

Journal

J. Biol. Chem.
Environ. Sci., 2019,
Vol. 13(1): 263-278
<http://biochemv.sci.eg>

PHYSIOLOGICAL STUDIES ON STEVIA (*STEVIA REBAUDIANA* BERTONI) PLANT

**Abu El-Liel Eman F.¹, A. A. Mahmoud²,
E. A. E. El-Ghadban¹, M. K. Khalil².**

¹*Medicinal and Aromatic Plants Dept., Hort. Res.
Inst., N.G.B., Agric. Res. Center, Giza, Egypt.*

²*Plant Physiology section, Agriculture Botany Dept.,
Faculty of Agric. Cairo University, Giza, Egypt.*

ABSTRACT

Pot experiment was conducted in greenhouse on stevia plant to study the effect of both ammonium and potassium nitrate under the treatments of BA., Kin, and TDZ on both growth and chemical constituents. Leaf chemical composition of P, N, K and protein as well as chlorophyll% were determined during 2016 and 2017 seasons. Ammonium nitrate excelled potassium nitrate fertilization on all growth characters except plant height. B.A. treatment tended to increase total fresh weight/ plant as compared to those of Kin and TDZ in some cases. K con. was higher concentration under potassium nitrate fertilization when compared to ammonium nitrate fertilization. Potassium nitrate addition resulted in slight increase in chlorophyll a & b and carotenoids over ammonium nitrate addition.

ABBREVIATIONS: TDZ= Thidiazuran, Kin= Kinetin, BA= 6-benzylaminopurine, Chl= Chlorophyll, cv. =Cultivar, Fig.=Figure, F.W.=Fresh Weight, l =Liter, mg= Milligram, min= Minute, MS media= Morashige and Skoog Media, µg= Microgram (10⁻⁶ g), °C= Degree Centigrade, %= Percent.

Keywords: ammonium nitrate, benzylaminopurine, chemical composition, Kinetin, fertilization, Physiological characters, potassium nitrate, *Stevia rebaudiana*, Thidiazuran.

INTRODUCTION

Stevia plants (*Stevia rebaudiana* Bertoni) are a perennial bush of the family Asteraceae, formerly Compositae (**Sapna et al., 2008**). The native occurrence of *S. rebaudiana* is between 22-24° S and 53-56° W in Paraguay and Brazil (**Soejarto et al., 1983**). Now, it is also cultivated in Japan, Korea, Thailand, China and India. The genus contain about 200 species are native to South America and Central America (**Oddone, 1997; Sapna et al., 2008**). It is herb with an extensive root system and brittle stems producing small, elliptic leaves. Plant height can reach up to more 1 m. The tiny white florets are perfect, borne in small corymbs of 2 – 6 flowers. Corymbs are arranged in loose panicles (**Oddone, 1997**). The plant growth requires mild temperature between 15° - 38°C and relative humidity of about 80 % (**Soejarto et al., 1983**). Stevia grows well on a wide range of soils given a consistent supply of moisture and adequate drainage (**Shock, 1982**).

Stevia grows well in sandy loam soils with an ample supply of water. Stevia prefers acidic to neutral soil with a pH range of 6.5-7.5 for its best growth. Saline soils should be avoided and Stevia plant is susceptible to water logged conditions. Stevia best grown is in sunny areas of the garden or in containers. Raised beds are the best choice for growing this herb if the soil is heavy or has high clay content. Ideal soil would be a friable garden loam high in organic matter. Stevia is not a drought tolerant herb; the soil should be kept continuously moist but not saturated (**Kinghorn, 2002; Tucker et al., 2009; Goettmoeller, J. and Ching 2010**). Stevia responds completely to photoperiod stimulation (short day flowering). It is self-incompatible, and therefore desires entomophilic fecundation (**Handro and Ferreira, 1989**).

Stevia is a low caloric natural source and can be used instead of alternative to artificially sugar (**Shock 1982**) reported that stevia contains eight glucoside compounds, each featuring a three-carbon ring central structure. Stevioside is the most abundant glucoside product. The leaves of stevia are the source of diterpene glycosides, viz stevioside and rebaudioside (**Ahmed et al., 2007**). Stevioside is considered as a valuable natural sweetening agent because of its relatively good taste and chemical stability (**Yamazaki and Flores, 1991; Toyoda and Matsui, 1997**). An extract of one or more of these compounds may be up to 300 times sweeter than sugar (**Duke, 1993**). Stevioside and rebaudioside-A are assessed to be, severally, 300 and 450 times sweeter than plant product however they are noncaloric. Stevia is helpful for hypoglycemia and

diabetes because it nourishes pancreas and thereby helps to restore its normal function (Soejarto *et al.*, 1983; Kanokporn *et al.*, 2006). Oviedo 1971 reported that 35.2% fall in normal blood sugar levels 6-8 hours following the ingestion of stevia leaf extract (Miyazaki *et al.*, 1978).

The work was to discriminate between two cultivars of Stevia are Spantia and China; was to establish optimal different nitrogen source and some growth regulators to achieve higher vegetative growth, yield and chemical composition.

MATERIALS AND METHODS

This study was carried out in the Medicinal and Aromatic plants Dept., Horticultural Research Institute, Agriculture Research Center (ARC), Giza, Egypt.

Plant material:

Acclimatized plants produced from tissue culture technique (El-Liel *et al.* 2019) were used in this experiment including both Spanti and China-1 Cultivars. Plants were transferred to black plastic bags (20 × 26 cm). The plant produced from acclimatization potted in open filed, the used experimental soil were clay, sand and peatmose (2:1:1).

Stevia plants in pots fertilized by two kinds of nitrogen fertilizer as follows:

1g calcium super phosphate (15.5% P_2O_5).

1-2 g Ammonium nitrate + 6 g Potassium sulphate/ pots (48% K_2O)

2-Or 6 g Potassium nitrate K_2O ./ pot

Plant growth regulators foliar application using super film (New Film BIO ®)

BA (2ppm, 4ppm and 6ppm)

Kinetin (2, 4 and 6ppm)

TDZ (2, 4 and 6 ppm)

The amount of calcium super phosphate was added during preparation of the soil. While the amount of N and K fertilizer were divided into six equal portions as side dressing at one each week of both season.

chemical composition:

Phosphorus was determined in stevia leaves according to (Olsen and Sommers, 1982). Potassium was determined in stevia leaves according to (Knudsen *et al.*, 1982). The amount of total nitrogen was determined in stevia leaves using Kjeldahel distillation using according to

(Bremner and Mulvaney, 1982).

Analysis of Chlorophyll:

Three attributes were subjected to analyze the pigment concentration are chlorophyll-a, chlorophyll-b and carotenoids concentration (Slack, *et al.* 1973; Arnon, 1949; Bruinsma, 1963). samples are accurately weighted at 0.5g of fresh plant leaf sample; then add 5 ml of dimethyl-sulphoxide (DMSO) to each tube. Samples were kept in the darkness for 24 hrs. The absorbance was read at 663, 645 and 470 nm using spectrophotometer model (JENWAY spectrophotometer).

Estimation of chlorophylls concentration were calculated using method of Arnon (1949), according to following equations:

$$\text{Chlorophyll a: } 12.47 (A_{663}) - 3.62 (A_{645}) = \text{mg/l}$$

$$\text{Chlorophyll b: } 25.06 (A_{645}) - 6.5 (A_{663}) = \text{mg/l}$$

$$\text{Carotenoids: } (1000A_{470} - 1.29Ca - 53.78Cb)/220 = \text{mg/l}$$

Data Analysis

The averages and standard error were computed for all data. The analysis of variance was carried out for the whole attributes using Complete Randomized Block Design (CRD) according to Gomez and Gomez (1984). Mean treatments were compared using Least Significant Difference (LSD 0.05). Three replicates were used for greenhouse experiment.

RESULTS AND DISCUSSION

Variability and Effects the different nitrogen source and some growth regulators on growth characters.

Average data in Table (1) shows the effect of different nitrogen fertilization source and some plant growth regulators on growth characters of Stevia plant var. Spanti Ammonium nitrate excelled potassium nitrate fertilization significantly, 27.21 and 24.27 g/ plant, respectively.

Similar data were obtained for dry weight/ stem. However, plant height gave an opposite trend. While number of leaves were higher under ammonium nitrate as compared to potassium nitrate fertilization.

As for plant growth regulators treatments, BA treatment under ammonium fertilization excelled both Kin and TDZ in total fresh weight plant. However, it was similar in total fresh/ plant under potassium nitrate when compared to both Kin and TDZ treatments. Similar data were obtained for F.W./stem, F.W./ leave. B.A. treatment increased plant height in both ammonium and potassium nitrate fertilization as compared

to Kin and TDZ treatments. Similar trend was obtained for China-1 var. in the first cut (**Table 2**). BA was found to be more efficient than other cytokinins with respect to initiation and subsequent proliferation of shoots. **Nigar and Mohammad (2011)** mentioned that on (*Withania somnifera* L.) Dunal in three different cytokinins tested (BA, Kin and 2ip).

Table (1): Effect of Chemical Fertilization and some plant growth regulators on morphological characteristics under study of *stevia rebaudiana* var. Spanti plant at first Cut in both seasons.

Treatment (b)	Amonium nitrate (a)					Potasium nitrate (a)									
	Plant height (cm)	No. of branches / plant	No. of leaves / Plant	F. W. / plant (g)	D. W./ plant (g)	F. W. / leaf (g)	F. W. / stem (g)	Plant height (cm)	No. of branches / plant	No. of leaves / Plant	F. W. / plant (g)	D. W. / plant (g)	F. W. / leaf (g)	F. W. / stem (g)	
Control	53.57	13.67	124	19.78	7.65	12.23	7.56	59.81	14.67	106	18.60	6.87	11.52	7.09	
BA (2mg/l)	64.18	33.00	234	26.90	9.83	16.63	10.27	53.57	28.83	155	22.81	8.29	14.13	8.68	
BA (4mg/l)	57.55	35.33	307	33.87	13.02	20.82	13.05	71.81	32.33	195	27.10	9.97	16.74	10.36	
BA (6mg/l)	61.45	41.00	381	40.97	15.34	25.18	15.79	70.63	37.33	251	29.07	10.69	17.96	11.11	
Mean	59.19	30.75	261	30.38	11.46	18.71	11.67	63.95	28.29	177	24.40	8.95	15.09	9.31	
Kin (2mg/l)	58.18	32.78	172	22.63	7.95	14.03	8.61	58.62	21.83	179	21.77	8.02	13.47	8.30	
Kin (4mg/l)	59.31	34.17	232	25.33	9.39	15.66	9.68	67.06	25.00	211	26.63	9.79	16.45	10.19	
Kin (6mg/l)	59.59	31.33	264	33.70	12.63	20.79	12.91	65.37	29.00	217	29.47	11.12	18.15	11.31	
Mean	57.66	27.99	198	25.36	9.41	15.68	9.69	62.71	22.63	178	24.12	8.95	14.89	9.22	
TDZ (20µM/l)	51.37	28.72	122	20.47	7.76	12.71	7.76	58.78	18.50	144	20.33	8.72	14.13	8.87	
TDZ (40 µM/l)	53.08	29.25	156	22.87	9.01	14.20	8.67	58.71	26.33	172	22.17	9.22	13.29	9.38	
TDZ (60 µM/l)	52.52	30.00	221	25.60	10.05	15.85	9.75	58.99	28.00	189	24.80	10.60	14.10	10.73	
Mean	52.63	25.41	156	22.18	8.62	13.75	8.43	59.07	21.88	153	21.48	8.85	13.26	9.02	
G. Mean	57.08	30.93	221	27.21	10.26	16.81	10.40	62.33	26.18	182	24.27	9.33	14.99	9.60	
L.S.D (0.05)															
Plant height	a = 2.93					b = 6.55					a*b = 9.26				
No. of branches	a = 1.01					b = 2.26					a*b = 3.20				
No. of leaves	a = 22					b = 50					a*b = 71				
F. W.	a = 1.74					b = 3.89					a*b = 5.50				
D. W.	a = 0.62					b = 1.38					a*b = 1.95				
F. W. leaf	a = 1.12					b = 2.50					a*b = 3.54				
F. W. stem	a = 0.79					b = 1.76					a*b = 2.49				

Table (2): Effect of Chemical Fertilization and some plant growth regulators on morphological characteristics under study of *stevia rebaudiana* var China-1 plant at first Cut in both seasons.

Treatment (b)	Amonium nitrate (a)						Potasium nitrate (a)							
	Plant height (cm)	No. of branches / plant	No. of leaves / Plant	F. W. / plant (g)	D. W. / plant (g)	F. W. / leaf (g)	F. W. / stem (g)	Plant height (cm)	No. of branches / plant	No. of leaves / Plant	F. W. / plant (g)	D. W. / plant (g)	F. W. / leaf (g)	F. W. / stem (g)
Control	44.96	9.00	89	11.07	5.12	6.84	4.22	49.57	7.00	77	8.46	3.11	5.22	3.24
BA (2mg/l)	55.24	14.33	110	17.03	7.39	10.52	6.51	55.92	9.73	125	19.06	6.90	11.67	7.39
BA (4mg/l)	46.08	16.50	248	24.99	10.35	15.31	9.68	59.32	13.50	150	20.67	7.48	12.65	8.02
BA (6mg/l)	56.25	27.33	338	30.34	11.65	18.65	11.69	73.65	24.33	252	23.37	8.46	14.31	9.06
Mean	50.63	16.79	196	20.86	8.63	12.83	8.03	59.61	13.64	151	17.89	6.49	10.96	6.93
Kin (2mg/l)	47.04	13.67	136	20.61	8.14	12.62	7.99	63.10	10.17	137	14.89	5.45	9.17	5.72
Kin (4mg/l)	50.05	16.00	187	20.72	8.72	12.68	8.04	62.47	12.17	163	19.90	7.21	12.19	7.72
Kin (6mg/l)	51.54	16.67	238	28.67	11.48	17.54	11.13	52.32	18.83	198	21.66	7.86	13.28	8.38
Mean	48.40	13.83	162	20.27	8.37	12.42	7.85	56.86	12.04	144	16.23	5.91	9.96	6.26
TDZ (20µM/l)	39.73	12.33	142	16.90	7.06	10.36	6.54	51.35	8.50	101	9.37	3.37	5.78	3.59
TDZ (40 µM/l)	56.53	13.17	173	21.02	8.48	12.90	8.12	56.56	10.67	110	12.50	4.56	7.69	4.82
TDZ (60 µM/l)	48.95	16.00	192	22.21	9.12	13.62	10.26	51.28	14.00	134	15.90	5.81	9.79	6.12
Mean	47.54	12.63	149	17.80	7.45	10.93	7.29	52.19	10.04	105	11.56	4.21	7.12	4.44
G. Mean	49.64	15.50	185	21.36	8.75	13.10	8.42	57.55	12.89	145	16.58	6.02	10.17	6.40

L.S.D (0.05)

Plant height

No. of branches

No. of leaves

F. W.

D. W.

F. W. leaf

F. W. stem

a = 2.70

a = 0.83

a = 21

a = 1.57

a = 0.53

a = 0.76

a = 0.98

b = 6.03

b = 1.85

b = 47

b = 3.52

b = 2.00

b = 1.71

b = 1.33

a*b = 8.53

a*b = 2.61

a*b = 67

a*b = 4.98

a*b = 2.83

a*b = 2.24

a*b = 2.29

As for second cut, data in **Table (3)** showed the effect of different nitrogen sources fertilization and some plant growth regulator treatments in var. Spanti. Similar data to those of the first cut were obtained as total fresh weight in the second cut under ammonium nitrate excelled that under potassium nitrate, 26.36 and 22.28 g/plant, respectively. However, no significant differences was realized in plant height under both type of N. fertilization in the second cut. As for China-1 var. in the second cut **Table (4)** was almost in the similar trend it was obtained to that of Spanti var. These result agree with **Habasy (2017)** on Navel Orange Trees sources of N had significant effect on these growth traits especially among the two sources namely ammonium nitrate and ammonium sulphate.

In general, Spanti var. excelled China-1 var. in growth (Total fresh weight / plant). B.A. treatment tended slightly increase Total fresh weight/ plant as compared to those of Kin and TDZ in some cases.

Table (3): Effect of Chemical Fertilization and some plant growth regulators on morphological characteristics under study of *stevia rebaudiana* var Spanti plant at second Cut in both seasons.

Treatment (b)	Amonium nitrate (a)						Potasium nitrate (a)							
	Plant height (cm)	No. of branches / plant	No. of leaves / Plant	F. W. / plant (g)	D. W./ plant (g)	F. W. / leaf (g)	F. W. / stem (g)	Plant height (cm)	No. of branches / plant	No. of leaves / Plant	F. W. / plant (g)	D. W. / plant (g)	F. W. / leaf (g)	F. W. / stem (g)
Control	52.26	18.10	176	19.36	6.78	12.39	6.97	58.08	16.10	128	17.78	6.22	11.28	6.50
BA (2mg/l)	55.79	33.67	215	29.53	10.33	18.67	10.86	59.69	27.53	183	22.21	7.77	14.07	8.14
BA (4mg/l)	66.61	36.13	287	31.45	11.01	19.88	11.57	64.39	34.27	226	24.05	8.42	15.24	8.81
BA (6mg/l)	80.64	42.23	456	38.91	13.62	24.56	14.35	83.10	38.93	340	28.60	10.01	18.15	10.46
Mean	63.82	32.53	283	29.81	10.43	18.88	10.93	66.31	29.21	219	23.16	8.11	14.69	8.48
Kin (2mg/l)	47.90	31.84	204	21.99	7.69	13.93	8.06	55.17	30.20	134	20.13	7.05	12.63	7.50
Kin (4mg/l)	61.91	32.77	226	27.25	9.54	17.23	11.02	62.91	30.40	197	23.36	8.18	14.71	8.66
Kin (6mg/l)	64.65	35.30	307	29.95	10.48	18.95	11.00	74.79	32.13	277	25.96	9.09	16.36	9.60
Mean	56.68	29.50	228	24.64	8.62	15.63	9.01	62.73	27.21	184	21.81	7.63	13.74	8.06
TDZ (20µM/l)	48.77	33.40	159	19.60	6.86	12.48	7.12	53.10	30.87	190	16.81	5.88	10.61	6.19
TDZ (40 µM/l)	52.89	31.37	195	23.55	8.24	15.01	8.54	64.23	34.53	190	19.99	7.00	12.60	7.40
TDZ (60 µM/l)	56.83	30.40	290	22.01	7.70	13.88	8.14	60.24	38.20	202	23.86	8.35	15.02	8.84
Mean	52.69	28.32	205	21.13	7.40	13.44	7.69	58.91	29.93	178	19.61	6.86	12.38	7.23
G. Mean	58.82	32.52	251	26.36	9.23	16.70	9.66	63.57	31.32	207	22.28	7.80	14.07	8.21

L.S.D (0.05)

Plant height

No. of branches

No. of leaves

F. W.

D. W.

F. W. leaf

F. W. stem

a = 3.73

a = 1.82

a = 43

a = 1.41

a = 0.49

a = 0.84

a = 0.57

b = 8.33

b = 3.90

b = 64

b = 3.15

b = 1.1

b = 1.89

b = 1.28

a*b = 11.78

a*b = 5.29

a*b = 91

a*b = 4.46

a*b = 1.56

a*b = 2.67

a*b = 1.81

Table (4): Effect of Chemical Fertilization and some plant growth regulators on morphological characteristics under study of *stevia rebaudiana* var China-1 plant at second Cut in both seasons.

Treatment (b)	Amonium nitrate (a)						Potasium nitrate (a)							
	Plant height (cm)	No. of branches / plant	No. of leaves / Plant	F. W. / plant (g)	D. W. / plant (g)	F. W. / leaf (g)	F. W. / stem (g)	Plant height (cm)	No. of branches / plant	No. of leaves / Plant	F. W. / plant (g)	D. W. / plant (g)	F. W. / leaf (g)	F. W. / stem (g)
Control	54.43	12.00	145	16.14	5.65	10.25	5.89	46.35	9.50	100	16.26	5.69	10.29	5.97
BA (2mg/l)	52.15	20.17	173	17.90	6.27	11.40	6.50	47.58	11.33	136	17.49	6.12	11.06	6.43
BA (4mg/l)	53.17	22.17	207	22.45	7.86	14.18	8.27	55.08	17.33	205	18.94	6.63	12.00	6.94
BA (6mg/l)	55.47	30.28	335	30.43	10.65	19.09	11.34	73.75	27.83	304	27.29	9.55	17.12	10.17
Mean	53.80	21.15	215	21.73	7.61	13.73	8.00	55.69	16.50	187	19.99	7.00	12.61	7.38
Kin (2mg/l)	51.82	17.50	168	20.51	7.18	12.87	7.64	45.13	14.50	102	16.36	5.72	10.26	6.10
Kin (4mg/l)	54.82	18.33	215	23.24	8.13	14.54	7.54	53.40	17.00	202	19.79	6.93	12.43	7.36
Kin (6mg/l)	58.46	22.50	278	27.52	9.63	17.23	8.62	62.38	19.50	248	23.56	9.12	16.29	9.77
Mean	54.88	17.58	202	21.85	7.65	13.72	7.42	51.81	15.13	163	18.99	6.87	12.32	7.30
TDZ (20µM/l)	45.39	12.50	226	19.51	6.83	12.16	7.36	43.00	12.33	150	15.63	5.47	9.78	5.85
TDZ (40 µM/l)	50.45	16.67	270	20.46	7.16	12.73	7.73	45.64	14.83	197	18.08	6.33	11.29	6.78
TDZ (60 µM/l)	48.52	21.67	271	23.42	8.20	14.62	8.80	54.68	18.00	246	19.56	6.85	12.23	7.33
Mean	49.70	15.71	228	19.88	6.96	12.44	7.44	47.42	13.67	173	17.38	6.08	10.90	6.49
G. Mean	52.47	19.38	229	22.16	7.75	13.91	7.97	52.70	16.22	189	19.29	6.84	12.27	7.27

L.S.D (0.05)

Plant height

No. of branches

No. of leaves

F. W.

D. W.

F. W. leaf

F. W. stem

b = N.S.

b = 3.89

b = 46

b = 3.22

b = 1.18

b = 2.05

b = 1.23

a[†]b = 8.17

a[†]b = 5.51

a[†]b = 65

a[†]b = 4.56

a[†]b = 1.67

a[†]b = 2.90

a[†]b = 1.88

Effects the different nitrogen sources and some growth regulators on Chemical composition (NPK and protein).

Data in **Table (5)** showed the effects of N fertilization sources on chemical composition (NPK) of stevia leaves at the second cut in Spanti var. Chemical composition of P, N, K and protein showed no difference between Ammonium and Potassium nitrate fertilization source. However, K was higher under Potassium nitrate fertilization when compared to ammonium nitrate fertilization at the second cut. B.A., Kin and TDZ treatments showed no difference in NPK in stevia leaves at the first cut.

Table 5: Effect of Chemical Fertilization and some plant growth regulator on phosphorus (%), potassium (%), nitrogen (%) and protein under study of *stevia rebaudiana* var Spanti plant at second Cut in second season.

Treatment (b)	Amonium nitrate (a)				Potasium nitrate (a)			
	P (%)	K (%)	N (%)	protein	P (%)	K (%)	N (%)	protein
Control	0.18	1.41	2.60	16.30	0.16	1.45	2.97	18.58
BA (2mg/l)	0.18	1.23	2.74	17.18	0.11	1.20	2.63	16.48
BA (4mg/l)	0.18	1.49	2.83	17.70	0.15	2.30	2.46	15.42
BA (6mg/l)	0.28	3.13	3.00	18.75	0.13	2.41	2.88	18.05
Mean	0.21	1.82	2.79	17.48	0.14	1.84	2.74	17.13
Kin (2mg/l)	0.16	1.83	2.66	16.65	0.20	1.63	2.52	15.78
Kin (4mg/l)	0.17	1.58	2.77	17.35	0.22	2.07	3.05	19.11
Kin (6mg/l)	0.21	1.54	3.00	18.75	0.23	3.27	3.30	20.68
Mean	0.18	1.65	2.81	17.58	0.22	2.32	2.96	18.52
TDZ (2mg/l)	0.17	1.13	2.74	17.18	0.12	2.84	2.94	18.40
TDZ (4 mg/l)	0.20	1.04	3.11	19.46	0.17	3.30	2.94	18.40
TDZ (6 mg/l)	0.23	2.29	3.16	19.81	0.27	3.22	3.44	21.56
Mean	0.20	1.49	3.00	18.82	0.19	3.12	3.11	19.45
G. Mean	0.20	1.67	2.86	17.91	0.18	2.37	2.91	18.25

Data in **Table (6)** showed the effects of N fertilization sources on chemical composition (NPK) of stevia leaves at the second cut in China-1 var. No difference in all constituents (P, N, K and crude protein) were noticed between the two N fertilization source. Treatment of B.A., Kin and TDZ showed no difference in stevia leaves chemical composition i.e., P, K, N and crude protein.

It could be conclude that chemical composition of stevia leaves (P, N, K and crude protein) were not different by the sources of N fertilization or B.A., Kin and TDZ Treatment in both Spanti and China-1 var. at second cut second season.

Table (6): Effect of Chemical Fertilization and some plant growth regulator phosphorus (%), potassium (%), nitrogen (%) and protein under study of *stevia rebaudiana* var China-1 plant at second Cut in second season.

Treatment (b)	Amonium nitrate (a)				Potasium nitrate (a)			
	P (%)	K (%)	N (%)	protein	P (%)	K (%)	N (%)	protein
Control	0.18	2.47	2.24	14.02	0.14	2.32	1.93	12.09
BA (2mg/l)	0.19	2.61	2.49	15.60	0.14	2.82	1.96	12.27
BA (4mg/l)	0.19	2.59	2.38	14.90	0.17	2.26	2.44	15.25
BA (6mg/l)	0.19	2.85	2.60	16.30	0.20	2.80	2.27	14.20
Mean	0.19	2.63	2.43	15.21	0.16	2.55	2.15	13.45
Kin (2mg/l)	0.21	2.80	2.72	17.00	0.18	3.04	2.21	13.85
Kin (4mg/l)	0.17	2.78	2.21	13.85	0.19	2.96	2.30	14.37
Kin (6mg/l)	0.20	2.86	2.38	14.90	0.22	3.19	2.13	13.32
Mean	0.19	2.81	2.44	15.25	0.20	3.06	2.21	13.85
TDZ (2mg/l)	0.22	3.35	3.08	19.28	0.19	2.47	2.07	12.97
TDZ (4 mg/l)	0.27	3.13	2.04	12.80	0.19	3.04	2.30	14.37
TDZ (6 mg/l)	0.09	1.76	1.90	11.92	0.17	3.29	2.21	13.85
Mean	0.19	2.75	2.34	14.67	0.18	2.93	2.19	13.73
G. Mean	0.19	2.72	2.40	15.06	0.18	2.82	2.18	13.65

Effect of some growth regulators on Stevia Pigments

Data in Fig (1 & 2) showed the effect of N fertilization sources and plant growth regulators on chlorophyll a & b and carotenoids concentrations at the second cut in Spanti var. of stevia plant. Potassium nitrate addition resulted in slight increase in cChlorophyll a & b and carotenoids over ammonium nitrate addition. Kinetin treatments tended to increase chlorophyll a & b and carotenoids concentrations under ammonium nitrate fertilization. While under Potassium nitrate fertilization, B.A. treatments tended to increase these previously mentioned characters.

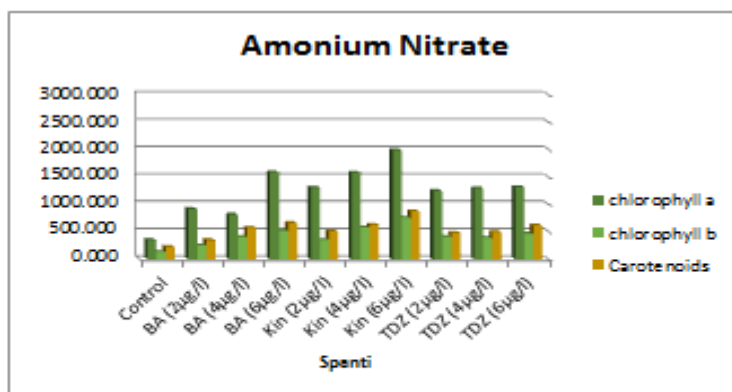


Fig.1. Chlorophylls (a & b) and carotenoids (µg/g F.W.) in *Stevia rebaudiana* var. Spanti under ammonium nitrate fertilization.

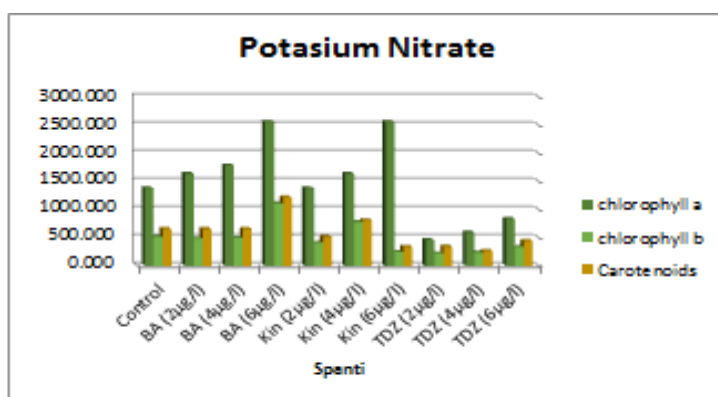


Fig.2. Chlorophylls (a &b) and carotenoids (µg/g F.W.) in *stevia rebaudiana* var. Spanti under potassium nitrate fertilization.

In China-1 var. in the second cut, kinetin treatment increased chlorophyll a & b and carotenoids under both N fertilization (Fig 3 & 4), when compared to B.A. and TDZ treatments. However, ammonium nitrate fertilization excelled potassium nitrate fertilization in all previously mentioned characters.

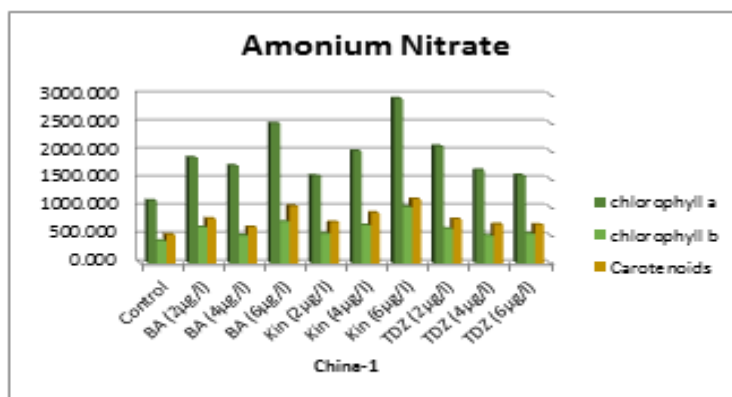


Fig.3. Chlorophylls (a &b) and carotenoids (µg/g F.W.) in *stevia rebaudiana* var. China-1 under ammonium nitrate fertilization.

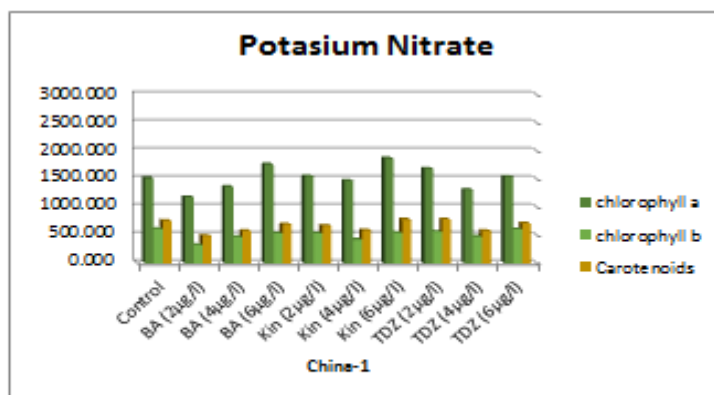


Fig.4. Chlorophylls (a & b) and carotenoids (µg/g F.W.) in *Stevia rebaudiana* var. China-1 under potassium nitrate fertilization.

REFERENCES

- Ahmed, M.B.; M. Salahin; R. Karim; M. A. Razvy; M. M. Hannan; R. Sultana; M. Hossain and R. Islam (2007): An Efficient method for in vitro clonal propagation of a newly introduced sweetener plant (*Stevia rebaudiana* Bertoni.) in Bangladesh. American-Eurasian J. Sci. Res., 2(2): 121-125.
- Arnon. D. I. (1949): Copper enzymes in isolated chloroplasts. Polyphenoloxidases. Plant Physiol. 24: 1-15.
- Bremner, J.M. and C. S. Mulvaney (1982): Nitrogen-Total: Methods of soil analysis. Part 2.
- Bruinsma, J. (1963): The quantitative analysis of chlorophylls (I and II) in plant extracts. Photochem. Photobiol. 2: 241-249.
- Duke, J. (1993): *Stevia rebaudiana* in: handbook of "Alternative Cash Crops". CRC Press, Boca Raton, (New York & London) 422-424.
- El-Liel, E. F.; A. A. Mahmoud; A. M. Salama; E. A. E. El-Ghadban; and M. K. Khalil, (2019): Improved Micropropagation of *Stevia rebaudiana* Bertoni) Plant. Journal ISSN 2166-0379 Vol.7 No (1).
- Goettemoeller, J. and A. Ching (2010): Seed germination in *Stevia rebaudiana* perspective on new crops and new uses. J. Janick (ed.), ASHS press, Alexandria, VA. pp: 510-511.
- Gomez, K.A. and A.A. Gomez (1984): Statistical procedures for agricultural research. John Wiley & Sons Ed., New York, 680 pp.

- Habasy, R. E.Y. (2017):** Effect of Different Levels and Sources of Nitrogen on Tree Growth, Yield and Fruit Quality of Navel Orange Trees. Middle East Journal of Agriculture Research, V: 06 P:639-645.
- Handro, W. and C.M. Ferreira (1989):** *Stevia rebaudiana* Bertoni: Production of natural sweeteners. Biotechnology in Agriculture and Forestry, (7): 468-487.
- Kanokporn, S.; A. Salika; S. Supap; M. Jeeradej and M. Aranya (2006):** Safety of extracts from *A. marmelos* and *Stevia rebaudiana* on reproduction of female rats. Southeast Asian J. Trop Med. Public Health, (37):203-205.
- Kinghorn, A.D. (2002):** *Stevia* (The genus *Stevia*). Department of Medicinal Chemistry and Pharmacognosy. University of Illinois at Chicago, USA.
- Knudsen, D.; G. A. Peterson Pratt and P. F. Lithium (1982):** sodium, and potassium, Methods of Soil Analysis, Part 2. :pp 225-264.
- Miyazaki, Y.; H. Watanabe and T. Watanabe (1978):** Studies on the cultivation of *Stevia rebaudiana* Bertoni. Yield and stevioside content of 2-year-old plants. Eisi Shikenjo hokoku, Tokyo, Japane, (96): 86-89.
- Nigar F. and A. Mohammad (2011):** Role of growth regulators on *in vitro* regeneration and histological analysis in Indian ginseng (*Withania somnifera* L.) Dunal. Physiol Mol Biol Plants. 2012 Jan; 18(1): 59–67.
- Oddone, B. (1997):** How to grow stevia. Technical manual. Guarani Botanicals, Pawtucket, CT. <http://www.scribd.com/doc/6390515/Advances-in-Agronomy-Volume-89>.
- Olsen, S. R. and L. E. Sommers (1982):** Phosphorus, Methods of Soil Analysis, Part 2. pp 403-430.
- Oviedo, C.A. (1971):** Accion hipoglicemiente de la *Stevia rebaudiana* Bertoni (Kaa-he-e). Excerpta Medica. (208): 92-93.
- Sapna, S.; A. Kondalkar; M. Tailang and A. K. Pathak (2008):** PHCOG MAG: Research Article Pharmacognostic and Phytochemical Investigation of *Stevia rebaudiana*. Phcog Mag. Vol 4, Issue 13 (Suppl), PHCOG MAG. An official Publication of Phcog. Net.
- Slack, K. V.; R. C. Avekett; P. E. Gieenson and R. G. Lipscomb (1973):** Methods for collection and analysis of aquatic biological and microbiological samples. U.S. Geol. Surv.. Tech. Water Resour. Invest. Book.

- Soejarto, D. D.; C. M. Compadre; P. J. Medon; S. K. Kamath and A. D. Kinghorn, (1983):** Potential sweetening agents of plant origin. Econ. Bot., 37(1): 71-79.
- Shock, C.C. (1982):** Experimental cultivation of Rebaudi's stevia in California. Univ. California, Davis Agron. Progr. Rep. 122.
- Toyoda, K. and H. Matsui (1997):** Assessment of the carcinogenicity of stevioside in F344 rats. Food Chem. Toxicol., 35(6): 597-603.
- Tucker, A.; J. Brandling; and P. Fox (2009):** Improved record keeping with reading handovers. Nursing Management (Harrow) 16(8): 30-34.
- Yamazaki, T. and H. E. Flores (1991):** Examination of steviol glucosides production by hairy root and shoot cultures of *Stevia rebaudiana*. J. Natural Products, 54(4): 986-992.

دراسات فسيولوجية على نبات الإستيفيا

(*Stevia rebaudiana* Bertoni)

*إيمان فاروق أبو الليل** عبير محمد عبد الرحمن* الموافي عبده الموافي الغضبان

** محمد خليل خليل الدعدع.

*قسم النباتات الطبية والعطرية- معهد بحوث البساتين- مركز البحوث الزراعية- الجيزة- مصر.

** فرع فسيولوجيا النبات- كلية الزراعة- جامعة القاهرة- الجيزة- مصر.

تم إجراء تجربة أصص في الصوبة على نبات الإستيفيا لدراسة تأثير نوعين من الأسمدة النيتروجينية وهما نترات الأمونيوم ونترات البوتاسيوم كما تم دراسة تأثير معاملات TDZ- Kin- B.A. تحت كل منها على صفات النمو والتركيب الكيماوي. تفوق صنف Spanti على صنف China-1 في النمو (الوزن الطازج للنبات). أدت المعاملة ب. B.A. إلي حدوث زيادة طفيفة في الوزن الطازج للنبات مقارنة بمعاملات كل من TDZ و Kin. زاد تركيز عنصر Kin في أوراق نبات الإستيفيا تحت ظروف التسميد نترات البوتاسيوم مقارنة نترات الأمونيوم. أدت إضافة سماد نترات البوتاسيوم إلي حدوث زيادة طفيفة في تركيزات كل من كلوروفيل (أ، ب) و الكاروتينويدات مقارنة بمعامل إضافة نترات الأمونيوم.