

EFFECT OF ORGANIC MANURE, BIOFERTILIZER AND DIFFERENT SOIL MIXTURES ON THE PLANT GROWTH, YIELD, CHEMICAL PROPERTIES AND NUTRIENT UPTAKE OF *Stevia rebaudiana* leaves.

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ABSTRACT

The present study was conducted during the two growing seasons 2002 and 2003 at El-Nubaria Agric. Res. Station, Agric. Res. Center, Bihira Governorate, in order to investigate the effect of two soil mixtures (clay + sand), two organic manures (chicken + compost) and two biofertilizers (*Azotobacter chroococcum* + *A. vinalendii*) on growth, yield, chemical composition and leaf mineral contents of *Stevia rebaudiana* plant. Results showed that all growing media and biofertilizers enhanced the growth and chemical composition (chlorophyll mg/g f.w., Carbohydrates %, Stevioside % and leaf nutrients uptake). The medium containing clay + sand + chicken2 plus inoculation with biofertilizer produced the tallest plants, increased the number of shoots and leaves (number, area and fresh and dry weight). Also, the maximum content of total chlorophyll, total carbohydrates, Stevioside, nitrogen, phosphorus and potassium were highest from the plants cultivated in the same medium. In general, using chicken manures gave results better than using compost manure. However, the medium of clay + sand produced the shortest plants and other characters in both seasons. Based on the obtained results, it is recommended to inoculate *Stevia* seedlings with the biofertilizer and addition of organic manures to improve growth, yield and leaves quality, as well as to reduce the amount of the mineral fertilizers.

INTRODUCTION

Sweet herb, *Stevia rebaudiana* (Bert) Compositae family is a herbaceous perennial native to the highlands of Paraguay. A number of compounds in the leaf tissue have a potential food use as low-calorie sweeteners. *Stevia* shows its greatest potential as a natural - source alternative to the synthetic sweetening agents now available to diet-conscious, consumers. [Metivier and Viana, 1979; Shock, 1982; Soejarto & Kinghorn, 1983 and Tamura & Tabata, 1984].

The four major glycosides are stevioside, rebaudioside A, C and dulcoside. Glycoside levels in leaf tissue can vary according to the method of propagation, day length, fertilizer and agronomic practices [Brandle and Gijzen 1988, and Knighor, 2002], Biofertilizers are biological preparations that contain primarily patent strains of microorganisms which are safe for human, animal and environment. They are capable of nitrogen fixation and enhancing availability of nutrients. (Ahmed *et al*, 1997). Joiner (1981) reported that good potting mixtures must be retentive of sufficient water, fertilizer and allowing for an excellent aeration.

Several types of compost are available depending on the locality. Warman (1991) found that the application of organic fertilizers - increased the vegetative growth on Asparagus and improved the soil fertility. Ali, *et al*, (2001) stated that the organic matter could improve the soil water holding capacity, cation exchange capacity, nutrient retention and soil microbial activity.

Table (1-a): Mechanical and chemical analysis and water constants for the different soil types .

Soil analysis	Soil types	Clay	Sand
Mechanical:			
Clay %		49.10	7.40
Silt %		23.57	15.70
Fine sand %		18.50	56.10
Coarse sand %		08.65	20.80
Chemical:			
pH (1: 2.5)		7.20	7.70
E.C dsm- at 25C°		0.44	0.47
CaCO ₃ %		0.95	3.80
Organic matter %		1.80	0.90
Water constants at 0-10 cm depth			
Field capacity %		39.0	32.0
Permanent wilting point %		14.0	10.0
Available water %		25.0	22.0
Soluble cations (mg/100g soil):			
Ca ⁺⁺		7.27	1.8
Mg ⁺⁺		2.10	1.4
K ⁺		0.58	0.4
Na ⁺		6.60	2.6
Soluble anions (mg/100g soil):			
HCO ₃ ⁻		8.19	2.6
Cl ⁻		4.57	3.4
SO ₄ ⁻		3.79	0.2

Table (1-b): Some chemical composition of the dried organic manures used in the experiment

Properties	Chicken manure	Aerial compost
Organic manure type		
EC (dsm ⁻¹)	6.3	4.03
pH	8.1	7.3
Organic matter %		38.9
Organic Carbon %		28.2
C/N Ratio	37.50	33.6
Bulk density, Kg/m ³	12.6	430
Total N %	527.0	0.84
Total P %	2.97	1.76
Total K %	5.4	1.14
Moisture Content %	3.7	55.7
Soluble Cations (meqL⁻¹)	23.60	
Na ⁺	35.40	28.4
Ca ²⁺	76.50	61.2
Mg ²⁺	29.40	23.6
K ⁺	25.30	19.3
Soluble anions (meqL⁻¹)		
HCO ₃ ⁻	30.70	22.2
CL ⁻	34.90	24.3
SO ₄ ⁻²	100.70	85.0
Available micronutrients mg/100g		
Fe ⁺⁺	31.6	11.3
Zn ⁺	46.2	9.2
Mn ⁺	38.3	5.4
Cu ⁺	16.5	1.6

Zaghloul and Maghazy (2001) pointed out that the application of organic manure (animal or chicken manure) enhanced the vegetative growth and flowering of gladiolus.

Horn (1995) mentioned that compost is used most commonly as a soil amendment to improve soil properties and to supply nutrients to crops. It can also be used in container mixes, as a mulch, or in other ways. Compost is typically applied to fields at rates of 3 to 6 ton/acre or more.

Compost is the biologically active material that results from microbial decomposition of organic matter under controlled conditions. The production and use of compost in agriculture has many potential benefits including nutrient recycling, soil improvement, and enhanced crop growth.

Plant growth and leaf numbers/ plant were increased with using FYM and liquid fertilizer, where the best results were obtained at 175 g/plant and 6.25% liquid fertilizer/ plant, respectively (Goenadi, 1985).

Cattle manure improved soil fertility. The best mixtures for seedling growth consisted of sand clay loam soil, hen manure (10%V/V) and lime. (Cameiro, *et al.*, 1997).

The aim of this research was to investigate the effect of different growing media as well as the addition of different organic manure and two biofertilizers on the vegetative growth, yield and chemical composition of sweet herb *Stevia rebaudiana* leaves to obtain the highest economic yield.

MATERIALS AND METHODS

Pot experiments under open filed condition, were conducted during the seasons of 2002 and 2003 at the experimental Station of El-Nubaria, Hort. Res. Inst. Agric. Res. Center, Egypt.

The experiment was consisted of combinations of two soil (Clay + Sand), two organic manures (Chicken + Compost) and two biofertilizers (*Azotobacter chroococcum* + *A. Vinelandii*).

Mechanical and chemical analyses of the experimental soil as well as organic manures (chicken and aerial compost) are presented in Tables (1a) and (1b). Soil analysis were estimated according to Jackson (1973). The chicken and compost manure were prepared and analyzed according to Page *et al.*, (1982; Stoffella & Kahn, 2001 and Kannaiyan, 2002). The source of compost was prepared from a mixture of animal wastes and plant residues.

The amount of organic manure were mixed with the soil before packing into the pots and applied one month before transplanting.

The commercial biofertilizer (HALEX), a non-symbiotic N-Fixers which contains a mixture of cultures of efficient strains of *Azotobacter chroococcum* and *A. vinelandii* carried on talc powder was used as a suspension by adding 5g fertilizer per one liter of water. The soil of the treated plants was inoculated by adding 500 ml of the fertilizer suspension immediately after the final transplanting. Gum Arabic solution 4% was used as an adhesive material.

The experimental pots were randomly assigned in a completely randomized block design with four replicates as follows:

A- Uninoculated without biofertilizer (Bo).

- 1- Clay: Sand (1:1) by Volume (C+S) as a control
 - 2- Clay: Sand: Chicken (1: 1 : 1) (C+ S+ Chic₁)
 - 3- Clay: Sand: Chicken (1: 1 : 2) (C+ S+ Chic₂)
 - 4- Clay: Sand: Compost (1:1:1) (C+S+ Comp₁)
 - 5- Clay: Sand: Compost (1:1:2) (C+S+ Comp₂)
- B- An inoculation with the mixture of two biofertilizers were used on the above media treatments

Uniformity seedlings in length (12 ± 3 cm) produced from tissue culture central laboratory, A.R.C., Giza were planted in plastic pots. The pots of 30 cm depth and 40 cm diameter was filled with 12-20 kg mixed soil and growing media. Ten stevia seedlings were transplanted in each pot.

Each pot was fertilized with 3g P (15.5 % P_2O_5) and 1.5 g k (48% K_2O). These fertilizers were mixed with the soil, as the basic fertilizer before packing the pots. Ammonium sulphate (20.5 %N) was applied at a rate of 5g/pot in two equal doses every three months.

Planting date was in February each season, 2002 and 2003 .The first cut was 5cm above soil at the end of April. The second cut at the the end of July before flowering, to obtain the maximum accumulation of stevioside content were applied in the two years.

The pots were lightly irrigated for two weeks before being planted with tap water, to establish a good microbial activity for decomposing chicken and compost material before transplanting.

Each pot was irrigated (on the basis of soil water holding capacity) to about 75% of it. The frequencies of irrigation periods every three days were decreased in parallel with increasing growth and weather conditions.

Before the flowering season (July each season), the following data were recorded plant height (cm), shoots number, leaves (number, area, cm^2 and fresh & dry weight (g), total Chlorophyll, mg/g. Fresh weights were determined in the fresh leaves according to Witham, *et al.*, (1971).

- 1 In the dry leaves, total carbohydrates % were estimated as outlined in A.O.A.C (1995).
- 2 A sample of leaves/ treatment were taken, oven- dried at $70^\circ C$, ground, then 0.3 g was digested with sulfuric acid and hydrogen peroxide.
- 3 Suitable aliquots were taken for the determination of N,P,K, Ca and Mg, according to the methods of (AOAC., 1995).
- 4 Extraction and determination of stevioside, rebaudisoide A,C% from dried leaves of stevia was conducted according to Kohda *et al.*, (1976).

Data obtained during the two seasons of the study were statistically analysed and treatment means were compared by LSD test at 0.05 level according to Gomez and Gomez(1984).

RESULTS AND DISCUSSION

1- Effect of biofertilizer and growing media on vegetative growth:

Data in Table (2) showed that all tested growing media had stimulating effect on vegetative growth of stevia plant. In this concern, the

medium containing clay + sand + chicken2 was the most effective on promoting the growth parameter. This treatment significantly produced the tallest plants at 83.2 and 81.3 cm in the first and second seasons, respectively.

Table (2): Effect of biofertilizer and growing media on the vegetative growth of *Stevia* plant during the seasons of 2002 and 2003.

Treatments	Plant height cm	Shoots number	leaves number	leaves area cm ²	leaves fresh wt.kg/m ²	leaves dry wt.kg/m ²
7/2002 season						
Bo (C+S) control	34.3	5.1	21.8	3.17	0.9	0.12
C+S+chic.1	49.5	12.3	44.6	6.13	2.6	0.25
C+S+chic.2	56.6	14.7	52.7	6.31	2.8	0.28
C+S+Comp.1	46.3	11.0	41.3	6.08	2.4	0.24
C+S+Comp.2	53.9	13.5	50.5	6.27	2.5	0.26
B1 (C+S)	42.4	9.3	38.2	4.32	1.6	0.14
C+S+Chic.1	768	14.7	56.1	8.43	3.1	0.34
C+S+Chic.2	83.2	18.2	63.7	9.55	3.3	0.39
C+S+Comp.1	72.7	12.3	51.3	7.84	3.0	0.31
C+S+Comp.2	74.6	14.1	58.5	8.26	3.2	0.32
Mean	59.03	12.52	47.87	6.53	2.54	0.26
LSD at 0.05	4.7	2.6	3.5	0.53	0.14	0.07
7/2003 season						
Bo (C+S) control	35.4	5.2	20.9	3.13	0.8	0.13
C+S+Chic.1	48.1	12.4	44.1	6.16	2.7	0.26
C+S+Chic.2	57.2	14.8	52.5	6.33	2.9	0.27
C+S+Comp.1	45.4	11.3	41.6	6.11	2.4	0.25
C+S+Comp.2	52.6	13.9	52.9	6.29	2.6	0.27
B1 (C+S)	44.5	19.8	39.6	4.29	1.4	0.16
C+S+Chic.1	73.9	14.8	57.2	8.46	3.2	0.35
C+S+Chic.2	81.3	17.9	59.4	9.42	3.6	0.38
C+S+Comp.1	75.4	12.6	57.0	7.81	3.1	0.34
C+S+Comp.2	78.6	14.3	59.9	8.29	3.3	0.36
Mean	59.24	12.7	48.46	6.62	2.6	0.27
LSD at 0.05	4.6	3.5	3.4	0.51	0.13	0.006

The highest number of shoots as 18.2 shoots/ plant in the first season and 17.9 in the second season were recorded from the potting medium containing clay + sand + chicken2 + biofertilizer. Similar trend was obtained with the number of leaves per plant, where as the greatest number of leaves per plant as 63.7 in the first season and 59.4 in the second one were obtained from the growing medium containing clay + sand + chicken2 + biofertilizer. Then came the medium from biofertilizer + clay + sand + compost2 which gave 58.5 and 59.5 leaves in the first and second seasons, respectively. As a general data in Table (2) it is evident that, during the two seasons, the maximum expansion of *Stevia* plant leaves was obtained by inoculation of biofertilizer to growing media compared with control. These results may be attributed to the increase in leaves number or size or both. Similar trend of results was found by (Dahlzell, 1987, Dick and McCoy, 1993; Horn, 1995 and Atta - Alla *et al.*, 2002).

It seemed that the different potting media generally increased the fresh and dry weights of stevia leaves. The best results in this regard were recorded from the medium containing biofertilizer + clay + sand + chicken2. However, the medium with clay + sand 1:1 (v/v) produced the shortest plants, the lowest number of (shoots and leaves), leaf area (cm²) and their fresh and dry weight in the both seasons.

The above results clearly indicate that the application of compost or chicken manures to the potting mixtures gave satisfactory vegetative growth. Besides, biofertilization may enhance the accumulation of carbohydrates and proteins, consequently leaves dry matter could be increased. In general using chicken manure obtained results better than using aerial compost. Moreover, Zaghoul and Moghazy (2001) reported that the composting caused a reduction in particle size that resulted in reducing air capacity and increased easily available water content which was reflected in improving the growth.

2- Effect on chemical composition:

Data presented in Table (3) showed that the different growing media increased the total chlorophyll (a+b) contents in the fresh leaves as well as increased the percentages of total carbohydrates in the dry leaves of *Stevia* plant in comparison to control.

The best results in this concern were obtained from the medium containing biofertilizer + clay + sand + chicken2 followed by the medium from biofertilizer + clay + sand +comps2 .The control medium (clay + sand) followed by the medium from biofertilizer + clay + sand gave the lowest values. The same trend was observed in both seasons. Generally, using the chicken manure gave results better than the application of compost manure to the potting mixture.

As for the interaction between biofertilizer and growing media, data in Table (3) clearly showed that the inoculation of biofertilizer combined with medium containing clay + sand + chicken2 produced the highest values of total chlorophyll and total carbohydrates, which were 2.8mg/g fresh weight and 22.86% in the first season and 2.7 mg/g and 22.9% in the second season, respectively.

This increment may be due to the ability of the microorganisms to produce growth regulator substances. These phytohormones play an important role in plant growth through promoting photosynthesis, translocation and accumulation of dry matter within different plant parts (Lampkin and Padel, 1994 and Kannaiyan, 2002).

These results may be due to the enhancing effect of the suitable rate of the organic manure on increasing the availability and absorption of the essential nutrient elements, specially (Fe⁺⁺), Mg⁺⁺ and NH₄⁺ cations, which are necessary for enzymes activation and formation of chloroplasts and chlorophyll as reported by Goenadi (1985) and Stoffela and Kahn(2001). Data presented in Table (3) showed that the different growing media increased the stevioside (%), reboudioside A and C (%) content in dry leaves of stevia plant. The best results were obtained from the medium containing clay + sand + chicken then followed by the media containing clay + sand + compost besides inoculation by biofertilizer. These results were the same in

the two seasons. Similar results were obtained by Kohda, *et al.* 1976; Metivier and Viana, 1979; Brandie and Gijzen, 1988; Carneiro, *et al.* 1997 and Kinghorn, 2002.)

Table (3): Effect of biofertilizer and growing media on the chemical composition of leaves of *Stevia* plant during the seasons of 2002 and 2003.

Treatments	Total chlorophyll mg/g	Total carbohydrates %	Stevio side	Rebaud side A %	Rebaud side C %
7/2002 Season					
Bo (C+S) control	1.1	8.25	4.15	1.3	0.31
C+S+Chic.1	1.7	12.30	7.6	2.5	0.43
C+S+Chic.2	1.8	14.73	9.9	2.8	0.47
C+S+Comp.1	1.5	10.25	6.5	2.4	0.41
C+S+Comp.2	1.6	13.40	8.7	2.7	0.42
B1 (C+S)	1.3	9.70	4.6	1.8	0.38
C+S+Chic.1	2.7	22.32	10.2	3.5	0.73
C+S+Chic.2	2.8	22.86	11.3	3.7	0.86
C+S+Comp.1	2.4	21.24	9.5	3.5	0.51
C+S+Comp.2	2.6	21.78	10.4	3.6	0.62
Mean	1.95	15.81	8.88	2.78	0.51
LSD	0.32	2.08	1.3	0.46	0.09
7/2003 Season					
Bo (C+S) control	1.1	7.89	4.18	1.4	0.32
C+S+Chic.1	1.7	11.96	7.70	2.6	0.45
C+S+Chic.2	1.8	14.68	9.83	2.8	0.48
C+S+Comp.1	1.5	10.01	7.18	2.4	0.42
C+S+Comp.2	1.6	14.10	8.92	2.6	0.41
B1 (C+S)	1.1	9.65	4.57	1.7	0.43
C+S+Chic.1	2.3	21.77	10.36	3.4	0.33
C+S+Chic.2	2.7	22.90	11.29	3.7	0.84
C+S+Comp.1	2.5	21.31	9.67	3.3	0.53
C+S+Comp.2	2.6	22.49	10.73	3.4	0.70
Mean	1.89	15.67	8.38	2.73	0.49
LSD at 0.05	0.31	2.08	1.2	0.43	0.08

3- Effect on major nutrients content:

Data in Table (4) showed that nitrogen, phosphorus, potassium, magnesium and calcium contents in dry leaves of stevia plant were increased to the maximum values with biofertilizer inoculation and organic manure application. The lowest values were noticed with the control and non inoculated plants. These results may be due to the efficiency of growing media on improving the soil acidity via enhancing the chemical conditions leading to the absorption of phosphorus.

It is clear that the application of chicken manure to the potting medium raised the contents of N, P and K, except Ca and Mg in leaves in the uninoculated treatments. The medium containing clay + sand + chicken2 plus inoculation with biofertilizer was the most effective treatment. These

results were similar in both seasons. This increment might be attributed to the promoting effect of inoculation on the fixation of atmospheric nitrogen and consequent uptake of major elements.

These results are in agreement with those found by Ahmad *et al*, (1997). They reported that bacteria present in the biofertilizer promote substances or organic acids that enhance nutrient uptake.

In conclusion, the application of organic manure (chicken + compost) to the mixture of soil (clay + sand) could be used as an alternative medium for growing the *Stevia* plant. In addition, using the biofertilizer (*Azotobacter chroococcum* + *A. vinalendii*) is important to increase the leaves growth and stevioside yield and quality.

Table (4): Effect of biofertilizer and growing media on the percentages of nitrogen, phosphorus, calcium and magnesium in the leaves of *Stevia* plant during the seasons of 2002 and 2003.

Treatments	N %	P %	K %	Ca %	Mg %
7/2002 Season					
Bo (C+S) control	0.73	1.35	0.86	0.26	0.19
C+S+Chic.1	2.14	3.42	1.33	0.39	0.31
C+S+Chic.2	2.19	3.47	1.45	0.51	0.34
C+S+Comp.1	2.12	3.22	1.24	0.42	0.30
C+S+Comp.2	2.15	3.39	1.36	0.48	0.31
B1 (C+S)	0.96	1.84	1.18	0.31	0.19
C+S+Chic.1	2.50	3.51	2.55	0.38	0.31
C+S+Chic.2	2.77	3.63	2.63	0.39	0.32
C+S+Comp.1	2.40	3.45	2.36	0.36	0.28
C+S+Comp.2	2.60	3.57	2.44	0.37	0.30
Mean	2.05	3.08	1.50	0.41	0.28
LSD at 0.05	0.45	0.52	0.74	0.26	0.17
7/2003 Season					
Bo (C+S) control	0.76	1.30	0.89	0.29	0.18
C+S+Chic.1	2.16	3.44	1.35	0.39	0.20
C+S+Chic.2	2.23	3.49	1.42	0.53	0.25
C+S+Comp.1	2.18	3.33	1.26	0.44	0.23
C+S+Comp.2	2.15	3.38	1.38	0.52	0.22
B1 (C+S)	1.10	1.86	1.16	0.31	0.20
C+S+Chic.1	2.8	3.54	2.53	0.36	0.30
C+S+Chic.2	2.81	3.62	2.62	0.39	0.31
C+S+Comp.1	2.77	3.43	2.35	0.36	0.25
C+S+Comp.2	2.79	3.61	2.48	0.38	0.27
Mean	2.17	3.1	1.74	0.39	0.24
LSD at 0.05	0.47	0.54	0.78	0.24	0.15

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تأثير الأسمدة العضوية والحيوية ومخاليط من التربة علي النمو والإنتاج والخصائص الكيميائية وامتصاص العناصر في أوراق نبات الإستيفيا .

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جيزة - مصر

أجريت هذه الدراسة خلال موسمي 2002، 2003 بمحطة البحوث الزراعية بالنوبارية بمحافظة البحيرة والتابعة لمركز البحوث الزراعية وذلك لدراسة تأثير خليط من التربة (الطينية والرملية)، (نوعان من الأسمدة العضوية) سماد الكتكوت، الكمبوست (نوعان من الأسمدة الحيوية) أزوتوبكتر (علي النمو والإنتاجية والتركيب الكيميائي للأوراق ومحتواها من العناصر الغذائية لنبات الإستيفيا .

وقد أوضحت النتائج أن كل البيئات المستخدمة زادت من نمو النباتات وخاصة بعد إضافة الأسمدة الحيوية إليها .

وأعطت البيئة المكونة من الطين والرمل وسماد الكتكوت بنسبة 2 : 1 : 1 بعد تلقحها بالأسمدة الحيوية أفضل النتائج حيث زاد معنوياً نمو الأوراق وكذلك التركيب الكيميائي لها مثل (الكلوروفيل - الكربوهيدرات - نسبة الإستيفوسيد، الريبوسيد أ، ج) علاوة علي زيادة امتصاص الأوراق للعناصر المعدنية الكبرى مثل النتروجين، الفوسفور، البوتاسيوم، الكالسيوم والماغسيوم بالمقارنة بالكنترول والتي أعطت أقل النتائج وذلك خلال موسمي الزراعة .

أكدت النتائج أن استخدام سماد الكتكوت أفضل في كثير من الحالات عن سماد الكمبوست . وبناء عليه يمكن التوصية باستخدام بيئة مكونة من الطين والرمل والأسمدة العضوية بعد تلقحها ببعض الأسمدة الحيوية لشتلات نبات الإستيفيا لزيادة النمو والمحصول والمحتوي من المواد الفعالة للحصول علي نباتات عالية الجودة وكذلك تخفيض المستخدم من الأسمدة المعدنية للحفاظ علي الصحة والبيئة .