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Website: http://www.aun.edu.eg/faculty_agriculture *E-mail: ajas@aun.edu.eg*

Efficacy of K-Humate, Compost and Biofertilizer Application as Well as Cutting Number on Yield and Quality of Stevia (*Stevia rebaudianaBertoni*) as Natural Sweetener

Rashwan, Basma R.A.[1]; Reem M. Abd-El Raouf[2]; Nagwa R. Ahmed[3] and H. Ferweez[4]

[1]Soil, Water and Environ. Res. Inst., A.R.C., Giza, Egypt.
[2]Horti. Res. Inst., Agric. Res. Centre, Giza, Egypt.
[3] Field Crops Rese. Inst., A.R.C., Giza, Egypt.
[4]Food Sci. and Techno. Dept., Fac. Agric. New Valley, Assiut University, Egypt.

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Abstract

Stevioside is natural sweetener isolated from the leaves of plant stevia and it is up to 300 times sweetener than sucrose, since it is a sweetener with no caloric value and with proven non-toxic effect on human health. Steviol glycosides are used as a sweetener in many industrial foods, such as soft drinks or fruit juices. So, two field experiments were conducted at Mallawi Agricultural Research Station, El-Minia Governorate during 2014 and 2015 seasons to deduce the effect of different sources of nutrient, i.e. Control (80 kg N fed^{-1}), K-humate + (40 kg N fed^{-1}), biofertilizer +(40 kg N fed^{-1}) and compost + (40 kg N fed^{-1})] and number of cutting , i.e. 1st , 2nd and 3rd cutting on yield and quality of stevia (*Stevia rebaudianaBertoni*) under Middle Egypt conditions. The obtained data pointed out that different sources of nutrient had a significant effect on plant height, fresh plant weight, dry plant weight, fresh leaves weight/plant, dry leaves weight/plant, N, P and K % of stevia leaf, fresh biomass yield, dry biomass yield , fresh leaves yield, dry leaves yield and fresh stem yield, total stevioside % (St %), rebaudioside A% (Rb%), stevioside yield and rebaudioside A yield of stevia in two growing seasons.

The studied cutting number of stevia had a significant influence on plant height, fresh plant weight, dry plant weight, fresh leaves weight/plant, dry leaves weight/plant, dry leaves weight: dry stem weight, P % of stevia leaf, fresh biomass yield, dry biomass yield, fresh leaves yield, dry leaves yield and fresh stem yield and dry stem yield of stevia, rebaudioside A%, total stevioside yield (kg/fed) and rebaudioside A yield (kg/fed) of stevia rebaudiana in two growing seasons. From the present study, it may be concluded that the application of compost at 2.0 ton/fed+(40 kg N fed-1) with 3rd cutting was the best treatment for improving the yield and quality of stevia (*Stevia rebaudianaBertoni*) and is advisable because it is achieved the highest value of rebaudioside A(102.80 kg/fed) of stevia. This helps in reducing the great gap in sugar production, which amounted to 0.7 million tons between production and sugar consumption at the national level production under the experimental conditions.

Keywords: *Stevia, source of nutrient, number of cutting, stevioside % and rabaudioside A.*

Introduction

Stevioside is natural sweetener isolated from the leaves of plant stevia and it is up to 300 times sweetener than sucrose, since it is a sweetener with no caloric value and with proven non-toxic effect on human health. Steviol glycosides are used as a sweetener in many industrial foods, such as soft drinks or fruit juices.

Stevia is grown commercially in many parts of Brazil, Paraguay, Uruguay, Central America, Israel, Thailand and China. It has been used for thousands of years as a source of intensively sweet-tasting compounds. The steviol glycosides, eight types of steviol glycosides in leaves were identified, stevioside, rebaudioside A, B, C, D, E and dulcoside A and B. Stevioside has been used as intensive more energetic sweeteners in many countries of South America and Asia. Steviosides had non- caloric property. Stevia is a major source of high potency sweetener in food market (Patil, 2010).

Fertilizers play an important role in increasing crop production. Its influence vegetative and reproductive phase of plant growth. In the developing countries such as Egypt, the increasing prices of fertilizers is affecting the poor farmers. Nutrient requirements of stevia are low to moderate because this crop is adaptable to poor- quality soils. Composts are prepared by biological degradation of plant and animal residues under controlled and aerobic conditions. virmicompost is described as biooxidation and stabilization of organic material involving the joint action of earthworms and mesoplilic microorganisms (Patil, 2010).

Bio-fertilizers are formulations of beneficial microorganisms, such as Bacillus megaterium, which upon application can increase the availability of nutrients by their biological activity and help to improve the soil health. Biofertilizers are useful substitutes to inorganic fertilizers which improves the soil quality. One of the recent achievements is the use of biofertilizer which retards nitrification for sufficiently longer time and increases the soil fertility. Integrated nutrient management using biofertilizer is found to increase yield of stevia (Patil, 2010). Gupta, *et al.* (2011) indicated that phosphorus-solubilizing bacteria treatment of stevia increased the growth and stevioside (St) and rebaudioside A (Rb) contents of plants.

The climatic conditions in most parts of Egypt are quite favorable for stevia cultivation. Hence, this work carried out to assess the changes in yield and quality of stevia (*Stevia rebaudianaBertony*) as affected by different sources of nutrient and number of cutting under Middle Egypt conditions.

Material and Method

Two field experiments were conducted at Mallawi Agricultural Research Station, El-Minia Governorate (latitude of 27.43^0N & longitude of 30.50^0 E) during 2014 and 2015 seasons to deduce the effect of different sources of nutrient (Control (80 kg N fed^{-1}), K-humate + 40 kg N fed^{-1}, biofertilizer +40 kg N $fed^{-1}$and compost + 40 kg N fed^{-1}])and number of cutting , i.e. 1^{st} , 2^{nd}or 3^{rd} cutting on yield and quality of stevia (*Stevia rebaudianaBertony*).

Biofertilizer (*Azotobacterchroococcum*and *Bacillus megaterium*) were provided from Microbiological Unit, Agriculture Research Center, Giza, Egypt. Compost was used by rate of 2 ton / fed in this work; it was incorporated 3 weeks before transplanting stevia in the plots and composed it as in Table 2. K-humatewas used at rate 2.0 liter/fed at twice, the first : the roots were soaked in solution (K-humate + water) before the transplanting in the 1st cutting, while in the phase of 2nd or 3rd cutting, the seedling were treated by K-humate as foliar(1.0 liter/100 water liters/fed). The second: the seedling were treated by K-humate as foliar(1.0 liter/100 water liters/fed) before irrigation and after 30 days from the first in the phase of 1st, 2nd or 3rd cutting.

A split-plot design in three replications was used. The different sources of nutrient [Control (80 kg N fed^{-1}), K-humate + (40 kg N fed^{-1}), biofertilizer +(40 kg N fed^{-1}) and compost + (40 kg N fed^{-1})] were allocated in the main plots and the number of cutting were randomly distributed in the sub-plots.

The seedlings of stevia, Spanish cultivar, were purchased from the sugar crops research institute and grown in the experimental farm of Mallawi Agric., Res. Station, on the beds (120 cm) at the two sides. Stevia seedlings were transplanted at a spacing of 35 ×60 cm at 25th and 23thMarch 2014 and 2015, respectively. It is perennial plant. Stevia plants was cut or harvested just prior to flowering when the concentration of steviol glycoside in the leaves is at its maximum, since glycoside synthesis is reduced at or just before flowering (Kumar, *et al.* 2014), leaving 10 cm up to ground level, periodically at 90 days(1st cutting), 140 days (2nd cutting) and 190 days (3rd cutting)of planting. Growth of stevia plants stops under the low temperatures, i.e. in winter season.

Nitrogen fertilizer as NH_4NO_3 was added at 80 kg Nfed^{-1}, Controlor 40 kg N fed^{-1} of control plus different sources of nutrient (K-humate, compostorbiofertilizer) were added in two equal doses, the first and the second before the second and third irrigation, respectively.

Phosphorus and potassium fertilizers were applied once before transplanting, (Phosphorus fertilizer the rate of 150 kg calcium super phosphate (15.5 % P_2O_5) and potassium fertilizer the rate of 50 kg Potassium Sulphate (48%K_2O). Seedlings of stevia were soaked before transplanting with the biofertilizers ((*Azotobacterchroococcum* and *Bacillus megaterium*), were provided from Microbiological Unit, Agriculture Research Center, Giza, Egypt. the number of bacteria reached about 1 x 10^{8}cell /ml and then, the inocula were used for inoculation of seedlings) and the biofertilizers was added with irrigation water two times (5 Liters per fed), the first was on after 30 days from planting seedlings and the second was after two weeks from the first.

Data recorded: Five plants of stevia / plots were selected randomly and cut at the ground level before flowering to measure the following parameters:

A. Vegetative traits :

1- Plant height (cm).

2- Fresh plant weight (g)

3- Dry plant weight (g), (the plants were dried in air oven at $60^{O}C$).

4- Fresh leaves weight (g)/plant

5- Dry leaves weight (g)/plant

6- Dry leaves weight: dry stem weight.

B. Leaves nutrient status:

Stevia leaves samples were done at 15 days interval followed by fertilizer treatments in the third and fourth leaf from the top to determine N P K of leaves collected from different treatments. Leaves dried in a forced oven at 60°C till constant of weight; ground to a fine powder and sub sample of 0.2 gm. was wet digested using sulphuric-perchloric acid mixture (1:1) as described by A.O.A.C. (2005), to determine the total N, P and K in the acidic extract as follows:

Table 1. Some physical and chemical properties of the experimental soil.

Properties	2014	2015
Particle size distribution (%)		
Sand (%)	8.03	8.11
Silt (%)	53.55	52.91
Clay (%)	38.43	38.98
Soil texture	Silty clay loam	
Organic matter (%)	1.14	1.15
pH soil – water suspension ratio (1:2.5)	8.20	8.15
EC(dsm-1)soil-water extract ratio (1:5)	1.24	1.26
Soluble cations (meq/L)		
Ca^{++}	7.35	7.15
Mg^{++}	2.13	2.16
Na^{+}	3.21	3.43
K^{+}	0.20	0.25
Soluble anions (meq/L)		
CO_3^{--}	---	---
HCO^{-}	3.20	3.44
Cl^{-}	4.14	4.08
SO_4^{--}	5.55	5.47
Available nutrients (mg kg^{-1})		
Available N (ppm)	18.20	18.31
Available P (ppm)	7.67	7.71
Available K (ppm)	155.50	155.82

- Total nitrogen (%) was determined by Kjeldahl method according to Jackson, (1967).

- Total Potassium (%) was determined using the flame photometer as described by Jackson, (1967).

- Total phosphorus (%) as described by Jackson, (1967).

Soil analysis was done according to the method described by Jackson (1967). The Physical and chemical properties of the experimental soil in Table I.

Table 2_a. Some characteristics of composted crop residues (CCR)

Properties	OM (%)	Organic carbon (%)	pH soil – water suspension ratio (1:10)	E.C soil-water extract (1:10). (dS/m\1)	C:N	Total macro-nutrients (%)			Total micro-nutrients (ppm)			Weight of one m^3 (kg)
						N	P	K	Zn	Fe	Mn	
Values	32.75	19.25	7.85	6.9	11.6:1	0.87	0.22	0.85	54	810	204	500

Table 2_b. Some characteristics of K- Humate

properties	Humic acid	Volvic acid	K	P	Fe	Zn	Mn	Mg
Values (%)	10	1	2.5	1	1	0.5	0.5	2

C. Yields:

Stevia plants of inner two ridges for each plot were harvested or cut from the bottom, leaving 10 cm up to ground level and weighed and converted for kg /fed to determine:

1. Fresh biomass yield in kg/fed,
2. Dry biomass yield in kg/fed, (the samples of stevia plants were dried in a hot-air oven at 60 ^{O} C).
3. Fresh leaves yield in kg/fed
4. Dry leaves yield in kg/fed
5. Fresh stem yield in kg/fed
6. Dry stem yield in kg/fed

D. Quality traits of stevia:

Quality traits of stevia in two field experiments were determined as follows:

1. Stevioside % of dry stevia leaves (St %) was estimated using the method described by A.O.A.C. (2005).
2. Rebaudioside A % (Rb%) of dry stevia leaves was estimated using the method described by A.O.A.C. (2005).
3. Steviosideyield (St yield) of dry stevia leaves (kg/fed) was calculated by the formula as follows:

St yield (kg /fed) = dry stevia leaves yield (kg/fed) X Stevioside % of dry leaves.

4. Rebaudioside A yield (Rb yield) of dry stevia leaves (kg/fed) was calculated by the formula as follows:

Rb yield (kg /fed) = dry stevia leaves yield (kg/fed) X Rebaudioside A% of dry leaves.

Sweet glycosides extraction:

Stevia leaves were collected from different treatments at harvest. Leaves were dried at 60 OC in hot air oven for 48 h. Hundred milligrams of air-dried powdered plant material (leaves) of stevia was macerated in methanol (10 ml) overnight and filtered. Plant material was re-extracted with same solvent twice (5ml each time) for 3 h each. theextractants were pooled together and concentrated up to dryness under reduced pressure. After defatting with n-hexane (2ml) thrice and vacuum drying, the extract was dissolved in 10 ml of HPLC grade acetonitrile and water (80:20) mobile phase degassed for 5 min. and filtered through 0.45 µm filter. The filtrated was used for HPLC analysis. Standard stock solutions (1mg/ 2ml) of standards of stevioside and rebaudioside A contents were calculated through high-performance liquid chromatography (HPLC).

Data collected were subjected to Analysis of Variance (ANOVA). The proper statistical of all data was carried out according. Differences among treatments were evaluated by the least significant difference test (LSD) according to procedure out lined by Gomez & Gomez (1984). Significant of differences was defined at 5 per cent level.

Results and Discussion

I-Vegetative traits:

It could be clarified from the results in Tables (3-8) that source of nutrients had a significant effect on plant height (cm), fresh plant weight, dry plant weight, fresh leaves weight/plant, dry leaves weight/plant of stevia rebaudianaBertoni in two growing seasons, except dry leaves: dry stem in the 1st season. The lowest values of plant height (48.67cm), fresh plant weight (624.33g), dry plant weight (212.67g), fresh leaves weight (230.61g)/plant, dry leaves weight (75.45g)/plant and dry leaves weight: dry stem weight fresh (1.17) was recorded with control(80 kg N fed^{-1}) treatment. While, compost+ 50% of the control treatment scored the highest values for plant height, fresh plant weight, dry plant weight, fresh leaves weight/plant and dry leaves weight/plant traits (62.52 cm, 670.28 g, 250.45 g, 274.67 g, and 93.95 g) of stevia. This result might be due to that the release of nutrients from compost and their absorption by plants and remineralization of immobilized N require time, which has become imperative to sustain high nutrient supply for greater productivity. However, application of control (80 kg N fed^{-1}) treatment alone might meet the lower nutrient demand .Similar data was recorded with those reported by Das *et al.* (2009) ; Khaled & Fawy (2011) and Kumar, *et al.* (2012 and 2013) who indicated biomass yield of stevia increased with application of compost over control (without biofertilizer or compost).

Table 3. Effect of different sources of nutrient and number of cutting on plant height (cm) of stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	37.67	44.67	60.00	47.44	39.00	47.67	63.00	49.89	48.67
K-Humate+((50%of cont.)	39.40	48.67	63.67	50.58	61.33	61.00	77.67	66.67	58.62
Biofertilizer+(50% of cont.)	41.20	58.53	66.00	55.24	61.67	60.28	71.67	64.54	59.89
Compost+ (50%of cont.)	43.07	60.53	70.00	57.87	62.78	64.05	74.67	67.17	62.52
Mean	40.33	53.10	64.92	52.78	56.19	58.25	71.75	62.06	57.78
LSD A	1. 46				3.27				
B	2.71				2.27				
AB	5.42				4.55				

Table 4. Effect of different sources of nutrient and number of cutting onfresh plant weight (g) of stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cut-ting	Mean	1st cutting	2nd cutting	3rd cut-ting	Mean	
Control (80 kg N fed^{-1})	577.33	632.33	648.67	619.44	586.67	642.33	658.67	629.22	624.33
K-Humate+((50% of cont.)	587.33	640.67	668.67	632.22	619.33	659.33	696.33	658.33	645.28
Biofertilizer+(50% of cont.)	606.67	630.00	671.00	635.89	640.33	665.00	688.00	664.44	650.17
Compost+ (50% of cont.)	641.33	659.67	689.00	663.33	651.33	671.00	709.33	677.22	670.28
Mean	603.17	640.67	669.33	637.72	624.42	659.42	688.08	657.31	647.52
LSD A	10.04				7.51				
B	8.36				7.61				
AB	16.72				15.22				

Table 5. Effect of different sources of nutrient and number of cutting ondry plant weight (g) of stevia plant during during 2014 and 2015 seasons.

Source of nutrient(A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	188.67	203.67	231.33	207.89	197.67	213.33	241.00	217.44	212.667
K-Humate+((50% of cont.)	193.00	206.00	237.00	212.00	224.00	240.00	261.00	241.67	226.834
Biofertilizer+(50% of cont.)	204.67	219.67	239.00	221.11	239.33	263.33	275.33	259.22	240.167
Compost+ (50% of cont.)	217.00	234.33	253.33	234.89	249.00	265.00	284.00	266.00	250.445
Mean	200.83	215.92	240.17	218.97	227.42	245.42	265.42	246.09	232.528
LSD A	2.70				4.72				
B	3.22				3.05				
AB	6.44				6.09				

Table 6. Effect of different sources of nutrient and number of cutting on freshleaves weight (g)/ plant ofstevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overal l mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	215.33	227.33	242.33	228.33	219.00	232.33	247.33	232.89	230.61
K-Humate+(50% of cont.)	218.00	230.00	246.67	231.56	243.00	262.33	275.67	260.33	245.95
Biofertilizer+(50% of cont.)	222.67	250.67	269.33	247.56	257.33	280.33	294.00	277.22	262.39
Compost+ (50% of cont.)	243.00	267.67	279.33	263.33	264.67	289.33	304.00	286.00	274.67
Mean	224.75	243.92	259.42	242.69	246.00	266.08	280.25	264.11	253.40
LSD A	0. 91				2.89				
B	1.86				1.96				
AB	3.71				3.92				

Table 7. Effect of different sources of nutrient and number of cutting on dry leavesweight (g)/ plant of stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	64.67	75.00	81.33	73.67	67.67	78.00	86.00	77.22	75.45
K-Humate+((50%of cont.)	68.33	78.67	84.00	77.00	79.00	93.33	82.67	85.00	81.00
Biofertilizer+(50% of cont.)	73.00	86.33	91.33	83.56	83.33	96.67	102.00	94.00	88.78
Compost+ (50%of cont.)	79.67	91.00	98.67	89.78	86.33	101.00	107.00	98.11	93.95
Mean	71.42	82.75	88.83	81.00	79.08	92.25	94.42	88.58	84.79
LSD A	2.17				2.63				
B	1.33				1.76				
AB	2.65				3.52				

Table 8. Effect of different sources of nutrient and number of cutting on dry leaves: dry stem of stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cut-ting	Mean	
Control (80 kg N fed^{-1})	1.70	1.65	0.98	1.44	1.00	0.90	0.78	0.89	1.17
K-Humate+((50%of cont.)	1.78	1.71	1.02	1.51	1.03	0.93	0.79	0.91	1.21
Biofertilizer+(50% of cont.)	1.82	1.77	1.04	1.54	1.19	0.95	0.85	1.00	1.27
Compost+ (50%of cont.)	1.92	1.61	0.91	1.48	1.17	0.91	0.78	0.95	1.22
Mean	1.81	1.68	0.99	1.49	1.09	0.92	0.85	1.08	1.28
LSD A	NS				0.07				
B	0.09				0.08				
AB	NS				0.17				

Data tabulated in Tables (3-8) show the effect of number of cutting on plant height , fresh plant weight, dry plant weight, fresh leaves weight/plant, dry leaves weight/plant and dry leaves weight: dry stem weight of stevia rebaudiana. Results cleared that number of cutting had a significant effect on the all studied traits in two growing seasons. The present results revealed that increasing number of cutting from 1st cutting to 2nd and 3rd cutting led to an increase in plant height from 48.26 to 55.68 and 68.33 cm, fresh leaves weight/plant from 613.79 to 650.04 and 678.71 g, dry leaves weight/plant from 214.13 to 230.67 and 252.79 g, fresh leaves weight/plant from 235.71 to 255.00 and 261.76 g ,weight was decreased from 1.45 to 1.30 and 0.92 % ,as an average value of the two studies seasons, respectively. where there were a reverse relationship between number of cutting and dry leaves weight: dry stem weight dry. This decrease might be due to the increase in dry stem weight with progess number of cutting .This increase might be due to increasing number of cutting from 1st cutting to 2nd and 3rd cutting led to improving the morphological characters and improved the root activity and enhanced the photosynthesis rate. These results are agreement with those found by Kumar, *et al.* (2012 and 2013).

A significant interaction was showed in Tables (3-8) between sources of nutrient (A) and number of cutting (B) with regard toplant height, fresh plant weight, dryplant weight, fresh leaves weight/plant in both seasons and the 2nd season for dry leaves weight: dry stem weight . It could be revealed from the results that application of compost + 50% of the control with 3rd cutting scored the highest values of fresh plant weight, dry plant weight, fresh leaves weight/plantand dry leaves weight/plant of stevia. Such findings are in accordance with those found by Kumar, *et al*. (2013).

II- Plant nutrient status:

Data for the major nutrients in leaf of stevia such as N, P and k, affected by the different sources of nutrient are given in Tables 9-11. The present results revealed that sources of nutrient had a significantly effect on N, K and P % in stevia leaf. The lowest values of nitrogen, phosphorus and potassium contents (1.50, 0.19 and 1.52%,) were found with control (80 kg N fed^{-1}), while, compost + 50% of the control treatment scored the highest values (1.63, 0.28 and 2.11%), respectively. The aforementioned findings correlated with those recorded by Gupta, (2010); Gupta, *et al.* (2011); Khaled & Fawy(2011) and Kumar, *et al.* (2012 and 2013) who reached the same results. In this subject, they revealed that increase in K% of stevia leaf by compost application might be due to enhancement in K availability by shifting the equilibrium among the forms of K from relatively exchangeable K to soluble K forms in the soil. They added that K-humateis technically not a fertilizer, although in some walks people do consider it. K-humatemay enhance the uptake of some nutrient, reduce the uptake of toxic elements and improve the plant.

Table 9. Effect of different sources of nutrient and number of cutting on nitrogen content (%) of fresh stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	1.44	1.50	1.52	1.48	1.47	1.53	1.58	1.52	1.50
K-Humate+((50%of cont.)	1.46	1.50	1.55	1.50	1.50	1.55	1.59	1.54	1.52
Biofertilizer+(50% of cont.)	1.62	1.55	1.56	1.58	1.68	1.57	1.64	1.63	1.60
Compost+ (50%of cont.)	1.64	1.62	1.59	1.62	1.66	1.66	1.61	1.64	1.63
Mean	1.54	1.54	1.56	1.55	1.60	1.58	1.60	1.59	1.57
LSD A	0.04				0.07				
B	NS				NS				
AB	Ns				NS				

Table 10. Effect of different sources of nutrient and number of cutting on phosphorus content (%) of fresh stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting				(B)				
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	0.13	0.19	0.23	0.18	0.14	0.19	0.27	0.20	0.19
K-Humate+((50%of cont.)	0.14	0.19	0.23	0.19	0.14	0.20	0.27	0.20	0.25
Biofertilizer+(50% of cont.)	0.13	0.20	0.26	0.20	0.13	0.23	0.30	0.22	0.26
Compost+ (50%of cont.)	0.13	0.19	0.23	0.18	0.14	0.24	.032	0.23	0.28
Mean	0.13	0.19	0.24	0.19	0.14	0.21	0.29	0.21	0.27
LSD A	0.01				0.01				
B	0.01				0.02				
AB	0.02				0.04				

Table 11. Effect of different sources of nutrient and number of cutting on potassium content (%) of fresh stevia plant during 2014 and 2015 seasons.

Source of nutrient(A)	2014 season				2015 season				Overall mean
	Number of cutting				(B)				
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	1.39	1.52	1.52	1.48	1.44	1.58	1.65	1.56	1.52
K-Humate+((50%of cont.)	1.46	1.58	1.68	1.57	1.47	1.60	1.69	1.59	1.58
Biofertilizer+(50% of cont.)	1.78	1.90	2.02	1.90	1.80	1.92	2.03	1.92	1.91
Compost+ (50%of cont.)	2.11	2.15	1.87	2.05	2.13	2.19	2.19	2.17	2.11
Mean	1.77	1.79	1.68	1.75	1.71	1.82	1.89	1.81	1.78
LSD A	0.14				0.05				
B	NS				0.03				
AB	NS				0.06				

The results given in Tables (9-11) indicated the differences in N % of stevia leaf did not reach the level of significance as affected by number of cutting. The present results revealed that number of cutting led to significant increase in P and K% of stevia leaf in the 2nd season only. Increasing number of cutting from 1st cutting to 2nd or 3rd cutting led to increase in P % of stevia leaf from 0.14 to 0.20 or 0.26% and K % of stevia leaf from1.74 to 1.81 or 1.79% ,as average value of the two studied seasons, respectively. This increase in might be due to improve the root activity and enhanced the P and K absorption from the soil. These results are agreement with those found by Kumar, *et al*. (2012 and 2013).

A significant interaction was scored in Tables (9-11) between sources of nutrient (A) and number of cutting (B) with regard to P% of stevia leaf in both seasons and K% of stevia leafin the 2nd season. It could be revealed from the results that application of compost+ 50% of the control with 2nd or 3rd cutting scored the highest value (0.32 and 2.19 %) of P and K % of stevia leaf. These findings are in the same line with those found by Rodriguez & Fraga (1999) and Kumar, *et al.* (2013) who revealed that biofertilizer containg *Bacillus megaterium* and *Azotobacterb chroococcum* allows an increase in K supply to the plant.

III- Yields of stevia rebaudiana :

Leaves of stevia are the main economic part, hence production of more leaf biomass with higher steviol glycosides (St and Rb) is the main criterion for performance. The recorded results in this work

(Tables,12-17) indicated that sources of nutrient had a highly significant effect on fresh biomass yield (kg/fed), dry biomass yield (kg/fed), fresh leaves yield (kg/fed), dry leaves yield (kg/fed) and fresh stem yield (kg/fed) of stevia, except dry stem yield (kg/fed) did not reach the level of significance in both seasons. It could be concluded from the results that the lowest values of fresh biomass yield (10289kg/fed), fresh leaves yield (6139 kg/fed), dry leaves yield (1971 kg/fed), fresh stem yield (4146kg/fed) as well as highest value of dry stem yield (1830 kg/fed) were found with K-Humateapplication, while, biofertilizer application scored the highest values of fresh biomass yield, dry biomass yield, fresh leaves yield and dry leaves yield of stevia (10551kg/fed, 3941.06 kg/fed, 6456kg/fed and 2122 kg/fed), as well as the lowest value of fresh stem yield (4095 kg/fed) respectively,. The aforementioned findings correlated with those recorded by Rodriguez & Fraga (1999) ;Gupta, (2010) ;Gupta, *et al.* (2011) and Kumar, *et al*. (2012, 2013 and 2014) who revealed that application of biofertilizer improved the root activity and enhanced the photosynthesis, finally the biomass of stevia was increased. They added that this increase might be due to bacterial inoculation may be attributed to their effect on nitrogen fixation. They indicated that stevia is many time sweetener than sugar cane and sugar beet and becomes an inevitable alternative to sugar, especially for the diabetic population of nearly 347 million across the world (WHO, 2013). Currently, stevia production is centered in China and the major market is Japan. In addition, they demonstrated that the principal mechanism for biofertilizer is the production of organic acids and acid phosphatases play a major role in the mineralization of organic phosphorous in soil.

Table 12. Effect of different sources of nutrient and number of cutting on fresh biomass yield (kg/ fed) of stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	8903	10585	11999	10496	8770	9843	11630	10081	10289
K-Humate+(50%of cont.)	9169	10581	11320	10356	8861	10475	11569	10302	10329
Biofertilizer+(50%of cont.)	9609	11368	11982	10986	8248	9426	10922	9532	10259
Compost + 50%of cont.)	9682	11279	12323	11095	8423	10050	11550	10007	10551
Mean	9340	10953	11906	10733	8576	9949	11418	9981	10357
LSD A	388.98				428.63				
B	339.94				317.25				
AB	NS				NS				

Table 13. Effect of different sources of nutrient and number of cutting on dry biomass yield(kg /fed) of fresh stevia plant during 2014 and 2015 seasons.

Source of nutrient(A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	2970	3102	4253	3442	2994	3122	4279	3465	3453
K-Humate+(50%of cont.)	3079	3215	4364	3552	3552	4049	4720	4107	3830
Biofertilizer+(50%of cont.)	3076	3420	4313	3603	3395	3904	4467	3975	3789
Compost + 50%of cont.)	32560	3560	4541	3787	3493	4055	4737	4095	3941
Mean	3096	3324	4368	3596	3480	4003	4641	3897	3747
LSD A	62.64				163.43				
B	144.44				129.17				
AB	NS				NS				

Table 14. Effect of different sources of nutrient and number of cutting on fresh leaves weight(kg/fed) of fresh stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	6524	6876	7142	6847	5006	5352	5933	5430	6139
K-Humate+(50%of cont.)	5866	6929	7311	6702	4958	5530	6259	5582	6142
Biofertilizer+(50%of cont.)	6467	7049	7578	7031	5066	5871	6707	5881	6456
Compost + 50%of cont.)	5993	7097	7052	6714	6010	7106	7073	6730	6722
Mean	6213	6988	7271	6824	5260	5965	6493	5631	6228
LSD A	NS				283.20				
B	327.40				298.02				
AB	NS				NS				

Table 15. Effect of different sources of nutrient and number of cutting on dry leaves weight(kg /fed) of fresh stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	1868	1925	2102	1965	1881	1935	2118	1978	1971
K-Humate+(50%of cont.)	1968	2025	2202	2065	1778	1945	2081	1935	2000
Biofertilizer+(50%of cont.)	2020	2105	2057	2061	1803	1860	1963	1876	1969
Compost + 50%of cont.)	2103	2272	2310	2228	1894	1971	2180	2015	2122
Mean	1990	2082	2168	2080	1839	1928	2086	1951	2030
LSD A	42.13				56.95				
B	66.42				67				
AB	NS				NS				

Table 16. Effect of different sources of nutrient and number of cutting on fresh stemyield (kg/fed) of stevia plant during 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	2837	3356	4488	3561	2851	3369	4495	3572	3566
K-Humate+(50%of cont.)	3037	3656	4689	3794	3813	4312	5371	4499	4146
Biofertilizer+(50%of cont.)	3216	4230	4745	4063	3357	4179	4843	4126	4095
Compost + 50%of cont.)	3084	4493	4840	4139	3242	4074	4988	4101	4120
Mean	3044	3934	4690	3889	3316	3983	4924	4075	3982
LSD A	179.16				208				
B	163.45				114				
AB	326.90				NS				

Table 17. Effect of different sources of nutrient and number of cutting on dry stemweight (kg/fed) of stevia plant during 2014 and 2015 seasons.

Source of nutrient(A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	1103	1177	2150	1477	1113	1187	2160	1487	1482
K-Humate+(50%of cont.)	1111	1189	2162	1487	1774	2104	2639	2172	1830
Biofertilizer+(50%of cont.)	1055	1315	2256	1542	1592	2044	2504	2046	1794
Compost + 50%of cont.)	1157	1288	2231	1559	1599	2084	2557	2080	1820
Mean	1107	1243	2200	1516	1655	2077	2566	1946	1731
LSD A	NS				122				
B	102				110				
AB	NS				221				

The data in Tables, (12-17) indicated that number of cutting had a highly significant effect on fresh biomass yield, dry biomass yield, fresh leaves yield, dry leaves yield and fresh stem yield and dry stem yield of stevia in both seasons. It could be concluded from the results that increasing number of cutting from 1st cutting to 2nd and 3rd cutting led to increase in fresh biomass yield from 8939.28 to 10425.00 and 11734.22 kg/fed, dry biomass yield from 3309.00 to 3750.00 and 4523.72 kg/fed, fresh leaves yield from 5647.83 to 6267.95 and 6821.67 kg/fed, dry leaves yield from 1927.67 to 2029.72 and 2132.33 kg/fed and fresh stem yield from 3291.44 to 4157.28 and 4912.56 kg/fed and dry stem yield of stevia from 1381.33 to 1720.83 and 2391.39 kg/fed, as average value of the two studied seasons, respectively.This increase in might be due to improve the root activity, which led to increasing the yields of leaves and stem. These results are agreement with those found by Das, *et al*. (2007).

A significant interaction was scored in Tables (12-17) between sources of nutrient (A) and number of cutting (B) with regard to fresh biomass yield, dry biomass yield, fresh leaves yield and fresh stem yield of stevia in both seasons, except fresh biomass yield in the 2nd season and fresh leaves yield of stevia in the 1st season. It could be revealed from the results that application of compost with 3rd cutting given the highest value of dry stevia leaf yield (2180.67 kg/fed). These results are in good accordance with those reported by Das, *et al*. (2007).

IV- Quality parameters of stevia :

Production of more dry leaf biomass with higher steviol glycosides (St and Rb) is the main criterion for performance. The present results in this work (Tables,18-21) clarified that sources of nutrient had a highly significant effect on total stevioside % (St %), rebaudioside A% (Rb%), total stevioside yield (kg/fed) and rebaudioside A yield (kg/fed) in both seasons of stevia. It could be concluded from the results that the lowest values of total stevioside (9.63%), rebaudioside A(4.51%), total stevioside yield (192.50 kg/fed) and rebaudioside A yield (90.26 kg/fed) were found with K-Humateapplication, while, compost treatment scored the highest values (10.63%, 5.23%, 208.95 kg/fed and 102.80 kg/fed), respectively. The aforementioned findings correlated with those recorded by Kumar, *et al.* (2012, 2013 and 2014) who reached glycoside content in stevia was greater in those plants which was supplied with compost due to improve root activity. They added that Rb is responsible for sweetness in stevia leaves, so higher Rb is desirable. Stevia crop give economically viable yield up to 4-5 years.

Table 18. Effect of different sources of nutrient and number of cutting on Stevioside % of dry stevia leavesduring 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting						(B)		
	1st cutting	2nd cut-ting	3rd cut-ting	Mean	1st cutting	2nd cut-ting	3rd cut-ting	Mean	
Control (80 kg N fed^{-1})	9.03	8.93	9.13	9.03	9.57	9.37	9.53	9.49	9.26
K-Humate+(50%of cont.)	9.23	9.17	9.50	9.30	9.87	9.93	10.10	9.97	9.64
Biofertilizer+(50%of cont.)	9.40	9.53	9.87	9.60	9.90	10.27	10.20	10.12	9.86
Compost + 50%of cont.)	10.10	10.30	10.60	10.33	10.63	10.93	11.20	10.92	10.63
Mean	9.44	9.48	9.78	9.57	9.99	10.13	10.26	10.13	9.85
LSD A	0.34				0.26				
B	NS				NS				
AB	NS				NS				

Table 19. Effect of different sources of nutrient and number of cutting on rebaudiside A % of dry stevia leavesduring 2014 and 2015 seasons .

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cut-ting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	3.87	3.93	4.20	4.00	4.30	4.40	4.70	4.47	4.23
K-Humate+(50%of cont.)	4.23	4.30	4.53	4.36	4.47	4.63	4.90	4.67	4.51
Biofertilizer+(50%of cont.)	4.57	4.53	4.80	4.63	4.80	4.97	5.07	4.94	4.79
Compost + 50%of cont.)	4.93	5.13	5.20	5.09	5.23	5.33	5.53	5.37	5.23
Mean	4.40	4. 48	4.68	4.52	4.70	4.83	5.05	4.86	4.69
LSD A	0.25				0.22				
B	0.16				0.14				
AB	0.32				0.28				

Table 20. Effect of different sources of nutrient and number of cutting on stevioside yield kg/fed. of dry stevia leavesduring 2014 and 2015 seasons.

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cutting	3rd cutting	Mean	1st cutting	2nd cutting	3rd cutting	Mean	
Control (80 kg N fed^{-1})	168.53	171.82	192.01	177.46	179.92	181.05	201.96	187.64	182.55
K-Humate+(50%of cont.)	181.50	185.47	209.24	192.07	175.37	193.16	210.28	192.93	192.50
Biofertilizer+(50%of cont.)	197.66	216.58	227.92	214.05	187.35	202.26	222.38	204.00	209.02
Compost + 50% of cont.)	204.06	216.69	217.98	212.91	191.76	203.42	219.81	204.99	208.95
Mean	187.94	197.64	211.79	199.12	183.60	194.97	213.60	197.39	197.39
LSD A	5.20				7.83				
B	5.54				5.95				
AB	11.08				11.90				

Table 21. Effect of different sources of nutrient and number of cutting on rebaudioside A yield kg/fed of dry stevia leaves plant during 2014 and 2015 seasons .

Source of nutrient (A)	2014 season				2015 season				Overall mean
	Number of cutting (B)								
	1st cutting	2nd cut-ting	3rd cut-ting	Mean	1st cutting	2nd cut-ting	3rd cut-ting	Mean	
Control (80 kg N fed^{-1})	72.15	75.80	88.30	78.75	80.78	85.27	99.57	88.54	83.64
K-Humate+(50%of cont.)	83.19	87.08	99.85	90.04	79.47	90.11	101.89	90.49	90.27
Biofertilizer+(50%of cont.)	96.02	102.98	110.88	103.29	90.87	97.76	110.45	99.69	101.49
Compost + 50%of cont.)	99.70	108.02	106.90	104.87	94.38	99.25	108.56	100.73	102.80
Mean	87.76	93.47	101.48	94.24	86.37	93.10	105.12	94.86	94.55
LSD A	4.95				1.74				
B	3.86				3.56				
AB	7.72				7.12				

In concern of number of cutting, data in Tables (18-21) revealed that increasing number of cutting from 1st cutting to 2nd and 3rd cutting led to increase in total stevioside % from 9.86 to 10.2 and 10.25 %, rebaudioside A% from 4.71 to 4.82 and 5.01%, total stevioside yield (189.62 to 202.93 and 217.93kg/fed) and rebaudioside A yield (90.61 to 97.53 and 106.97 kg/fed) of stevia as average value of the two studied seasons, respectively. These results are agreement with those found by Kumar, *et al*. (2013 and 2014) who indicated that production of leaf biomass along with higher steviol glycosides is the main criteria for technologist.

Significant interaction was scored in Tables (19-21) between sources of nutrient (A) and number of cutting (B) with regard to rebaudioside A%, total stevioside yield (kg/fed) and rebaudioside A yield (kg/fed) of stevia leaf in both seasons. It could be revealed from the results that application of compost with 3rd cutting scored the highest values of total stevioside %, rebaudioside A%, total stevioside yield (kg/fed) and rebaudioside A yield (kg/fed) of stevia leaf. Such data are in the same trend with those found by Kumar, *et al*. (2012).

Chemical fertilizers are not only costly but also adversely affect the soil microbial population and are prohibited for the production of medicinal plants. Use of compost or biofertilizers has become imperative in medicinal plants to meet the nutrition demand of the crop. Compost provide a good substate for the growth of microorganisms and maintain a favorable nutrition balance and soil properties. Compost has a special place because of the presence of readily available plant nutritions, growth enhancing substances and number of beneficial microorganisms such as nitrogen (N)-fixing, P-solubility, potential to support the growth of microorganisms Kumar, *et al*. (2013).

In general, human health is greatly endangered as various complex diseases such as obesity and diabetes, developed in the last decade due to excessive intake of harmful sugars present in foods, beverages and in wide range of food prpducts. Stevioside is natural sweetener isolated from the leaves of plant stevia and it is up to 300 times sweetener than sucrose, where it is sweetener with no caloric value and with proven non-toxic effect on human health. steviol glycosides are used as a sweetener in many industrial foods, such as soft drinks or fruit juices (Dushyant, *etal*.2014).

From the present study, it may be concluded that the application either the biofertilizer or compost at 2.0 ton/fed was the best treatment for improving the yield and quality of stevia (Stevia rebaudiana Bertoni) with 3rd cutting and is advisable because it is achieved the highest value of rebaudioside A (110.67 or 107.74 kg/fed). The use of biofertilizer or compost ineludible to minimise the environmental population, caused by the chemical ones. This helps in reducing the great gap in sugar production, which amounted to 0.7 million tons between production and sugar consumption at the national level production .

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تأثير استخدام هيومات البوتاسيوم ، الكمبوست والسماد الحيوى وايضا رقم الحشة على ناتج و جودة نبات الاستيفيا كمحلى طبيعي

بسمة رشوان احمد رشوان[١]، ريم محمد الحسيني عبد الرءوف[٢]، نجوي رفعت احمد حسانين[٣] وحسين فرويز محمد حسن[٤]

[١]قسم تغذية النبات ، معهد بحوث الأراضي، المياه والبيئة ، مركز البحوث الزراعية ، جمهورية مصر العربية

[٢]قسم النباتات الطبية والعطرية ، معهد بحوث البساتين ، مركز البحوث الزراعية ، جمهورية مصر العربية

[٣]قسم بحوث التكيف المحصول معهد بحوث المحاصيل الحقلية مركز البحوث الزراعية، جمهورية مصر العربية

[٤]قسم علوم وتكنولوجيا والاغذية ، كلية الزراعة بالوادي الجديد، جامعة اسيوط ، جمهورية مصر العربية.

الملخص

فصل الاستيفيوسيد المحلى الطبيعي من اوراق نبات الاستيفيا والذى تصل حلاوتـه الـى اكثر من ٣٠٠ مرة عن حلاوة السكر (السكروز) ولا ينتج عن هذا المحلى طاقة او تـاثير سـام لصحة الانسان، ويستخدم استيفيول الجليكوسيدات كمحلى طبيعى في العديد من الاغذية المصنعة مثل المشروبات وعصائر الفاكهة. لهذا الشأن أقيمت تجربة حقلية بمحطـة البحـوث الزراعيـة بملوى، محافظة المنيا ،مصر خلال عامي ٢٠١٤ و ٢٠١٥ لدراسة تأثير مـصادر العناصـر الغذائية { كنترول (٨٠ كجم نتروجين) ، هيومات البوتاسيوم + ٥٠% مـن الكنتـرول ، سـماد حيوي + ٥٠% من الكنترول و الكمبوست + ٥٠% من الكنترول} ورقم الحشة (الاولى ، الثانية أو الثالثة) على ناتج وجودة نبات الاستيفيا.

أوضحت النتائج المتحصل عليها الأتى :

١ – أحدثت مصادر العناصر الغذائية تاثير معنوى على ارتفـاع النبـات ، وزن النبـات الطازج ، وزن النبات الجاف ، وزن الاوراق الطازجة/ نبات ، وزن الاوراق جافـة/ نبـات ، نسبة البوتاسيوم فى الاوراق ، الناتج الكلى الطازج ، النـاتج الكلـى الجـاف ، نـاتج الاوراق الطازجة ، ناتج الاوراق الجافة ، ناتج السيقان الطازجة ، نسبة الاستيفيوسيد فى الاوراق ، نسبة الريباديوسيد أ فى الاوراق ، ناتج الاستيفيوسيد ونـاتج الريباديوسـيد أ فـي كـلا الموسـمين الزراعيين.

٢ – سجل رقم الحشة لنبات الاستيفيا تأثير معنوي على ارتفـاع النبـات ، وزن النبـات الطازج ، وزن النبات الجاف ، وزن الاوراق الطازجة/ نبات ، وزن الاوراق جافـة / نبـات ، نسبة البوتاسيوم فى الاوراق ، الناتج الكلى الطازج ، النـاتج الكلـى الجـاف ، نـاتج الاوراق الطازجة ، ناتج الاوراق الجافة، ناتج السيقان الطازجة ، ناتج السيقان الجافة ، نسبة الريباديوسيد أ فى الاوراق ، ناتج الاستيفيوسيد وناتج الريباديوسيد أ في كلا الموسمين الزراعيين.

٣– بناء على ذلك وجد إن استخدام السماد الحيوى او الكمبوست مـع نـصف الجرعـة السمادية فى الحشة الثالثة كانت الأفضل تحت ظروف التجربة الذي حقق القيمة الاعلـى لنـاتج الريباديوسيد أ (١١٠,٦٧ و ١٠٧,٧٤ كجم / فدان) ، وهذا يساعد فى خفض الفجوة الضخمة في انتاج السكر التي وصلت الى ٠,٧ مليون طن بين أنتاج و أستهلاك السكر على المستوى الوطنى وهى تعادل انتاج السكر من ٢٠ فدان قصب سكر او ٣٠ فدان بنجر سكر.