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GROWTH, YIELD COMPONENTS AND CHEMICAL CONSTITUENTS OF *Stevia rebaudiana* Bert. AS AFFECTED BY HUMIC ACID AND NPK FERTILIZATION RATES

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ABSTRACT: Two field experiments were carried out during 2016 and 2017 consecutive seasons at a Private Farm at Mit-Ghamr Distrect, Dakahlia Governorate, Egypt, to study the effect of humic acid rates (0.0, 325.60, 651.2 and 976.80 g/fad.), NPK fertilization rates [0.0, 25, 50, 75 and 100% of the recommended rate (RR)] and their interactions on growth, leaves and herb yield and some chemical constituents as well as active ingredients of stevia plants. The obtained results referred that the maximum value for each of plant height, branch and leaf number/plant, total dry weight/plant, dry weight of leaves and herb/fad., total N, P, K and total sugar percentages as well as total chlorophyll content (a+b) were detected when plants were applied with the highest rate of humic acid (976.80 g/fad.) and fertilized with 75% RR of NPK, in most cases. The main constituents of *Stevia rebaudiana* leaves as detected by high performance liquid chromatography-mass spectrometry (LC-MS) were Rebaudioside A, Rebaudioside B, Rebaudioside C, Dulcoside and Steviolbioside which increased with treatment of 100% RR of NPK fertilizers combined with the highest rate of humic acid (976.80 g/fad.) compared to control.

Key words: Stevia, humic acid, NPK, growth, yield, chemical constituents, stevioside.

INTRODUCTION

Stevia rebaudiana Bert. is one of 154 members of the genus *Stevia* and one of only two that produce sweet steviol glycosides (**Soejarto *et al.*, 1983**). The leaves of *Stevia rebaudiana* originating in Paraguay and Northeastern Brazil containing glycosides (steviosides) which can be extracted and used as sweeteners. It requires liberal watering after transplanting, before and after harvesting of leaves. These leaves can be harvested in one cut before flowering or in more cuts (**Andolfi *et al.*, 2002**).

The highest amount of stevioside was found in the upper young actively growing branches sections, whereas lowest senescent branches sections exhibited the lowest amount of such compounds. During ontogeny, a gradual enhance in the stevioside concentration was observed in both mature leaves and stems of stevia and this process lasted up to the budding phase and the onset of flowering (**Bondarev *et al.*, 2003**).

The macronutrients, N, P, and K, are often classified as 'primary' macronutrients, because deficiencies of N, P and K are more common than the 'secondary' macronutrients, Ca, Mg, and S. Most of the macronutrients represent 0.1 to 5%, or 100 to 5000 (ppm), of dry plant tissue, whereas the micronutrients generally comprise less than 0.025%, or 250 ppm, of dry plant tissue (**Wiedenhoeft, 2006**).

Chalapathi *et al.* (1999) examined influence levels of NPK fertilizers on stevia (*Stevia rebaudiana* Bertoni). They showed that stevia growth and yield were significantly increased with increasing fertilization rates of NPK up to

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40:20:30 kg/ha and exhibited marginal increase with increasing NPK up to 60:30:45 kg/ha. The yield of dry leaves was high at a rate of 40:30:20 kg/ha of NPK fertilization.

Humic acid is a part of the humus compounds which plays an important role in balance plant nutrition by improving physical, chemical and biological properties of soil. **Mikkelsen (2005)** reported that humic acid has a high molecular weight and high complexation ability. **Sangeetha *et al.* (2006)** reported that humic material have two direct and indirect effects on physiological and biochemical processes in plant and on physical, chemical, and biological properties of soil.

The most important aim of this study was to investigate the effect of humic acid and NPK fertilization rates on growth, yield and chemical constituents of *Stevia rebaudiana* plant under Dakahlia Governorate conditions.

MATERIALS AND METHODS

Two field experiments were carried out in a Private Farm at Mit-Ghamr Distrect, Dakahlia Governorate, Egypt, throughout the two summer seasons of 2016 and 2017. This work was conducted to investigate the effect of different rates of humic acid (0.0, 162.8, 325.6 and 488.4 g/fad./cut), NPK fertilization at different rates [0.0, 25, 50, 75 and 100% of recommended rate (RR)] and their interactions on vegetative growth, herb yield and chemical composition of stevia plant (*Stevia rebaudiana*, Bertoni). The recommended rates of NPK were 24, 12 and 18 kg/faddan, respectively. Sources of NPK were ammonium nitrate (33.5% N), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48.5% K_2O), respectively, as reported by **Mohamed (2013)**. Stevia seedlings were obtained from the Sugar Crops Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt.

The seedlings were planted in the experimental plots on 15th April during the two seasons. The experimental plot area was 3 × 2.4 m^2 containing four ridges, with 60 cm between them. The distance between plants in the ridge was 50 cm, so each plot area contained 24 plants, under drip irrigation system. Amounts of humic acid and NPK fertilization rates per cut and per faddan during growing season are presented in Schedule 1.

Vegetarian humic acid fertilizer (Abo Zaabal Company to Fertilizers) contains 86% humic acid. Humic acid rates were divided equally and one portion was applied in each cut to the plant root area during the vegetative period after being dissolved in fixed amount of irrigation water, when each addition. Nitrogen, phosphorus and potassium fertilizers were applied in two equal portions in each cut. However, all the plants received normal agricultural practices whenever they needed. The mechanical and chemical properties of the used soil are shown in Table 1 according to **Chapman and Pratt (1978)**.

This experiment was set up in a split-plot design with three replicates. The main plots were occupied by four humic acid rates. The sub plots were entitled to five NPK fertilization rates. The interaction treatments between NPK fertilization and humic acid rates were 20 treatments.

Data Recorded

Plant growth

In both seasons, the plants were harvested twice yearly by cutting the aerial parts of each plant (5 cm) above the soil surface. The two cuts were done on 15th July and 15th October in both seasons. Four plants were randomly chosen from each experimental unit at the two cuts, in both seasons. The following data were recorded in each cut; plant height (cm), branch and leaf number per stevia plant. Different plant parts were oven at 70°C till constant weight then total herb dry weight/ plant (g) was recorded.

Yield components

At harvesting of the two cuts, the central two ridges of each plot were used for yield components determination of stevia plants. Dry leaves yield/fad. (kg) and dry herb yield/fad. (kg) were calculated.

Chemical constituents and active ingredients

All chemical constituents content were determined in stevia leaves at the second cut during the two seasons. Total nitrogen, total phosphorus and potassium percentages, total sugar percentage (reducing and non reducing

Schedule 1. Amounts of humic acid and NPK fertilization rates per cut and per faddan during growing season

Amounts of NPK fertilization rates (kg/fad.)		
NPK/fad*	**NPK 1st cut**	**NPK 2nd cut**
N= 48	N= 24	N= 24
P_2O_5= 24	P_2O_5= 12	P_2O_5= 12
K_2O= 36	K_2O= 18	K_2O= 18
Amounts of humic acid rates (g/fad.)		
Fad.	**1st cut**	**2nd cut**
0.00	0.00	0.00
325.60	162.8	162.8
651.20	325.60	325.60
976.80	488.40	488.40

* Recommended rate of NPK

Table 1. Physical and chemical properties of experimental farm soil (average of two seasons)

Mechanical analysis			Soil texture
Clay (%)	**Silt (%)**	**Coarse sand (%)**	**Loamy**
44.60	34.10	21.30	

Chemical analysis										
pH	**E.C. (m.mohs/cm)**	**Soluble cations (meq./ l)**			**Soluble anions (meq./ l)**			**Available (ppm)**		
		Ca^{++}	Mg^{++}	Na^{+}	Cl^{-}	HCO_3^{-}	SO_4^{--}	N	P	K
7.59	**0.95**	11.50	5.00	3.12	4.5	1.9	2.88	170	83	71

sugars) and total chlorophyll content a + b (mg/ g fresh weight) were determined according to **Brown and Lilleland (1946), Dubois *et al* (1956)**, **Naguib (1969)**, **Hucker and Catroux (1980)** as well as **Mazumdar and Majumder (2003)**, respectively. In addition, stevioside content in leaves was determined in interaction treatments between humic acid rate at 488.4 g/fad., and different NPK fertilization rates in the 2nd cut during 2nd season as recorded by **Steinmann and Ganzera (2011**).

Statistical Analysis

Data of the present work were statically analyzed and the differences between the means of the treatments (humic acid rates and NPK fertilization rates) were considered significant when they were more than the least significant differences (LSD) at the 5% level by using computer program of Statistix Version 9 (**Analytical Software, 2008**).

RESULTS AND DISCUSSION

Growth Parameters

Effect of humic acid rates

The results in Table 2 show that, most of humic acid rates significantly increased stevia plant height, branch and leaf number per plant and total dry weight per plant compared to control. Moreover, humic acid at the rate of 976.80 g/fad., recorded higher increase in stevia growth parameters compared with the other treatments under study with significant differences with the treatments of control and 325.60 g/fad., in most cases, in the two cuts during the two seasons. Similar results were stated by **Mohammad (2009)** on *Catharansus roseus* and **Nasiri *et al.* (2015)** on geranium plant.

These results might be due to the role of humic acid which is a product contains many elements which improve soil fertility and increase the availability of nutrient elements by

Table 2. Effect of humic acid rates on some growth parameters of *Stevia rebaudiana* in the two cuts during the two seasons of 2016 and 2017

Humic acid rate (g/fad.)	Plant height (cm)		Branch No./plant		Leaf No./plant		Total herb dry weight/plant (g)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
				2016 season				
Control	29.8	52.9	4.33	5.27	31.3	82.9	4.00	9.8
325.60	35.8	64.1	4.40	7.33	40.3	99.3	7.00	14.8
651.20	37.8	72.1	6.80	10.2	56.5	171.7	11.3	22.1
976.80	39.4	95.1	7.13	10.4	55.0	180.0	12.3	26.9
LSD at 5%	2.24	10.42	0.59	1.85	9.90	38.1	1.84	6.97
				2017 season				
Control	48.3	57.3	8.47	7.47	66.2	190.0	9.70	21.4
325.60	53.0	62.0	8.87	8.07	86.8	251.0	13.3	26.2
651.20	63.7	71.5	11.9	9.80	154.3	316.8	18.0	29.4
976.80	60.8	73.3	9.87	10.8	177.7	316.7	17.7	29.6
LSD at 5%	4.02	2.78	0.82	0.94	24.6	22.5	3.46	2.02

holding them on mineral surfaces and consequently affect plant growth leading to taller, more branches and leaves and heaviest plants (**Akinci *et al.*, 2009**).

Effect of NPK fertilization rates

Results presented in Table 3 indicate that, plant height, number of branches and leaves per plant as well as total dry weight of plant were increased with increasing NPK fertilization rates in the two cuts in both seasons, in most cases. However, in the two seasons in both cuts, the different rates of NPK fertilization gave significant increase compared to control, in most cases. In addition, 75 and 100 % of RR of NPK gave high values in this regard at the first and second cuts during both seasons without significant differences between them, in most cases. These results are in agreements with those stated by **Singh *et al.* (2015)** on stevia plant.

It is well known that chemical fertilization could enhance plant growth due to the role of nitrogen in nucleic acids and protein synthesis, and phosphorus as essential component of energy compounds (ATP and ADP) and phosphoprotein, also, the role of potassium as an activator of many enzymes (**Helgi and Rolfe, 2005**).

Effect of the interaction between humic acid and NPK fertilization rates

Results under discussion in Tables 4 and 5 indicate that, plant height of stevia, branch and leaf number/plant and total dry weight per plant were significantly increased with combination between humic acid and NPK rates compared to control in the two seasons in both cuts, in most cases. Furthermore, the combination treatment (100% or 75% of RR of NPK + 651.20 or 976.80 g humic acid/fad., respectively) gave the highest values in this connection with significant increase compared to other treatments in the two cuts in the first and second seasons, in most cases. Also, all combination treatments were higher than individual humic acid rates or individual NPK levels.

Similar results were obtained by **El-Bassiony *et al.* (2010)** on snap bean and **Ali *et al.* (2014)** on *Tulipa agesneriana*. Moreover, as mentioned just before, both humic and NPK rates treatments (each alone) increased growth parameters of stevia plant, in turn, they together might maximize their effects leading to taller, more branches and leaves and heaviest plants.

Table 3. Effect of NPK fertilization rates on some growth parameters of *Stevia rebaudiana* in the two cuts during the two seasons of 2016 and 2017

NPK fertilization rate	Plant height (cm)		Branch No./plant		Leaf No./plant		Total herb dry weight/plant (g)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
2016 season								
Control	34.70	63.90	4.58	7.17	44.40	123.50	7.60	16.00
25% RR	34.60	65.30	5.08	8.17	46.70	127.80	8.70	18.90
50% RR	36.30	74.60	5.58	8.25	49.60	133.50	9.90	21.40
75% RR	36.50	74.20	6.33	8.83	44.80	132.10	8.30	18.10
100% RR*	36.50	77.30	6.75	9.25	43.50	150.40	8.70	17.80
LSD at 5%	2.09	4.97	0.81	0.82	NS	23.30	0.87	4.13
2017 season								
Control	48.80	49.10	7.67	6.42	73.10	129.00	8.27	14.50
25% RR	52.00	55.60	10.10	8.25	103.50	213.10	12.05	21.30
50% RR	59.50	66.90	10.00	9.25	141.30	266.90	15.45	26.10
75% RR	61.00	78.50	10.30	10.70	151.50	354.80	20.05	33.80
100% RR*	61.00	80.00	10.70	10.50	136.90	379.40	19.80	37.60
LSD at 5%	3.12	2.02	1.06	0.79	29.70	11.30	2.46	1.11

*Recommended rate (RR): 48, 24 and 36 kg/fad. of N, P_2O_5 and K_2O, respectively.

Table 4. Effect of the interaction between humic acid and NPK fertilization rates on plant height (cm) and branch number per plant of *Stevia rebaudiana* in the two cuts during the two seasons of 2016 and 2017

Humic acid rate (g/fad.)	NPK fertilization rates (kg/fad.)									
	Control	25%RR	50%RR	75%RR	100%RR*	Control	25%RR	50%RR	75%RR	100%RR*
	1st cut					2nd cut				
	Plant height (cm)									
2016 season										
Control	26.7	28.0	32.0	32.0	30.3	46.7	45.3	58.3	59.3	55.0
325.60	34.7	34.3	36.3	36.3	37.3	55.7	54.3	66.7	69.7	74.0
651.20	37.7	37.0	36.7	39.0	38.7	66.7	65.3	77.0	74.0	77.7
976.80	39.7	39.0	40.0	38.7	39.7	86.7	96.0	96.3	93.7	102.7
LSD at 5%			**4.34**					**4.97**		
2017 season										
Control	43.7	47.3	47.3	51.8	51.2	47.8	52.3	58.2	62.0	66.3
325.60	49.7	52.8	51.7	53.7	57.0	48.7	54.8	61.5	71.0	74.0
651.20	49.7	51.2	74.0	72.3	71.3	49.7	56.0	71.0	90.7	90.0
976.80	52.0	56.8	65.0	66.0	64.3	50.2	59.3	77.0	90.3	89.7
LSD at 5%			**6.85**					**4.54**		
	Branch number/plant									
2016 season										
Control	3.00	3.67	3.67	5.67	5.67	4.00	5.00	5.33	6.00	6.00
325.60	3.33	4.00	4.33	5.00	5.33	6.33	7.33	8.00	6.67	8.33
651.20	6.33	6.00	7.00	7.00	7.67	10.0	10.0	9.00	12.0	10.3
976.80	5.67	6.67	7.33	7.67	8.33	8.33	10.3	10.7	10.7	12.3
LSD at 5%			**1.56**					**2.36**		
2017 season										
Control	6.67	8.67	9.00	9.33	8.67	5.67	6.67	8.00	8.33	8.67
325.60	7.67	9.67	8.7	8.70	9.67	6.00	8.00	8.33	8.67	9.33
651.20	9.00	12.0	12.7	12.7	13.0	6.33	8.67	9.67	13.0	11.3
976.80	7.33	10.0	9.7	10.7	11.7	7.67	9.67	11.0	13.0	12.7
LSD at 5%			**2.06**					**1.69**		

*Recommended rate (RR): 48, 24 and 36 kg/fad. of N, P_2O_5 and K_2O, respectively.

Table 5. Effect of the interaction between humic acid and NPK fertilization rates on leaf number per plant and total dry weight (g) of *Stevia rebaudiana* in the two cuts during the two seasons of 2016 and 2017

Humic acid rate (g/fad.)	NPK fertilization rates									
	Control	25%RR	50%RR	75%RR	100%RR*	Control	25%RR	50%RR	75%RR	100%RR*
	1st cut					2nd cut				
	Leaf number / plant									
	2016 season									
Control	38.3	20.8	26.7	36.7	34.7	51.6	75.3	94.2	93.3	100.0
325.60	33.3	38.3	38.3	46.7	45.0	91.7	87.5	89.2	94.2	134.2
651.20	47.5	70.8	73.3	47.5	43.3	175.0	210.8	175.0	157.5	140.0
976.80	58.3	56.7	60.0	48.3	51.7	175.8	137.5	175.8	183.3	227.5
LSD at 5%			**17.00**					**56.20**		
	2017 season									
Control	46.7	55.8	72.5	88.3	67.5	102.5	160.8	213.3	211.7	261.7
325.60	75.8	72.5	80.8	99.2	105.8	110.8	205.0	251.7	311.7	375.8
651.20	62.5	124.2	200.8	214.2	170.0	137.5	247.5	294.2	460.0	445.0
976.80	107.5	161.7	210.8	204.2	204.2	165.0	239.6	308.3	435.8	435.0
LSD at 5%			**58.60**					**30.10**		
	Total herb dry weight / plant (g)									
	2016 season									
Control	3.70	3.80	3.70	4.10	4.80	8.40	8.50	8.40	10.6	13.2
325.60	5.40	6.60	7.00	7.60	8.30	13.0	13.8	14.3	15.4	17.7
651.20	9.50	11.9	15.4	10.4	9.20	17.7	22.9	31.1	21.4	17.6
976.80	11.8	12.6	13.6	11.2	12.3	24.8	30.2	31.7	25.1	22.7
LSD at 5%			**2.40**					**14.40**		
	2017 season									
Control	5.50	9.4	9.10	12.4	12.3	12.8	17.5	20.9	24.6	31.1
325.60	9.00	10.5	13.2	16.0	17.7	13.5	21.1	25.6	31.6	39.0
651.20	9.00	13.9	20.0	26.8	20.4	14.7	23.8	27.7	39.7	41.4
976.80	9.60	14.4	19.5	24.9	19.8	17.1	22.8	30.4	39.2	38.8
LSD at 5%			**7.84**					**2.82**		

*Recommended rate (RR): 48, 24 and 36 kg/fad. of N, P_2O_5 and K_2O, respectively.

Dry Leaves and Herb Yield/Faddan

Effect of humic acid rates

Results tabulated in Table 6 show that, dry yield of stevia leaves and herb per faddan increased by increasing humic acid rate, in most cases. Moreover, the humic acid rates 651.20 and 976.80 g/fad., gave high significant increases compared to control and other treatments with no significant difference among them at both cuts in the two seasons, in most cases. Also, the highest value for each of dry leaves and herb yield/fad., was obtained from humic acid rate at 976.80 g/fad., with high significant differences compared to the other treatments. This trend was repeated in the two cuts during both seasons. These results are in accordance with **Parakash *et al.* (2011)** on *Spirulina plantisis* plant.

The use of humic acid is a promising natural resource to be utilized as an alternative for increasing crop production. Humic acid make important contributions to improve soil stability, fertility, improves flower quality that lead to exceptional plant growth and micronutrient uptake which reflected in herb dry weight (**Knicker *et al.*, 1993**).

Effect of NPK fertilization rates

It is evident from the obtained results in Table 7 that, there was an increase in dry yield of stevia leaves and herb per faddan with increasing NPK rates in the two cuts in both seasons, in most cases. While, in the first season in both cuts, concerning the dry yield leaves, there were no significant differences among all rates of NPK, except treatment of 50% RR of NPK which gave significant increase compared to control in the first cut. The highest dry leaves yield obtained from the treatments of 50% and 100% of RR of NPK in the first and second cuts, respectively. In the same season, the treatment of 50% of RR of NPK gave the highest and significant differences with all treatments in both cuts with dry yield of herb/fad. However, all rates of NPK in both cuts in the second season with dry yield of leaves and herb/fad., gave high significant increases compared to control. Also, the highest values in this connection in first cut recorded from 75% of RR of NPK with high significant differences compared to control and 25% of RR. In the second cut, 100% of RR of NPK gave the highest values of leaves and herb dry yield per faddan with high significant increases compared to other treatments under study. The results are in conformity with the findings of **Khourang *et al.* (2012)** which reflected on total dry yield of medicinal flax.

Effect of the interaction between humic acid and NPK fertilization rates

Results recorded in Table 8 indicate that, under two humic acid rates at 651.20 and 976.80 g/fad., different rates of NPK gave high significant increases in dry yield of leaves and hreb per faddan compared to control in the two cuts in both seasons, in most cases. Moreover, the combination treatments between 651.20 g/fad.+ 50% of RR of NPK and 976.80 g/fad. +100% of RR of NPK gave highest values with high significant increases compared to other interaction treatments in the 1st and 2nd cuts, respectively, in the first season in most cases, which raised over the control with 267.0 and 340.9%, respectively. While, in the second season, combination treatment between 651.20 g/fad., and 75% of RR of NPK gave high values with high significant increases compared to other interaction treatments in both cuts in most cases, which yielded 119.9 and 257.7 Kg/fad., respectively. Also, herb dry weight per faddan was increased with interaction treatments with high significant increases compared to control in both cuts of the two seasons, in most cases.

Such results might be attributed to humic acid which is a bioactive organic biological slow-release fertilizer and together with the chemical fertilizers, forms an organic-inorganic complex fertilizer which holds the humic acid as the core. This can effectively improve the supply of nutrition leading to more yield of stevia plant (**Wang and Qin, 2009**).

Chemical Constituents

Effect of humic acid rates

It is quite clear from the results in Table 9 that, N, P, K and total sugars (%) in stevia leaves increased by increasing humic acid rate. Also, all humic acid rates gave significant increases compared to control. In addition, the highest values in this concern in the first and second seasons were obtained from the humic acid rate of 976.80 g/fad., followed by 651.20 g/fad., with no significant differences between

Table 6. Effect of humic acid rates on yield components in the two cuts during the two seasons of 2016 and 2017

Humic acid rate (g/fad.)	Dry leaves yield/ faddan (kg)		Dry herb yield/ faddan (kg)		Dry leaves yield/ faddan (kg)		Dry herb yield/ faddan (kg)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
	2016 season				2017 season			
Control	15.50	46.40	56.40	131.20	37.1	106.4	129.9	299.3
325.60	22.60	55.60	97.90	197.80	48.6	140.6	177.0	366.4
651.20	31.60	96.10	157.70	295.10	86.4	177.3	240.3	412.3
976.80	30.80	100.80	172.00	358.90	99.5	177.5	235.5	415.0
LSD at 5%	**5.67**	**21.30**	**26.27**	**65.70**	**13.70**	**12.00**	**32.60**	**28.30**

Table 7. Effect of NPK fertilization rates on yield components of *Stevia rebaudiana* in the two cuts during the two seasons of 2016 and 2017

NPK fertilization rate	Dry leaves yield/ faddan (kg)		Dry herb yield/ faddan (kg)		Dry leaves yield/ faddan (kg)		Dry herb yield/ faddan (kg)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
	2016 season				2017 season			
Control	22.30	69.20	106.30	213.10	41.00	72.20	110.70	203.10
25% RR	26.10	71.60	122.00	251.70	58.00	119.50	160.70	298.10
50% RR	27.80	74.80	138.80	284.90	79.10	149.50	206.00	366.10
75% RR	25.10	74.00	116.70	241.50	84.80	149.20	266.90	472.90
100% RR*	24.40	84.20	121.20	237.50	76.70	198.70	234.00	525.90
LSD at 5%	**4.41**	**NS**	**12.25**	**23.10**	**13.00**	**6.36**	**38.80**	**15.60**

*Recommended rate (RR): 48, 24 and 36 kg/fad. of N, P_2O_5 and K_2O, respectively.

Table 8. Effect of the combination between humic acid and NPK fertilization rates on some yield components of *Stevia rebaudiana* in the two cuts during the two seasons of 2016 and 2017

Humic acid rate (g/fad.)	NPK fertilization rates (kg/fad.)									
	Control	25%RR	50%RR	75%RR	100%RR*	Control	25%RR	50%RR	75%RR	100%RR*
	1st cut					2nd cut				
	Dry leaves yield/faddan (kg)									
	2016 season									
Control	11.2	11.7	14.9	20.5	19.1	28.9	42.2	52.7	52.3	56.0
325.60	18.7	21.5	21.5	26.1	25.2	51.3	49.0	49.9	52.7	75.1
651.20	26.6	39.7	41.1	26.6	24.3	98.0	118.1	98.0	88.2	78.4
976.80	32.7	31.7	33.6	27.1	28.9	98.5	77.0	98.5	102.7	127.4
LSD at 5%			**9.69**					**31.5**		
	2017 season									
Control	26.1	31.3	40.6	49.5	37.8	56.8	90.6	119.2	118.9	146.7
325.60	42.5	40.6	45.3	55.5	59.3	62.5	114.8	140.8	174.3	210.6
651.20	35.0	69.5	112.5	119.9	95.2	76.9	138.6	164.3	257.7	249.1
976.80	60.2	90.5	118.1	114.3	114.3	92.6	134.1	172.7	244.1	243.8
LSD at 5%			**32.8**					**16.5**		
	Dry herb yield/faddan (kg)									
	2016 season									
Control	51.3	53.7	51.3	57.9	67.7	111.9	113.9	112.0	141.8	176.4
325.60	75.6	91.9	98.5	106.9	116.7	173.3	184.0	190.2	204.9	236.4
651.20	133.5	166.1	215.6	145.1	128.3	235.9	305.7	414.2	285.3	234.2
976.80	164.7	176.4	189.9	156.8	172.2	331.1	403.1	423.1	334.2	302.8
LSD at 5%			**34.1**					**95.2**		
	2017 season									
Control	73.8	124.9	120.9	165.3	164.4	178.8	244.7	292.7	344.9	435.4
325.60	120.4	139.9	175.9	212.9	235.5	188.71	295.7	358.4	442.7	546.4
651.20	119.9	185.3	267.1	356.8	271.9	205.1	333.3	388.1	555.8	579.1
976.80	128.4	192.4	259.9	332.4	263.9	239.9	318.9	425.8	548.3	542.7
LSD at 5%			**52.4**					**39.5**		

*Recommended rate (RR): 48, 24 and 36 kg/fad. of N, P_2O_5 and K_2O, respectively.

Table 9. Effect of humic acid rates on chemical constituents of *Stevia rebaudiana* in the second cut during the two seasons of 2016 and 2017

Humic acid rate (g/fad.)	N (%)	P (%)	K (%)	Total sugars (%)	Total chlorophyll (a+b)	N (%)	P (%)	K (%)	Total sugars (%)	Total chlorophyll (a+b)
	2016 season					2017 season				
Control	2.91	0.330	1.90	11.96	1.396	2.75	0.312	1.92	11.2	1.381
325.60	3.21	0.362	2.23	13.21	1.450	3.19	0.352	2.14	12.5	1.442
651.20	3.21	0.371	2.40	13.44	1.528	3.34	0.372	2.27	13.1	1.488
976.80	3.34	0.381	2.30	14.08	1.551	3.32	0.380	2.27	13.6	1.485
LSD at 5%	**0.019**	**0.003**	**0.27**	**1.64**	**0.034**	**0.179**	**0.020**	**0.29**	**0.68**	**0.078**

them in the two seasons, in most cases. These resulted are in harmony with those reported by **Mohammad (2009)** on *Catharanthus roseus*. Also, in the same Table, total chlorophyll content (a+b) in stevia leaves was increased by increasing humic acid rate in both seasons, in most cases. Moreover, all humic acid rates gave high significant increase compared to control in first season. While, in second season, humic acid rates at (651.20 and 976.80 g/fad.) recorded significant increases compared to control, with no significant difference between them.

Effect of NPK fertilization rates

The results given in Table 10 show that, the percentages of NPK and total sugars as well as total chlorophyll content in stevia leaves gradually increased with increasing rates of NPK fertilization. Moreover, all treatments gave high significant differences compared to control, in most cases. Furthermore, 100% of RR of NPK recorded the highest percentages compared to the other treatments. In addition, there was no significant difference between the treatments of 75 and 100% of RR of NPK, in most cases.

These results are in agreement with those reported by **Aladakatti *et al.* (2012)**, **Inugraha *et al.* (2014)** and **Maniruzzaman *et al.* (2015)** on stevia as well as **Sabra (2014)** on khella plant.

Effect of the combination between humic acid and NPK fertilization rates

From data recorded in Tables 11 and 12, the different combination treatments gave significant increases in total chlorophyll content, N, P, K and total sugars percentages compared to control in both seasons, in most cases. Also, the highest values of N, P and total sugars in stevia leaves were obtained from the combination treatment between humic acid rate (488.4 g/fad.) with 100% of RR of NPK in the two seasons, in most cases. While, the interaction treatment of 325.6 g of humic acid/fad. + 100% of RR of NPK recorded the highest percentages of potassium and total chlorophyll (a+b) during both seasons, in most cases. Furthermore, there was no significant difference between both combination treatments with all chemical constituents in the two seasons.

Glycosides content in leaves

The bioactive glycosides identified in stevia leaves are listed in Fig. 1 and Table 13. Five glycosides were represented. The main stevia glycosides were Rebaudioside A, Rebaudioside B, Rebaudioside C, Dulcoside and Steviolbioside. It was clear that the molecular weight of these glycosides ranged between 641.6 and 965.1. The more intensity was presented at molecular mass 641.6 Daltons in stevia leaves extract.

Table 10. Effect of NPK fertilization rates on chemical constituents of *Stevia rebaudiana* in the second cut of the second season

NPK fertilization Rate	N (%)	P (%)	K (%)	Total sugars (%)	Total chlorophyll (a+b)	N (%)	P (%)	K (%)	Total sugars (%)	Total chlorophyll (a+b)
	2016 season					2017 season				
Control	2.72	0.309	1.55	11.65	1.284	2.80	0.297	1.55	10.80	1.270
25% RR	2.91	0.335	1.81	12.65	1.339	3.01	0.332	1.77	11.80	1.302
50% RR	3.25	0.359	2.33	13.40	1.472	3.12	0.361	2.30	12.90	1.451
75% RR	3.49	0.397	2.63	14.13	1.629	3.35	0.386	2.49	13.40	1.577
100% RR*	3.55	0.407	2.72	14.08	1.683	3.46	0.393	2.63	14.00	1.645
LSD at 5%	**0.05**	**0.007**	**0.16**	**0.87**	**0.034**	**0.20**	**0.014**	**0.21**	**0.59**	**0.057**

*Recommended rate (RR): 48, 24 and 36 kg/fad. of N, P_2O_5 and K_2O, respectively.

Table 11. Effect of the interaction between humic acid and NPK fertilization rates on chemical constituents of *Stevia rebaudiana* in the second cut during the two seasons of 2016 and 2017

Humic acid rate (g/fad.)	NPK fertilization rates (kg/fad.)									
	Control	25%RR	50%RR	75%RR	100%RR*	Control	25%RR	50%RR	75%RR	100%RR*
	2016 season					2017 season				
	Nitrogen percentage in leaves									
Control	2.60	2.67	2.87	3.17	3.21	2.64	2.67	2.47	2.78	3.19
325.60	2.71	2.93	3.37	3.47	3.55	2.90	3.07	3.21	3.47	3.29
651.20	2.76	2.97	3.34	3.64	3.71	2.85	3.23	3.37	3.57	3.67
976.80	2.81	3.05	3.42	3.69	3.74	2.81	3.05	3.42	3.59	3.71
LSD at 5%			**0.099**					**0.405**		
	Phosphorus percentage in leaves									
Control	0.292	0.299	0.329	0.360	0.370	0.269	0.296	0.312	0.337	0.347
325.60	0.310	0.339	0.355	0.399	0.407	0.286	0.323	0.365	0.389	0.400
651.20	0.312	0.348	0.359	0.414	0.424	0.315	0.348	0.376	0.408	0.411
976.80	0.320	0.353	0.391	0.416	0.427	0.320	0.363	0.391	0.410	0.415
LSD at 5%			**0.013**					**0.032**		
	Potassium percentage in leaves									
Control	1.50	1.59	1.89	2.24	2.28	1.53	1.59	1.83	2.24	2.41
325.60	1.47	1.91	2.37	2.64	2.75	1.57	1.74	2.31	2.44	2.62
651.20	1.75	2.05	2.45	2.81	2.97	1.58	1.87	2.45	2.64	2.80
976.80	1.49	1.72	2.60	2.84	2.88	1.53	1.90	2.60	2.64	2.68
LSD at 5%			**0.399**					**0.478**		
	Total sugars percentage in leaves									
Control	10.29	11.31	12.43	12.67	13.10	9.4	10.1	11.9	11.9	12.6
325.60	12.62	12.13	13.51	13.92	13.86	10.8	11.9	12.2	13.3	14.1
651.20	11.86	13.71	13.14	14.50	13.99	11.3	12.4	13.5	13.8	14.6
976.80	11.84	13.43	14.51	15.43	15.36	11.8	12.6	14.1	14. 6	14.8
LSD at 5%			**1.73**					**1.25**		

*Recommended rate (RR): 48, 24 and 36 kg/fad., of N, P_2O_5 and K_2O, respectively.

Table 12. Effect of the interaction between humic acid rate and NPK fertilizers levels on total chlorophyll content (mg/g) of *Stevia rebaudiana* in second cut during the second season of 2017

Humic acid rate (g/fad.)	NPK fertilization rates (kg/fad.)				
	Control	25%RR	50%RR	75%RR	100%RR*
			2016 season		
Control	1.220	1.238	1.367	1.526	1.630
325.60	1.266	1.313	1.451	1.579	1.643
651.20	1.317	1.373	1.536	1.684	1.732
976.80	1.333	1.433	1.535	1.728	1.727
LSD at 5%			**0.069**		
			2017 season		
Control	1.233	1.250	1.356	1.510	1.553
325.60	1.247	1.307	1.449	1.570	1.640
651.20	1.282	1.354	1.493	1.607	1.703
976.80	1.317	1.297	1.507	1.620	1.683
LSD at 5%			**0.128**		

*Recommended rate (RR): 48, 24 and 36 kg/fad., of N, P_2O_5 and K_2O, respectively.

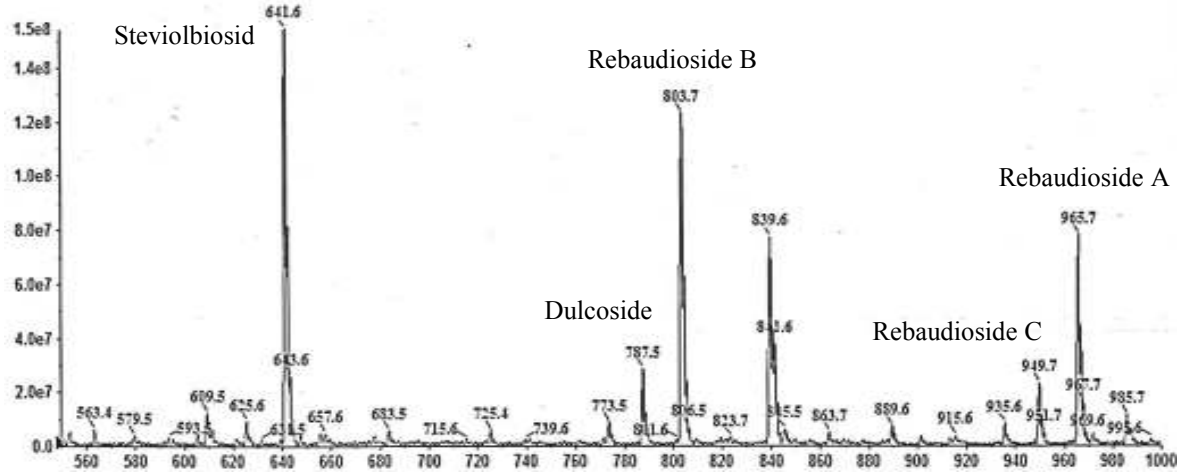

Fig. 1. LC-MS chromatogram of *Stevia rebaudiana* leaves in the second cut of the second season (2017)

Table 13. LC-MS chromatogram of *Stevia rebaudiana* leaves in the second cut of the second season (2017)

Compound No.	Stevia glycoside	Formula	Mw (Da)	R_1	R_2[a]	Intensity, cps
1	Steviolbioside	$C_{32}H_{50}O_{13}$	641.6	H	glc–glc	≈ 1.5e8
2	Rebaudioside B	$C_{38}H_{60}O_{18}$	803.7	H	glc(glc)$_2$	≈ 1.25e8
3	Rebaudioside C	$C_{44}H_{70}O_{22}$	949.7	glc	glc(rham)(glc)	≈ 2.0e7
4	Rebaudioside A	$C_{44}H_{70}O_{23}$	965.1	glc	glc(glc)$_2$	≈8.0e7
5	Dulcoside	$C_{38}H_{60}O_{17}$	787.5	glc	glc–rham	≈ 3.0e7

aglc = glucose, rham = rhamnose, xyl = xylose.

LC-MS: liquid chromatography-mass spectrometry.

Conclusion

Taking these results into account, it was generally concluded that growth, yield and its component of *Stevia rebaudiana* plant are widely affected by applying humic acid and chemical fertilizers. In general, the increase in growth and productivity of plants as well as stevioside percentage is closely related to the amount of the applied 976.80 g/fad. in combined with 75 and 100% of RR of NPK, which led to the increase in dry leaves and herb yields that are considered as the main components of growth and development of most of medicinal plants.

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تأثير معدلات حمض الهيوميك والتسميد بالنيتروجين والفسفور والبوتاسيوم على النمو ومكونات المحصول والمحتوى الكيميائي لنبات الإستيفيا

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أجريت تجربتان حقليتان خلال موسمين متتالين لأعوام ٢٠١٦ و ٢٠١٧ بمزرعة خاصة بمنطقة ميت غمر، محافظة الدقهلية، مصر، لدراسة تأثير معدل إضافة حمض الهيوميك (صفر، ٣٢٥,٦٠ ، ٦٥١,٢٠ و٩٧٦,٨٠ جم/فدان)، ومعدلات التسميد النيتروجيني والفوسفاتي والبوتاسي (صفر، ٢٥، ٥٠، ٧٥ و ١٠٠% من الموصى به) والتفاعل بينهما على النمو ومحصول الأوراق والعشب وبعض المكونات الكيميائية والمواد الفعالة لنبات الإستيفيا. أظهرت النتائج المتحصل عليها أن أعلى القيم لكل من إرتفاع النبات وعدد الأفرع والأوراق/نبات والوزن الجاف الكلي للنبات ومحصول الأوراق والعشب الجاف للفدان والنسب المئوية لكل من النيتروجين والفوسفور والبوتاسيوم والسكريات الكلية والمحتوى الكلى للكلوروفيل (أ+ب) تم الحصول عليها عند المعاملة بأعلى معدل من حمض الهيوميك (٩٧٦,٨٠جم/فدان) والتسميد بـ ٧٥% من المعدل الموصي به من النتروجين والفوسفور والبوتاسيوم، في معظم الأحيان، وقد تم الحصول على المكونات الرئيسية بأوراق الاستيفيا باستخدام التحليل الكروماتوجرافي للكثافة وهي الربيوسيد أ و الربيوسيد ب والربيوسيد ج والديكلوسيد والاستيفيولبيوسيد والتي زادت عند التسميد بـ ١٠٠% من المعدل الموصى به من النيتروجين والفسفور والبوتاسيوم مع المعدل الأعلى من حمض الهيوميك (٩٧٦,٨٠ جم/فدان) مقارنة بالكنترول.

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