, 2010. The State of Food Insecurity in the World. Food and Agriculture Organization of The United Nations Rome, www.fao.org.
23. Blackmore, A.D., T.D. Davis and R.H. Walser, 1972. Methods of Chemical Analysis of Soils. Newzealand. Soil Dureau.P A2.1, Dep., pp: 10.
24. Black, C.A., D.D. Evans, L.E. Ensminger, G.L. White, F.E. Clarck, 1982. 'Methods of Soil Analysis', Part 2. Agron. Inc. Madison Wise.
25. Cottenie, A., M. Verloo, L. Kiekens, G. Velgh and R. Camerlynk, 1982. Chemical Analysis of Plants and Soils. pp: 44-45. State Univ. Ghent, Belgium, pp: 63.
26. Chang, S.S. and J.M. Cook, 1983. Journal of Agriculture and Food Chemistry, 31: 409-412.
27. FAO, 1980. Soil and plant testing as a basis of fertilizer recommendations. Soil Bull., 3812.
28. Gabal, M.R., I.M. Abd-Allah, F.M. Hass and S. Hassannen, 1984. Annals of Agriculture Science Moshtohor, 22: 487-500.
29. Wood, C.W., P.W. Tracy, Receves, K.L. Edmisten, 1982. J. Plant Nut., 15: 1439-1442.
30. A.O.A.C. 1995. Method of analysis. Association of Official Agriculture Chemists. 16th Ed. Washington, D.C. USA.
31. Kohda, H., R. Kasai, K. Murakami and O. Tanaka, 1976. Phytochemistery, 15: 981-983.
32. Lui, J. And S.F.Y. Li, 1995. J. Lig Chromatography, 18: 1703-1719.
33. Nishiyama, P., M. Alrarez and L.G. Vieira, 1992. J. Sci. Food Agric., 59: 277-281.
34. SAS, 1996. Statistical analysis system, SAS users guide: statistics. SAS Institute Inc. Edition, Cary, NC.
35. Snedecor, G.W. and W.G. Cochran, 1982. Statistical methods. 7th Edition Iowa State Univ. Press. Ames. Iowa, USA.
36. Bisht, J.C., 1991. Interrelations between mineral plant tissues, iron and cobalt. Pescui, Agropecu. Bras, 16: 739-746

37. Nadia, Gad and N. Hassan, 2013. World Applied Sciences Journal, 2(5): 760-765.
38. Nadia, Gad and H. Kandil, 2008. Australian J. Basic and Applied Sci., 2(4): 949-955.
39. Griffiths, H.R. and J. Lunec, 2001. Enivron. Toxicol and Pharmacol., 10: 173-182.
40. Franceschi, V.R. and N.M. Tarlun, 2002. Plant Physiol., 130: 649-656.